DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

EFFECTS OF ANAEROBIC AND AEROBIC SEQUENTIALS IN THE TREATMENT OF POLYAROMATIC HYDROCARBONS (PAHs) FROM A PETROCHEMICAL INDUSTRY WASTEWATER

by
Oğuzhan GÖK

October, 2012
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EFFECTS OF ANAEROBIC AND AEROBIC SEQUENTIALS IN THE TREATMENT OF POLYAROMATIC HYDROCARBONS (PAHs) FROM A PETROCHEMICAL INDUSTRY WASTEWATER

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Ph.D. THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"EFFECTS OF ANAEROBIC AND AEROBIC SEQUENTIALS IN THE TREATMENT OF POLYAROMATIC HYDROCARBONS (PAHs) FROM A PETROCHEMICAL INDUSTRY WASTEWATER"** completed by **OĞUZHAN GÖK** under supervision of **PROF. DR. DELIA TERESA SPONZA** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy.

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Oğuzhan GÖK

EFFECTS OF ANAEROBIC AND AEROBIC SEQUENTIALS IN THE TREATMENT OF POLYAROMATIC HYDROCARBONS (PAHs) FROM A PETROCHEMICAL INDUSTRY WASTEWATER

ABSTRACT

The aerobic and sequential anaerobic/aerobic treatment of fifteen PAHs [acenaphthene (ACT), fluorene (FLN), phenanthrene (PHE), anthracene (ANT), carbazole (CRB), fluoranthene (FL), pyrene (PY), benz[a]anthracene (BaA), chrysene (CHR), benz[b]fluoranthene (BbF), benz[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (IcdP), dibenz[a,h]anthracene (DahA), benzo[g,h,i]perylene (BghiP)] were studied in the aerobic continuous stirred tank reactor (CSTR) and sequential anaerobic inverse turbulent bed reactor (ITBR)/aerobic CSTR system from the real petrochemical industry wastewater. Among the biosurfactants [(Rhamnolipid (RD, Emulsan (EM) and Surfactin (SR)] used it was found that the maximum PAH yields were obtained with RD biosurfactant at optimum sludge retention times (SRTs) and hydraulic retention times (HRTs). RD decreased significantly the inert chemical oxygen demand (COD) and the slowly degradable COD concentrations in the effluent of CSTR at optimum SRT and HRT. The main removal mechanism of the total PAHs was biodegradation. The maximum total PAH yields were observed with RD biosurfactant in the anaerobic ITBR system at optimum HRTs. The contribution of aerobic reactor to the removal of PAH in the sequential system was the biodegradation of the PAHs, of the PAH metabolites and of the dissolved COD remaining from the anaerobic reactor. The PAHs were mainly biodegraded according to the Monod and to the Modified Stover Kincannon in aerobic and anaerobic reactors, respectively. The PAHs exhibited inhibitions according to the competitive kinetic at high biosurfactant concentrations. High acute toxicity removals were observed in sequential reactor at optimum HRT and RD concentration using the *Daphnia magna* and *Vibrio fischeri*. The electric energy obtained from the methane in the anaerobic reactor can be used to recover partly the total electricity expenses for the sequential reactor.

Keywords: anaerobic inverse turbulent bed reactor, aerobic continuous stirred tank reactor, biosurfactant, polycyclic aromatic hydrocarbons, petrochemical industry wastewater, toxicity

ANAEROBİK VE AEROBİK KADEMELERİN BİR PETROKİMYA ENDÜSTRİSİ ATIKSUYUNDAKİ POLİAROMATİK HİDROKARBONLARIN (PAH) GİDERİMİNE ETKİLERİ

ÖZ

Gerçek bir petrokimya endüstrisi atıksuyunundaki onbeş adet çok halkalı aromatik hidrokarbon (PAH) [asenaftilen (ACT), floren (FLN), fenantren (PHE), antrasen (ANT), karbazol (CRB), floranten (FL), piren (PY), benz[a]antrasene (BaA), krizen (CHR), benz[b]floranten (BbF), benz[k]floranten (BkF), benzo[a]pirene (BaP), indeno[1,2,3-cd]pirene (IcdP), dibenz[a,h]antrasen (DahA), benzo[g,h,i]perilen (BghiP)]'ların, aerobik sürekli karışımlı tank reaktör (SKTR) ve ardışık anaerobik ters türbülanslı yatak reaktör (TTYR)/aerobik SKTR sisteminde, aerobik ve ardışık anaerobik/aerobik arıtımı çalışılmıştır. En yüksek PAH giderimi, optimum çamur bekletme süresi (ÇBS) ve hidrolik bekletme süresi (HBS)'nde, kullanılan biosurfaktanlardan [Rhamnolipid (RD), Emulsan (EM) ve Sürfaktin (SR)] arasından RD ile elde edilmiştir. SKTR sisteminin çıkış atıksuyunda inert kimyasal oksijen ihtiyacı (KOİ) ve yavaş ayrışabilen KOİ konsantrasyonunu optimum ÇBS ve HBS'de, RD biyosürfaktanı ile önemli ölçüde azaltmıştır. Toplam PAH'ların ana giderim mekanizması biyolojik parçalanma ile gerçekleşmiştir. Anaerobik TTYR sistem içerisinde optimum HBS'de RD biyosürfaktanı ile en yüksek toplam PAH giderimleri elde edilmiştir. Anaerobik reaktörde arıtılamayan PAH'lar, PAH'ların ara ürünleri ve çözünmüş KOİ'nin biyolojik olarak ayrışmasına ardışık sistemde yer alan aerobik reaktör katkı sağlamıştır. PAH'ların biyolojik ayrışması, aerobik reaktörde Monod ve anaerobik reaktörde modifiye edilmiş Stover Kincannon kinetik modeline göre gerçekleşmiştir. Yüksek biyosürfaktan konsantrasyonlarında, PAH'ların inhibisyonu competitive kinetik modeline göre gerçekleşmiştir. Ardışık reaktörde optimum HBS ve RD konsantrasyonunda *Daphnia magna* ve *Vibrio fischeri* kullanılarak yapılan akut toksisite testlerinde yüksek toksisite giderim verimleri gözlenmiştir. Ardışık reaktörde tüketilen elektrik enerjisi maliyetinin bir kısmı anaerobik reaktörde elde edilen metan gazından karşılanmıştır.

Anahtar kelimeler: anaerobik ters türbülanslı yatak reaktör, aerobik sürekli karışımlı tank reaktör, biosurfaktan, poliaromatik hidrokarbonlar, petrokimya endüstrisi atıksuyu, toksisite

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CHAPTER ONE
INTRODUCTION

1.1 Introduction

Wastewater treatment plants, especially those serving both urban and industrial areas, consistently receive complex mixtures and a wide variety of organic pollutants. Groups of compounds present in these mixtures include polycyclic aromatic hydrocarbons (PAHs), which are listed by the US-EPA and the EU as priority pollutants (Busetti et al., 2006; European Commission, 2001; Manoli and Samara, 2008). Their concentrations, therefore, need to be controlled in treated wastewater effluents due to their toxic, mutagenic and carcinogenic properties (Busetti et al., 2006). The International Agency for Research on Cancer (IARC) has identified 16 PAHs [Naphthalene (NAP), acenaphthene (ACT), fluorene (FLN), phenanthrene (PHE), anthracene (ANT), carbazole (CRB), fluoranthene (FL), pyrene (PY), benz[a]anthracene (BaA), chrysene (CHR), benz[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (IcdP), dibenz[a,h]anthracene (DahA), benzo[g,h,i]perylene (BghiP)] including 6 of the 16 Environmental Production Agency (EPA) regulated PAHs, as potential carcinogens (Zhang et al., 2012a; Guo et al., 2007). They have detrimental effects on the flora and fauna of affected habitats, resulting in the uptake and accumulation of toxic chemicals in the receiving bodies, serious health problems and/or genetic defects in humans (Fatone et al., 2011; Chauhan et al., 2008; Chen and Liao, 2006). Due to the carcinogenicity and/or mutagenicity of certain members of the PAH class, their presence in treated wastewater has been subjected to legislative control and some standards have already been established for PAH pollutants (European Commission, 2001). New draft directives of the aforementioned Councils and commissions have been released with the goal of regulating the maximum allowable concentrations of PAHs in the sewage sludge and in industrial effluents discharged to the receiving bodies.

The fate of PAHs in the environment is associated with both abiotic and biotic factors including volatilization, adsorption and microbial transformation (Pathak et al., 2009). The low solubility and high hydrophobicity of PAHs limit their ability to be transported into microbial cells and thus be biodegraded. Microbial processes are considered as the most significant route for PAH removal. Relatively few studies have been published in which the fate of PAHs in activated sludge treatment systems has been examined (Stringfellow and Alvarez-Cohen, 1999; Dobbs et al., 1988). Some investigators have considered the fate of PAHs through the biological reaction stage only in an aeration basin (Namkung and Rittmann, 1987), whilst others (e.g. Clark et al., 1995) have investigated the removal of PAHs in a primary clarifier. Most studies suggest that PAHs sorption onto biosolids present in activated sludge is an important removal mechanism (Namkung and Rittmann, 1987). Several studies have examined the relative role of biodegradation in the fate of PAHs in activated sludge systems (Manoli and Samara, 2008; Artola-Garicano et al., 2003; Stringfellow and Alvarez-Cohen, 1999). Activated sludge is the most widely used biological wastewater treatment process to treat petroleum refinery industry wastewaters in Turkey. However, the removal efficiencies of PAHs are low, for instance 25%-40%, in the conventional aerobic activated sludge reactor system treating this wastewater in Izmir-Turkey, since the Turkish Water Pollution Control regulation (Water Pollution and Control regulation, 2004) has no limitation for PAHs concentrations in the effluent discharges (Water Pollution and Control Regulation, 2004; Regulation for Control of Pollution Causing by the Toxic Substances around Water and Environment, 2005).

The greater parts of the PAHs are sent to the receiving bodies without treatment and accumulate in the aquatic ecosystem. Currently, available information regarding the effects of biosurfactant addition on enhanced biodegradation of petrochemical industry wastewater containing mixtures of PAHs with high rings are sparse for a CSTR system. As aforementioned, the operation of the aerobic activated sludge processes treating petrochemical industry wastewaters should be managed effectively to remove all the PAHs in İzmir Turkey.

Potential advantages of biosurfactants include their unusual structural diversity that may lead to unique properties, the possibility of cost-effective production, and their biodegradability (Mulligan et al., 2001). These properties make biosurfactants a promising choice for applications in enhancing PAH degradation. Many batch and continuous reactor studies have been conducted to investigate the use of surfactants to increase PAHs degradability (Zhou and Zhu, 2007; Yu et al., 2007). Surfactants have been shown to enhance both biodegradation and reaction rates (Yu et al., 2007). The last two researchers reported that biosurfactants like Rhamnolipid (RD), Emulsan (EM), Surfactin (SR) and glycolipid are surface-active molecules that have both hydrophobic and hydrophilic domains and are capable of lowering the surface tension of PAHs with high benzene rings. Therefore, the surfactants increased the hydrophobic substrate solubility and provide a less aggressive environment for bacterial cells. Zhou and Zhu, (2007) and Yu et al., (2007) also reported that the biosurfactants mentioned above are able to shorten the extended lag phase of bacteria for PAH biotransformation. Furthermore, they mentioned that the biochemical pathways of the biodegradation of PAHs depend, mainly, on aerobic conditions with the aforementioned biosurfactants. These biosurfactants can be used for degradation of most PAHs with four, five rings and an aerobic catabolism of a PAH molecule by bacteria occurs via oxidation of the PAH with dioxygenase enzyme system. Studies concerning the positive effects of biosurfactants on the biodegradation of different PAHs, however, dealt mostly with only naphthalene (Chauhan et al., 2008) PY, PHE (MacNally et al., 1998), FLN, BaP (Busetti et al., 2006), and ANT (Santos et al., 2008) in synthetic wastewater samples.

The anaerobic inverse turbulent bed reactor (ITBR) system show several advantages compared to classical up-flow and down-flow fluidization (Buffiere et al., 2000; Cresson et al., 2007). These advantages are the bed height which is capable of controlling the results automatically from the location of the injection device, simpler gas injection that reduce clogging problems and the low energy requirement, that is allowed by the low fluidization velocities (Buffiere et al., 2000; Cresson et al., 2007). The main advantages of the ITBR were: the down-flow configuration enables over coated particles to be recovered in the bottom of the bed. Moreover, the liquid

and the biogas are flowing in opposite directions, which help for bed expansion the expansion of a floating carrier is also possible under an up-flow current of gas only. This phenomenon called pseudo-fluidization. The gas bubbles generate downward liquid motions and apparent bed expansion. There are several studies on the anaerobic biodegradation of aliphatic and mono aromatic hydrocarbons (Widdel and Rabus, 2001; Hongwei et al., 2004; Callaghan et al., 2006; Mohamed et al., 2006; Delgadillo-Mirquez et al., 2011) and few studies are available on the anaerobic biodegradation of PAHs with low molecular weight (Zhang and Bennett, 2005; Tsai et al., 2009; Lu et al., 2011). No study was found in the recent literature investigating the aerobic CSTR, anaerobic ITBR and sequential aerobic CSTR/anaerobic ITBR for biodegradability of 15 PAHs with biosurfactants.

1.2 The Reasons of this Ph. D. Study

The studies performed until now contained only the removals of some PAHs under aerobic conditions with the utilization of the surfactants (Zheng and Obbard, (2002), Bautista et al., 2009; Li and Chen, 2009; Grimberg et al., 1995; Volkering et al., 1995; Laha and Luthy, 1992; Jin et al., 2007) excluding biosurfactants (Zhang et al., 2012b; Haritash and Kaushik, 2009; Habe and Omiri 2003; Grund et al., 1992). The literature surveys showed that the studies containing the anaerobic biodegradation of PAHs are very few (Dou et al., 2010; Chakraborty and Coates, 2004; Annweiler et al., 2000; Meckenstock et al., 2000; Makkar and Rockne, 2003; Zhang et al., 1997; McNally et al., 1998; Coates et al., 1996). No study was found investigating the anaerobic biodegradability of PAHs in the presence of biosurfactants. Similarly, a study was not found investigating the sequential anaerobic/aerobic reactor treatability of the petrochemical industry wastewaters in the recent literature. Furthermore, the ITBR reactor was not investigated before.

1.2.1 For Aerobic Continuous Stirred Tank Reactor (CSTR)

No study was found investigating the removal of 15 PAHs (ACT, FLN, PHE, ANT, CRB, FL, PY, BaA, CHR, BbF, BkF, BaP, IcdP, DahA and BghiP) with the

addition of some biosurfactants [Rhamnolipid (RD), Emulsan (EM) and Surfactin (SR)] throughout aerobic CSTR system. The effects of sludge retention times (SRTs) and biosurfactants concentration on the removal of the 15 PAHs have not investigated for a real petrochemical industry wastewater before.

No study was found effects of increasing SRTs on the removals of COD_{dis} and COD subcategories [dissolved COD (COD_{dis}, readily degradable COD (COD_{rd}), slowly degradable COD_{sd}, inert COD (COD_i) and inert microbial product COD (COD_{mp})] in a real petrochemical industry wastewater with RD biosurfactant in the aerobic CSTR system.

No study was found effects of some environmental conditions (temperature, dissolved oxygen, electron acceptors and pH) on the removals of total PAHs in a real petrochemical industry wastewater in the aerobic ITBR system.

No study was found to explain the main removal mechanisms for total PAHs in a real petrochemical industry wastewater with/without RD biosurfactant under aerobic conditions.

No study was found investigating the metabolites of some PAHs namely ACT, PHE, FLN, BaP, DahA and IcdP in a real petrochemical industry wastewater under aerobic conditions with and without RD biosurfactant.

No study was found investigating the acute toxicity responses of total PAHs in a real petrochemical industry wastewater to bacteria (*Vibrio fischeri*) in Microtox test and to water flea (*Daphnia magna*) in *Daphnia magna* acute toxicity tests under aerobic conditions with and without RD.

No study was found investigating the biodegradation and the inhibition kinetics of PAHs in a real petrochemical industry wastewater in the presence of biosurfactants (RD, EM and SR).

No study was found evaluating the cost analysis, in a real petrochemical industry wastewater under aerobic conditions.

1.2.2 For Anaerobic Inverse Turbulent Bed Reactor (ITBR)

No study was found investigating the removal of COD_{dis}, total and individual 15 PAHs (ACT, FLN, PHE, ANT, CRB, FL, PY, BaA, CHR, BbF, BkF, BaP, IcdP, DahA and BghiP) with the addition of biosurfactant throughout anaerobic ITBR. The effects of hydraulic retention times (HRT) and increasing biosurfactant concentrations on the removal of the 15 PAHs have not investigated for a real petrochemical industry wastewater before. No study was found investigating the measurement of biofilm thickness on the carrier material of a real petrochemical industry wastewater containing PAHs in the presence of biosurfactants in the anaerobic ITBR system.

No study was found investigating the acute toxicity responses of total PAHs in a real petrochemical industry wastewater to bacteria (*Vibrio fischeri*) in Microtox test and to water flea (*Daphnia magna*) in *Daphnia magna* acute toxicity tests under anaerobic conditions with and without RD.

No study was found investigating the biodegradation and gas kinetic models of COD_{dis} and PAHs in a real petrochemical industry wastewater in the presence of biosurfactants under anaerobic conditions. No study was found evaluating the cost analysis, specific energy estimation in a real petrochemical industry wastewater in the presence of biosurfactant under anaerobic conditions.

1.2.3 For Sequential Anaerobic Inverse Turbulent Bed Reactor (ITBR)/Aerobic Continuous Stirred Tank Reactor (CSTR)

No study was found investigating the removal of 15 PAHs (ACT, FLN, PHE, ANT, CRB, FL, PY, BaA, CHR, BbF, BkF, BaP, IcdP, DahA and BghiP) with the addition of biosurfactant throughout sequential anaerobic ITBR/aerobic CSTR

system. The effects of hydraulic retention times (HRT), organic loading rates (OLRs) on the removal of the 15 PAHs have not investigated for a real petrochemical industry wastewater before.

No study was found investigating the acute toxicity responses of total PAHs in a real petrochemical industry wastewater to bacteria (*Vibrio fischeri*) in Microtox test and to water flea (*Daphnia magna*) in *Daphnia magna* acute toxicity tests in the sequential anaerobic ITBR/aerobic CSTR system with biosurfactant. No study was found evaluating the cost analysis and specific energy estimation in a real petrochemical industry wastewater in the presence of biosurfactant in the sequential anaerobic ITBR/aerobic CSTR system with biosurfactant. The lacks in the literature mentioned above were the subject of this Ph.D. thesis.

1.3 The Objectives of this Ph.D Thesis

The general objective of this Ph.D. thesis was to evaluate the performance of the aerobic continuous stirred tank reactor (CSTR), anaerobic inverse turbulent bed reactor (ITBR) and sequential anaerobic ITBR/aerobic CSTR process on the treatment efficiencies of a real petrochemical industry wastewater. The specific objectives of this study are as follows:

1.3.1 For Aerobic Continuous Stirred Tank Reactor (CSTR)

To determine the COD_{dis} and PAHs removal efficiencies from a real petrochemical industry wastewater in an aerobic CSTR system at increasing hydraulic retention times (HRTs) (1.38-1.83-2.75-5.5-11 days) and sludge retention times (SRTs) (5-10-25-40 days).

To determine the effects of biosurfactants namely as rhamnolipid (RD), Surfactin (SR) and Emulsan (EM) on COD_{dis} and PAHs biodegradation in an aerobic CSTR system at increasing SRTs.

To determine the removal efficiencies COD subcategories [dissolved COD (COD_{dis}, readily degradable COD (COD_{rd}), slowly degradable COD_{sd} , inert COD (COD_i) and inert microbial product COD (COD_{mp})] at a SRT of 25 days with and without RD under aerobic conditions.

To determine the effects of some environmental conditions (temperature, dissolved oxygen, electron acceptors and pH) on the removals of total PAHs in a real petrochemical industry wastewater in the aerobic ITBR system.

To determine the main removal mechanisms (biodegradation, adsorption and volatilization) of PAHs under batch aerobic conditions.

To determine the metabolites of some PAHs namely ACT, PHE, FLN, BaP, DahA and IcdP in a real petrochemical industry wastewater under aerobic conditions with and without RD biosurfactant.

To determine the acute toxicities of PAHs to *Daphnia magna* and *Vibrio fischeri* under aerobic conditions.

To determine biodegradation kinetic models of COD and PAH and inhibition kinetics of PAHs under aerobic conditions with and without RD.

1.3.2 For Anaerobic Inverse Turbulent Bed Reactor (ITBR)

To determine the removal efficiencies of COD_{dis}, total PAHs and individual 15 PAHs (ACT, FLN, PHE, ANT, CRB, FL, PY, BaA, CHR, BbF, BkF, BaP, IcdP, DahA and BghiP) with the addition of biosurfactant throughout anaerobic ITBR. Furthermore, to determine of the effects of hydraulic retention times (HRT) and biosurfactants concentration on the removal of the total PAHs have not investigated for a real petrochemical industry wastewater.

To determine the measurement of biofilm thickness on the carrier materials of a real petrochemical wastewater containing PAHs in the presence of biosurfactants in the anaerobic ITBR system.

To determine the acute toxicity responses of total PAHs in a real petrochemical industry wastewater to bacteria (*Vibrio fischeri*) in Microtox test and to water flea (*Daphnia magna*) in *Daphnia magna* acute toxicity tests under anaerobic conditions with and without RD.

To determine the biodegradation COD_{dis}, total PAH and gas kinetic models of in a real petrochemical industry wastewater in the presence of biosurfactants under anaerobic conditions. Furthermore, to determine the cost analysis, specific energy estimation in a real petrochemical industry wastewater in the presence of biosurfactant under anaerobic conditions.

1.3.3 For Sequential Anaerobic Inverse Turbulent Bed Reactor (ITBR)/Aerobic Continuous Stirred Tank Reactor (CSTR) System

To determine the removal of 15 PAHs (ACT, FLN, PHE, ANT, CRB, FL, PY, BaA, CHR, BbF, BkF, BaP, IcdP, DahA and BghiP) with the addition of biosurfactant throughout sequential anaerobic ITBR/aerobic CSTR system. The effects of hydraulic retention times (HRTs) on the removal of the 15 PAHs have not investigated for a real petrochemical industry wastewater before.

To determine the acute toxicity responses of total PAHs in a real petrochemical industry wastewater to bacteria (*Vibrio fischeri*) in Microtox test and to water flea (*Daphnia magna*) in *Daphnia magna* acute toxicity tests in the sequential anaerobic ITBR/aerobic CSTR system with biosurfactant.

To determine the cost analysis and specific energy estimation in a real petrochemical industry wastewater in the presence of biosurfactant in the sequential anaerobic ITBR/aerobic CSTR system with biosurfactant.

CHAPTER TWO
PROPERTIES OF THE PETROCHEMICAL INDUSTRY WASTEWATER

2.1 Properties of the Petrochemical Industry Wastewater

The petrochemical industry is organized in four sectors: exploration and production of crude oil and natural gas, transport, refining and marketing and distribution. The petrochemical industry uses petroleum and natural gas based feed stocks such as naphtha, LPG, gas oil to produce plastics, rubber and fiber raw materials and other intermediates which are consumed by several sectors such as packaging, electronics, automotive, construction, textile and agriculture. Petrochemical industries use large quantities of water. Wastewater production strongly depends on the process configuration (Petkim, 2011). The properties of petrochemical industry wastewaters are given in Table 2.1.

Table 2.1 The properties of petrochemical industry wastewaters from literature (Lin et al., 2001; Patel and Madamwar, 2002; Ma et al., 2008; Khaing et al., 2010; Verma et al., 2010; Tobiszewski et al., 2012).

Parameter	**Values**	**Parameter**	**Values**
pH	5.0-9.6	Cd (mg/L)	0.003-0.005
Temperature ($^{\circ}$C)	18.9-24.6	Cr (mg/L)	0.004-0.008
Dissolved oxygen (DO)	1.5-2.9	Ni (mg/L)	0.02-0.04
Total COD (COD_{total})	600-4000	Pb (mg/L)	0.001-0.01
Dissolved COD (COD_{dis})	300-3500	Zn (mg/L)	0.30-0.10
BOD_5 (mg/L)	150-600	Fe (mg/L)	0.05-2.877
BOD_5/COD ratio	0.20-0.50	Cd (mg/L)	0.003-0.01
Total N (mg/L)	5-32	Cr (mg/L)	0.005-0.01
Total P (mg/L)	0.1-22	Ni (mg/L)	0.025-0.06
Ammonium (mg/L)	1.5-50	Pb (mg/L)	0.01-0.03
Nitrate (mg/L)	1.90-2.70	Mn (mg/L)	0.001-0.01
Nitrite (mg/L)	0.04-0.10	Co (mg/L)	0.001-0.004
Oil-grease (mg/L)	20-900	Mg (mg/L)	0.045-0.10
TSS (mg/L)	20-310	K (mg/L)	0.981-0.18
Total 15 PAHs (ng/mL)	65-300		

2.2 Polycyclic aromatic Hydrocarbons (PAHs)

2.2.1 Sources of PAHs

Polycyclic aromatic hydrocarbons (PAHs) are the widespread ubiquitous contaminants in the different compartments of the environments (Kastner et al., 1998; Juhasz and Naidu, 2000). These compounds are generally generated by natural and anthropogenic processes and can be introduced into the environments through various routes. Anthropogenic input from incomplete combustion, oil spills, urban runoff, domestic and industrial wastewater discharges, as well as atmospheric fallout of vehicle exhaust and industrial stack emission have caused significant accumulation of these compounds in the environments (Witt, 1995; Charlesworth et al., 2002; Domeio and Nerin, 2003; Doong and Lin, 2004; Crisafully et al., 2008; Delgado-Saborit et al., 2011). Due to their toxic, mutagenic, and carcinogenic characteristics, PAHs are considered to be hazardous to the biota and environments (Manoli and Samara, 1999; Pereira Netto et al., 2002).

The total concentrations of 16 PAHs in the influents to the five Norwegian wastewater treatment plants were within 0.2–1.3 μg/L, which is in the low range of what was reported (0.05–625 μg/L) in an European Community urban wastewater survey (Thornnton et al., 2001) and generally somewhat lower than the levels (1.3–8.0 μg/L) found in wastewaters in the Paris area (Blanchard et al., 2004). The most potent of the carcinogenic PAHs (BaP) was detected in the inlet to all but one of the wastewater treatment plants, with concentrations varying from 0.005 up to 0.028 mg/L with a mean of 0.010 μg/L. The observed BaP concentrations were low range of reported influent levels; varying between 0.002 and 0.104 μg/L for the Seine Aval treatment plant in Paris (Blanchard et al., 2004), varying between 0.020 and 0.077 μg/L for the wastewater treatment plant in Montreal (Pham and Proulx, 1997), 0.022 μg/L for the Thessaloniki municipal treatment plant (Manoli and Samara, 1999) and 0.297 μg/L for the Fusina WWTP in Venice (Busetti et al., 2006).

2.2.2 The Physical and Chemical Characteristics of the PAHs

Polycyclic aromatic hydrocarbons constitute a large and diverse class of organic compounds consisting of three or more fused aromatic rings in various structural configurations (Cerniglia, 1992; Arulazhagan and Vasudevan, 2011). The EPA classifies 16 PAHs as priority pollutants (EPA, 2002; Pierre et al., 2006 ; Khadhar et al., 2010). The chemical structures of the 16 PAHs are given in Figure 2.1. The physicochemical properties of the most common PAHs are presented in Table 2.2.

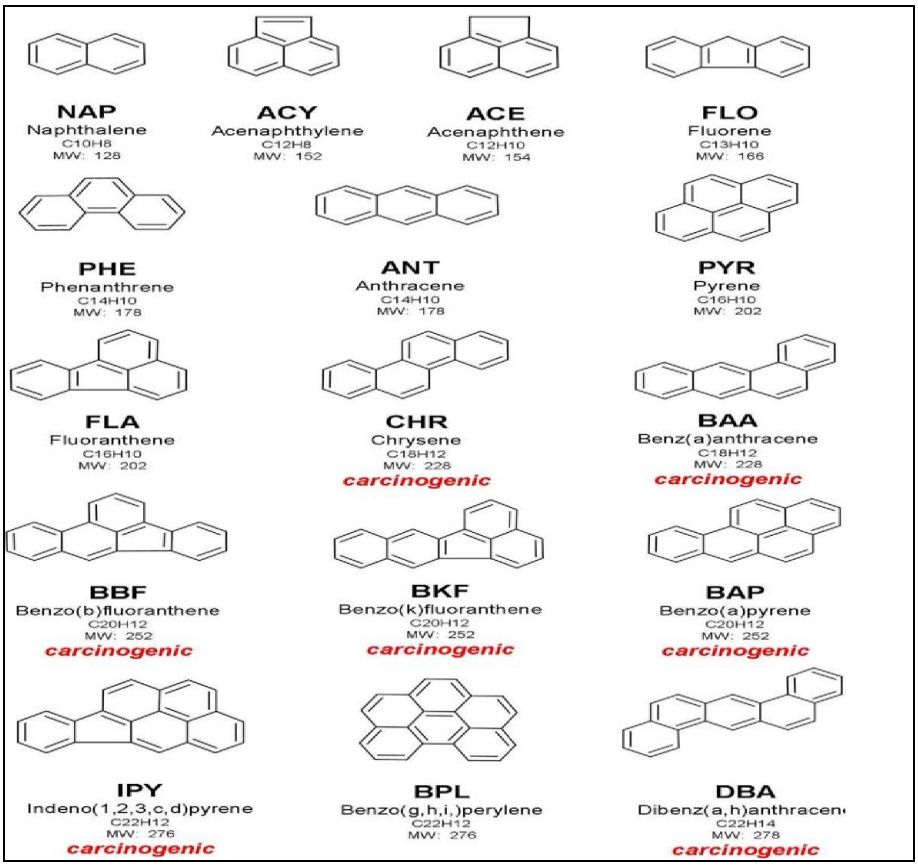

Figure 2.1 The chemical structures of the 16 PAHs

All PAHs are solids ranging from colorless to pale green-yellow. Molecular weights of PAHs ranged between 128 (Naphthalene; $C_{10}H_8$) and 278.4 [Dibenz(a,h)anthracene; $C_{22}H_{14}$] (See Figure 2.1 and Table 2.2). Higher molecular weight (HMW) PAHs (>3 aromatic rings) are relatively immobile because of their large molecular volumes. They are less water-soluble, less volatile and more lipophilic than lower molecular weight (LMW) PAHs (< 3 aromatic rings).

Table 2.2 Physicochemical properties of the PAHs

PAHs	Cas-No	Molecular Formula	MW (g/mol)	T_M (°C)	T_B (°C)	S_W (25 °C) (mg/L)	VP (25 °C) (mm Hg)	H (25 °C) (atm m^3 /mol)	log K_{OA} (25 °C)	log K_{OW}
ACT	83-32-9	$C_{12}H_{10}$	154	93	279	390E-02	2.15E-03	1.84E-04	6.52	3.94
FLN	86-73-7	$C_{13}H_{10}$	166	115	295	169E-02	6.00E-04	9.62E-05	6.9	4.18
PHE	85-01-8	$C_{14}H_{10}$	178	99	340	115E-02	1.21E-04	3.35E-05	7.68	4.46
ANT	120-12-7	$C_{14}H_{10}$	178	215	340	4.34E-02	2.67E-06	5.56E-05	7.71	4.45
CRB	86-74-8	$C_{12}H_9N$	167	246	355	1.80E-01	7.50E-07	1.16E06	8.03	3.72
FL	206-44-0	$C_{16}H_{10}$	202	108	384	2.60E-01	9.22E-06	8.86E-06	8.76	5.16
PY	129-00-0	$C_{16}H_{10}$	202	151	404	13.5E-02	4.50E-06	1.19E-06	8.81	4.88
BaA	56-55-3	$C_{18}H_{12}$	228	84	438	9.40E-03	2.10E-07	1.20E-05	10.28	5.76
CHR	218-01-9	$C_{18}H_{12}$	228	258	448	2.00E-03	6.23E-09	5.23E-06	10.30	5.81
BbF	205-99-2	$C_{20}H_{12}$	252	168	467	1.50E-03	5.00E-07	6.57E-07	11.34	5.78
BkF	207-08-9	$C_{20}H_{12}$	252	217	480	8.00E-04	9.70E-10	5.84E-07	11.37	6.11
BaP	50-32-8	$C_{20}H_{12}$	252	177	495	1.62E-03	5.49E-09	4.57E-07	11.56	6.13
IcdP	193-39-5	$C_{22}H_{12}$	276	164	536	1.90E-04	1.25E-10	3.48E-07	12.43	6.70
DahA	53-70-3	$C_{22}H_{14}$	278	270	524	2.49E-03	1.00E-10	1.23E-07	12.59	6.75
BghiP	191-24-2	$C_{22}H_{12}$	276	278	>500	2.60E-04	1.00E-10	3.31E-07	12.55	6.63

MW: Molecular weight, T_M: Melting point, T_B: Boiling point, S_W: Solubility in water, **VP:** Vapor pressure, **H:** Henry's law constant, **log K_{OW}**: Octanol-water coefficient, **log K_{OA}**: Octanol-air coefficient

PAHs have low to extremely low water solubility and also moderate to low vapour pressures. As a general rule, the hydrophobicity increases and the aqueous solubility decreases with an increase in the number of aromatic rings (Manoli and Samara, 1999).

2.2.3 Health Effects of PAHs

The most significant endpoint of PAH toxicity is cancer. PAHs generally have a low degree of acute toxicity to humans. Some studies have shown noncarcinogenic effects that are based on PAH exposure dose (Perera et al., 2012; Gupta et al. 1991). After chronic exposure, the non-carcinogenic effects of PAHs involve primarily the pulmonary, gastrointestinal, renal, and dermatologic systems (Crepeaux et al., 2012). Many PAHs are only slightly mutagenic or even nonmutagenic in vitro; however, their metabolites or derivatives can be potent mutagens (Kaivosoja et al., 2012). The carcinogenicity of certain PAHs is well established in laboratory animals. Researchers have reported increased incidences of skin, lung, bladder, liver, and stomach cancers, as well as injection-site sarcomas, in animals (Cernohorska et al., 2012). Animal studies show that certain PAHs also can affect the hematopoietic and immune systems and can produce reproductive, neurologic, and developmental effects (Winans et al., 2011; Zhang et al., 2012a; Xia et al., 2010; Dorea, 2008; Tsai et al., 2001). PAHs toxicity is very structurally dependent, with isomers (PAHs with the same formula and number of rings) varying from being nontoxic to being extremely toxic (Calderon-Segura et al., 2004). Thus, highly carcinogenic PAHs may be small or large. There are hundreds of PAHs compounds in the environment, but only seventeen of them are included in the priority pollutants list of United States Environmental Protection Agency (EPA, 2002; IARC, 2010).

2.3 Biosurfactants

2.3.1 Properties of Biosurfactants

Most of the biosurfactants are high molecular-weight lipid complexes, which are normally produced under aerobic conditions. This is achievable in their ex situ production in aerated bioreactors (Kosaric, 2001). The properties of biosurfactants are interest in: changing surface active phenomena, such as lowering of surface and interfacial tensions, wetting and penetrating actions, spreading, hydrophylicity and hydrophobicity actions, microbial growth enhancement, metal sequestration and anti-microbial action (Kosaric, 2001). The biosurfactant sources, classes and properties have been reviewed (Bognolo, 1999; Healy et al., 1996; Urum and Pekdemir, 2004). In general, biosurfactants can be classified as: glycolipids, hydroxylated and cross-linked fatty acids (mycolic acids), polysaccharide-lipid complexes, lipoprotein-lipopeptides, phospholipids, complete cell surface itself. Chemical structures of biosurfactants (RD, EM and SR) is given in Figure 2.2.

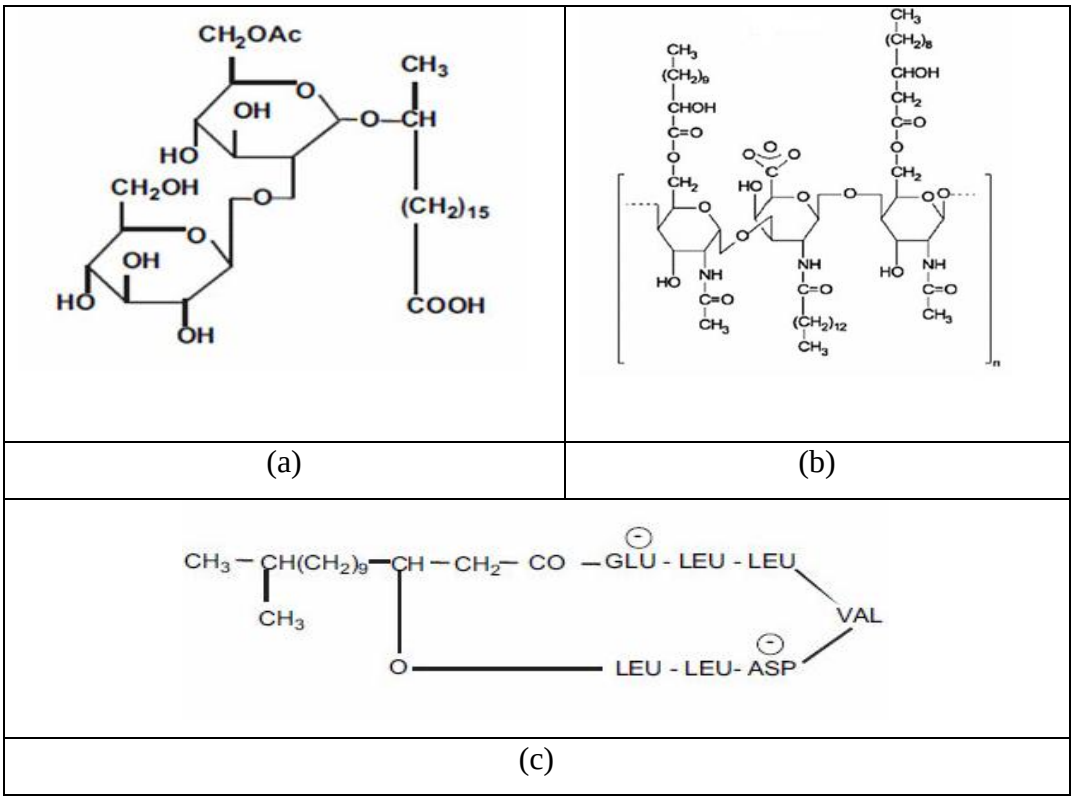

Figure 2.2 Chemical structures of biosurfactants (a) Rhamnolipid, (b) Emulsan and (c) Surfactin

The effectiveness of a biosurfactant is determined by its ability to lower the surface tension, which is a measure of the surface free energy per unit area required to bring a molecule from the bulk phase to the surface (Mulligan, 2005). In the presence of a biosurfactant, less work is required to bring a molecule to the surface and the surface tension is reduced. For example, a good biosurfactant can lower the surface tension of water from 72 to 35 mN/m and the interfacial tension (tension between non-polar and polar liquids) for water against n-hexadecane from 40 to 1 mN/m. The surface tension correlates with the concentration of the surface-active compound until the critical micelle concentration (CMC) is reached. Efficient biosurfactants have a low critical micelle concentration (i.e. less surfactant is necessary to decrease the surface tension). The CMC is defined as the minimum concentration necessary to initiate micelle formation (Mulligan, 2005, Mulligan and Gibbs, 1993). In practice, the CMC is also the maximum concentration of surfactant monomers in water and is influenced by pH, temperature and ionic strength. Some surfactants, known as biosufactants, are biologically produced by yeast or bacteria from various including sugar, oils, alkanes, and wastes (Wang and Mulligan, 2004; Rahman et al., 2003). Biosurfactants could easily be produced from renewable resources via microbial fermentation, making them have an additional advantage over chemically synthetic surfactants. The important challenges for the competitive production of biosurfactants include high yields, alternative low-cost substrates and cost-effective bioprocesses (Pornsunthorntawee et al., 2008). Some of the biosurfactants that have been studied using alternative low-cost substrates (Cameotra and Makkar 2004; Banat et al., 2000), soapstock and a by-product of the vegetable oil refining processes (Salihu et al., 2009), are surfactin produced by *Bacillus subtilis* and rhamnolipids produced by *P. aeruginosa*.

2.3.2 Advantages of the Biosurfactants

Surfactants are amphipathic molecules, which reduce the interfacial tensions between liquids, solids and gases and confer excellent detergency, emulsifying, foaming and other versatile chemical process (Hirata et al., 2009; Thanomsub et al., 2007). There are many advantages of biosurfacants if compared to their chemically synthesized

counterparts. They are less toxic, relatively low cost, more biodegradable, thus more environmentally friendly, and do not lose physicochemical properties at different temperatures, salinity and pH levels (Mulligan, 2005, Banat et al., 2000).

2.3.3 Environmental Application of Biosurfactants

Biosurfactants can be efficiently used in handling industrial applications (Kitamoto et al., 2009), control of oil spills (Yan et al., 2012; Bao et al., 2012; Urum and Pekdemir, 2004), biodegradation (Cerqueira et al., 2011) and detoxification of industrial wastewaters (Yin et al., 2009; Whang et al., 2008) and in bioremediation of contaminated soils (Pornsunthorntawee et al., 2008; Mulligan, 2009). The unique properties and diversity among biosurfactants make them likely candidates for replacement of chemically synthesized surfactants in biodegradation and bioremediation efforts and a broad range of other industrial applications as well. Zhang et al., (2009) investigated the removal of oil and grease using an activated sludge system. In this study, oil and grease and COD were removed with 95% and 94% yields, respectively, at a HRT of 30 hours in the presence of 90 mg/L RD. In the study performed by Whang et al. (2008) it was found that the diesel solubility increased with SR and RD addition up to 45 and 50 mg/L, respectively while the diesel biodegradation percentage increased up to 94% and 99% with SR and RD addition, respectively in aerobic batch reactor. Yin et al. (2009) investigated the solubilization of PHE in an aerobic condition with RD biosurfactant. The 50 mg/L RD biosurfactant were exhibited high performance of PHE solubilization with about 23 times higher solubility of PHE in wastewater than the control experiment.

2.3.4 Uptaken of Biosurfactants by Microorganisms in Wastewaters

The low water solubility of many hydrocarbons, especially the polycyclic aromatic hydrocarbons (PAHs), is believed to limit their availability to microorganisms, which is a potential problem for biodegradation of contaminated sites (Ron and Rosenberg, 2002). Schippers et al. (2000) suggested three approaches for the promotion of the biodegradation of PAHs by biosurfactants (Figure 2.3A–C).

In the first approach, bacteria are able to take up the pollutant from the micellar core (Miller and Bartha, 1989). In the second approach, biosurfactants increase the mass transfer of pollutants to the aqueous phase for further use by the bacteria. In the third approach, addition of surfactants changes the cell hydrophobicity, facilitating the direct contact between cells and aqueous phase. A fourth possible mechanism has been suggested by others in which surfactants help bacteria adsorb to particles occupied by pollutants, thus decreasing the diffusion path length between the site of adsorption and site of bio-uptake by the bacteria (Tang et al, 1998; Poeton et al., 1999; Makkar and Rockne, 2003) (See Figure 2.3-D).

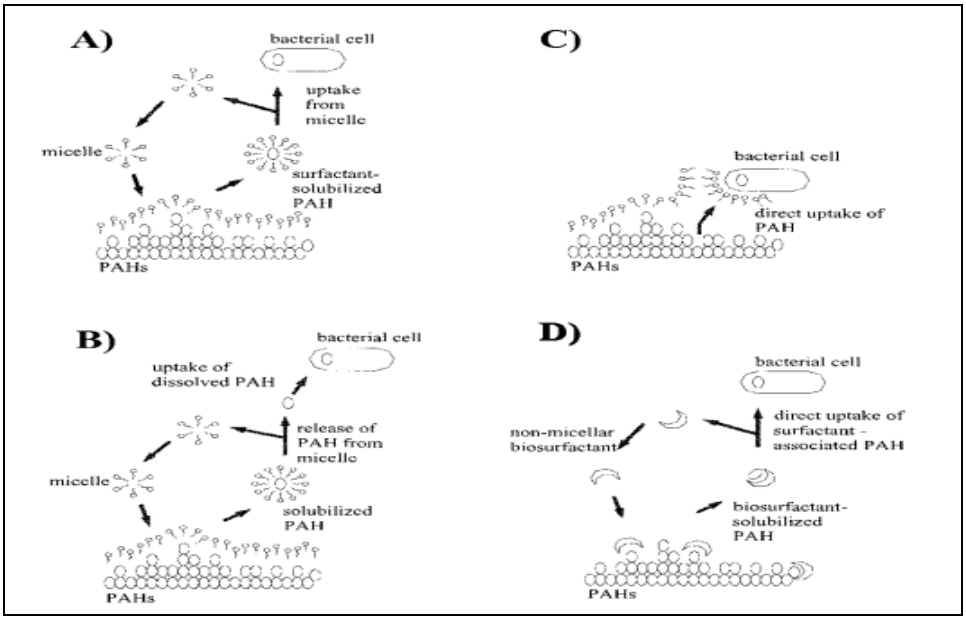

Figure 2.3 Mechanisms for PAHs bioavailability enhancement using biosurfactants (Makkar and Rockne, 2003)

2.3.5 Disadvantages of the Biosurfactants

Although the biosurfactants are cheap, for treatment of petrochemical industry wastewaters containing high PAH concentrations the utilized biosurfactant dose can be high. This can increase the cost spending for biosurfactant as reported by Haritash and Kaushik (2009). However, if the biosurfactants can be recovered and purified can be used again for the treatments will be performed in the future as reported by Li et al. (2009).

CHAPTER THREE
LITERATURE REVIEW FOR THE TREATMENT OF PAHs IN THE AEROBIC AND ANAEROBIC SYSTEM

3.1 Literature Review for the Treatment of PAHs in the Aerobic System

In a study performed by Dimoglo et al. (2004) the treatment process of a petrochemical industry wastewater consisted of a mechanical and physicochemical treatment such as oil–water separation and coagulation, followed by biological treatment within the integrated activated sludge treatment plant. Ahmed et al., (2011) investigated the performance of aerobic and anaerobic sequencing batch reactor (SBR) in the treatment of petroleum refinery wastewater. The average COD removal for the aerobic reactor, combined anaerobic-aerobic reactors and aerobic mixed with domestic wastewater achieved were found approximately, 91%, 91%, and 88%, respectively, at a influent COD concentration of 1066 mg/L, at HRT of 24 h and SRT of 2 days.

Zhang et al. (2011) investigated the performance of aerobic sequencing batch reactor (SBR) in the treatment of a petrochemical wastewater. Up to 64% COD and 30% total nitrogen removal were observed at an OLR of 2 g/L.day in the SBR was fed with real petrochemical wastewater containing 282 mg/L COD and 43 mg/L total nitrogen. The treatment performance of wastewater containing PAHs was investigated in an activated sludge system (Manoli and Samara, 2008). They found that removals of PAHs ranged between 28-67% in the primary, between 1 and 60%, and between 37 and 89% in the whole process and 92% COD_{dis} were removed at a HRT of 3 h and at an influent COD_{dis} concentration of 55 g/L in activated sludge system treating municipal and industry wastewater.

Bautista et al. (2009) investigated the effect of several surfactants (Tween-80, Triton X-100 and Tergitol NP-10) on the ability of different bacteria (*Enterobacter sp., Pseudomonas sp. and Stenotrophomonas sp.)* to degrade PAHs. High degree of PAHs degradation (>90%) was reached in 15 days in all experiments. On the other

hand, Triton X-100 and Tergitol NP-10 were not biodegraded and toxicity kept constant along time. However, PAHs-degradation rate was higher, especially by the action of *Enterobacter sp.* with Tween-80 or Triton X-100. In a study performed by Zhang et al (2009) it was reported that 63% COD and 68% total petroleum hydrocarbon was achieved in an oil field wastewater at a HRT of 32 h and an OLR of 0.28 kgCOD/m^3.day in activated sludge system.

The treatment performance of wastewater containing PAHs was investigated in an aerobic process (Zheng et al., 2007). Maximum PAH removal efficiencies were 95% at a SRT of 21 days. Three 3-ring PAHs were rapidly removed with more than 90% of reduction after only two weeks of treatment. The removal of 4, 5 and 6 ring PAHs started generally after 5 days of period and the final removal yields were weaker than for 3-ring PAHs. Yuan et al. (2000) studied that biodegradation of PAHs by a mixed culture. PHE degradation was enhanced by the individual addition of yeast extract, acetate, glucose or private. The mixed culture completely degraded PHE, ACT and PY alone at 28 h, 10 days and 12 days following treatment, respectively, but was ineffective in degrading either ANT or FLN. Average degradation rates were calculated at 0.18 mg/L.h for PHE, 0.5 mg/L.day for ACT, and 0.42 mg/L.h for PY.

Zhao et al. (2011) studied that efficiency of RD by PAH degrading bacteria using RD concentrations varying between 20 and 400 mg/L. In this study, it was found that the PHE solubility increased with RD addition at 400 mg/L in an chemical industry wastewater containing 250 mg/L PHE ending with PHE removal efficiencies varying between 82.2 % and 92.7% at a SRT of 30 days. Sanchez-Avila et al. (2009) found a total PAH removal efficiency of 73% at a HRT of 22 h in an activated sludge treatment system treating industrial wastewaters from the area of Maresme (Catalonia, Spain) at an initial total PAH concentrations of 94.9 ng/mL. Sartoros et al., (2005) found that the addition of a surfactant (100 mg/L Tergitol NP-10) at 25 ^oC increased the overall mineralization of ANT and PY yields to 50 from 33%.

Guieysse et al. (2000) investigated the removal of ACT, PHE and PY using an aerobic packet bed reactor. In this study, ACT, PHE and PY were removed with

yields of 99%, 99% and 90%, respectively, at a HRT of 10 hours, at a SRT of 20 days with initial ACT, PHE and PY concentrations of 3.9, 1.3 and 0.014 mg/L, respectively.

The older studies showed that the biosurfactants enhance the solubility of PAHs resulting in with absorption of PAHs by biomass in aerobic reactor (Rosenberg et al., 1999). They showed that the addition of tween-80 increased the FLN solubility and the biodegradation rate 5-20 folds. Biosurfactants are preferred over synthetic surfactants because they are more cost-effective, less toxic and easily biodegradable. Applications of surfactants increase the solubility of PAHs, and thus, facilitate their biodegradation. In the study performed by Whang et al., (2008) it was found that the diesel solubility increased with surfactin addition up to 40 mg/L while the diesel biodegradation percentage increased up to 94%. Some reports showed that surfactants are also able to exhibit negative effects on biodegradation of PAHs, due either to the surfactant toxicity or to the increased toxicity to microorganisms of PAHs at very high surfactant concentrations.

3.2 Literature Review for the Treatment of PAHs in the Anaerobic System

Anaerobic process as a biological process in which organic matter is converted to CH_4 and CO_2, is a more attractive technology for industrial wastewater treatment owing to its low cost compared with other technologies available physicochemical and aerobic biological treatments (Tabatabaei et al., 2010; Chan et al., 2009). The process has many advantages, including a low space requirement, much less waste sludge production, no need for aeration and most importantly, the production of biogas (50-80 % methane), as an useable fuel (Yoo et al., 2012; Kassab et al., 2010).

Laboratory scale fixed film anaerobic systems were used by Patel and Madamvar (2001) to treat the petrochemical industrial wastewater. The effect of operational parameters such as HRT and OLR were investigated in anaerobic multichamber systems. Significant degradation of aromatic compounds was observed in the

acidogenic phase. The maximum COD reduction was of 96% while the methane yield was 0.37 m^3/m^3.day at an OLR of 20.4 kg COD/m^3.day and at a HRT of 4 days.

Fuchedzhieva et al. (2008) studied the anaerobic biodegradation of FLN containing simulate wastewater under methanogenic conditions in presence of surface-active compounds [(linear alkyl benzene sulphonates (LAS) and RD biosurfactant)]. The results for FLN biodegradation under strict anaerobic methanogenic conditions showed that 30% FLN removal efficiency obtained after 7th day in the presence of 100 mg/L LAS and 100 mg/L RD concentrations at an initial concentration of 120 mg/L FL and at a SRT of 14 days.

Tsai et al. (2009) investigated the treatability of FLN (5 mg/L) and PHE (5 mg/L) PAHs containing simulate wastewater in an anaerobic contact reactor. FLN and PHE PAHs removal efficiencies were found as 88% FLN and 65% PHE, respectively at a SRT of 21 days in the anaerobic reactor. Maillacheruvu and Pathan (2009) used an enrichment anaerobic culture which was able to degrade 30 mg/L NAP, 1.8 mg/L PHE and 0.2 mg/L PY. It was found NAP, PHE and PY removal efficiencies of 96%, 95% and 90%, respectively at an OLR of 0.50 gCOD/L.day, at a SRT of 20 days and at a HRT of 4 days in an anaerobic batch reactor.

Delgadillo-Mirquez et al. (2011) reported 74% PAH, 60% COD and 89.4% volatile fatty acid (VFA) removals and 70% methane content at an applied HRT of 20 days at a OLR of 1.2 gCOD/L.day in an anaerobic reactor treating PAHs. Lu et al. (2011) investigated the effect of temperature, pH, NAP and nitrate concentrations on the NAP degradation under denitrification conditions. 10 mg/L NAP was removed with an efficiency of 93% at pH 7 and 25 ^oC.

Arnaiz et al. (2007) showed that the anaerobic ITBR system was appeared to be a good option for anaerobic treatment of high strength wastewater, for the treatment of wine distillery wastewater. The systems attained high OLR with good COD removal rates and it exhibited a good stability to the variations in OLR. They found that COD removal was obtained 70 and 92% at OLR from 9.5 to 30.6 kg COD/m^3day.

Alvarado-Lassman et al. (2008) found that anaerobic ITBR technology was suitable for organic matter removal present in brewery wastewater with COD removal efficiencies greater than 90%. The reactor with triturated polyethylene support showed an excellent COD removal with OLR values up to 10 g COD/L day, whereas the reactor with extendosphere support had an excellent hydrodynamic and biologic behavior working with OLR values up to 70 g COD/L day. Cresson et al. (2007) studied the influence of hydrodynamic conditions on the start-up phase of an anaerobic ITBR treating wine based industry wastewater. The COD removal efficiency increased from 60% to 80% when the OLR was increased stepwise from 0.5 to over 6 g COD/ L.day, respectively, at a HRT of 1 day in the anaerobic ITBR.

Rockne and Strand (1998) investigated the biodegrability of NAP and PHE PAHs and biphenyl in an anaerobic inverse fluidized bed reactor at SRTs varying between 100 and 200 days under anaerobic conditions with nitrate and sulfate as the sole potential electron acceptors. NAP and PHE removal efficiencies were 93% and 85% respectively. The treatment performance of wastewater containing 18 PAHs was investigated in a sequential anaerobic and aerobic wastewater treatment plant (Zhang et al., 2012b). They found that the removals of 18 PAHs ranged between 47-92% in a sequential anaerobic and aerobic system. 97% PAHs and 99% COD were removed in the whole reactor system at a HRT of 4 h at influent COD_{dis} and PAHs concentrations of 77 g/L and 5470 µg/L, respectively. In a study performed by Tian et al. (2012) 89% total PAHs removal efficiencies in a modified anaerobic-anoxic-oxic biological wastewater treatment plant, at a HRT of 9.2 h, at an initial total PAH concentrations of 1147 ng/L. Although the anaerobic ITBR were excessively used in the treatment of wine distillery, brewery, food processing and dairy industry wastewaters it was not found a research investigating the anaerobic treatabilities of PAHs and the petrochemical industry wastewaters in recent literature. Furthermore, it was not found a study investigating the effects of biosurfactants on the removal of PAHs in the ITBR system at increasing HRTs.

CHAPTER FOUR
PROPERTIES OF AEROBIC CONTINUOUS STIRRED TANK REACTOR (CSTR), ANAEROBIC INVERSE TURBULENT BED REACTOR (ITBR) AND SEQUENTIAL ANAEROBIC/AEROBIC REACTOR SYSTEM

4.1 Aerobic Continuous Stirred Tank Reactor (CSTR) System

Aerobic treatment is a biological process, the principle of which is the use of free or dissolved oxygen by microorganisms (aerobes) in the degradation of organic material. Since oxygen is available to working aerobes as an electron acceptor, the biodegradation process can be significantly accelerated, leading to increased throughput capacity of a treatment system (Bonakdarpour et al., 2011; Chan et al., 2009). Continuous stirred tank reactor (CSTR) is the most commonly used process for waste water treatment. The feed is pumped into the reactor (known as mixed liquor tank) containing acclimatized aerobic cultures. A mechanical stirrer along with aeration provides an aerobic environment for the cultures. After the desired retention time, the cells are separated from the treated waste water and partially recycled. The quantity of cells recycled and the retention time depend on the type of waste water and treatment efficiency. The mean cell retention time in the reactor depends on the flocculation and the settling property of microorganisms.

The aeration tank is a completely mixed bioreactor where specific concentration of biomass (measured as mixed liquor suspended solids (MLSS) or mixed liquor volatile suspended solids (MLVSS) is maintained along with sufficient dissolved oxygen (DO) concentration to effect biodegradation of soluble organic impurities measured as biochemical oxygen demand (BOD_5) or chemical oxygen demand (COD). The aeration tank is provided with fine bubble diffused aeration pipework at the bottom to transfer required oxygen to the biomass and also ensure completely mixed reactor.

Aerobic treatment has many advantages including: 1) minimum odor when properly loaded and maintained; 2) large biochemical oxygen demand (BOD)

removals providing a good quality effluent; 3) Tolerates shock load caused by organic and hydraulic load variability; 4) Inherent ability to remove nutrients without chemical addition, by controlling the oxygen demand and supply; 5) the final discharge may contain dissolved oxygen which reduces the immediate oxygen demand on receiving water (Mehrdadi et al., 2006; Von Sperling et al., 2001).

The main disadvantage of aerobic treatment system is the energy cost of aeration at an adequate rate to maintain the dissolved oxygen concentrations for aerobic conditions in the treated wastewater for aerobic growth. In addition, some organics cannot be efficiently decomposed aerobically. These biologically non-reactive components mainly composed of insoluble materials can account for up to 70% of the COD (Kushwaha et al., 2011; Ma et al., 2008).

4.2 Anaerobic Inverse Turbulent Bed Reactor (ITBR)

4.2.1 History and Current Uses of Anaerobic Inverse Turbulent Bed Reactor (ITBR)

Anaerobic fluidized bed reactors are relatively new tools in the environmental engineering field. The first fluidized bed gas reactor was developed by Fritz Winkler in Germany in the 1920s (Tavoulareas, 1991). One of the first USA fluidized bed reactors used in the petroleum industry was the Catalytic Cracking Unit, created in Baton Rouge, LA in 1942 by the Standard Oil Company of New Jersey (now ExxonMobil) (Thornhill, 2007). This anaerobic ITBR was developed to treat the wastewaters from the oil and petrochemical industries. Here catalysts were used to produce fuel from the wastewater of petroleum with a cracking process. The invention of this technology made it possible to significantly increase the production of various fuels in the United States (Thornhill, 2007). Several systems have been investigated to adapt the fluidization process for the anaerobic treatment of wastewater such as the inverse fluidized beds or down-flow fluidized beds (Campos-Diaz et al., 2012; Alvarado-Lassman et al., 2008) and more recently, the inverse turbulent bed (ITB) (Sowmeyan,and Swaminathan, 2008; Arnaiz et al., 2003).

4.2.2 Advantages of Anaerobic Inverse Turbulent Bed Reactor (ITBR)

Today's the increase in the utilization of inverse turbulent bed reactor for the treatment of industrial wastewaters in worldwide is largely due to the inherent advantages of the technology (Parthiban et al 2007; Arnaiz et al., 2007; Saravanane, R. and Murthy, 2000; Buffiere et al., 2000; Arnaiz et al., 2003; Nicolella et al. 2000).

Among the advantages presented by the latest are high mass transfer rate, minimum carry-over of coated microorganism due to less solid attrition and efficient control of biofilm thickness (Das et al., 2010). The main advantages of ITBR system is the gas injection is simpler than liquid recycling, requires less energy because of low fluidization velocities and allows controlling the height of fluidization (Arnaiz et al., 2005; Buffiere and Molatte 2000). The bed height is constant between the liquid surface and the point of gas injection, whatever the particle density is high in the ITBR. This system can be operated under severe conditions including high strength influents and short hydraulic retention times (HRTs) (Fernandez et al., 2008; Aiyuk et al., 2006; Michaud et al., 2003).

4.2.3 Disadvantages of Anaerobic Inverse Turbulent Bed Reactor (ITBR)

The inverse turbulent bed reactor does have it draw-backs (Fernandez et al., 2008; Arnaiz et al., 2005). In the ITBR system to suspend the solid material necessitates a higher fluid velocity. In order to achieve this, more pumping power and higher energy costs are needed. If fluidization pressure is suddenly lost, the surface area of the bed may be suddenly reduced (Cresson et al., 2007).

CHAPTER FIVE
MATERIALS AND METHODS

5.1 Experimental Set-ups in Batch Studies

5.1.1 Configuration of the Aerobic Batch Reactor for the Aerobic Treatability of PAHs

The first set of batch experiments were performed by using a batch scale completely mixed reactor model. The bioreactor system was made from glass with a working volume of 2 L. The properties of batch bioreactors are: an external length of 19.5 cm, an external diameter of 14.5 cm, an internal water height of 10 cm and a volume of 2 L.

5.1.2 Configuration of Batch Reactors for Biodegradation, Volatilization and Adsorption Studies

The closed glass batch reactors with a tight teflon-coated cap with a volume of 2.5 L were used in this step. Diffusers and magnetic stirrers coated with teflon were used to aerate and mix the mixtures in these reactors. Air was passed from columns containing activated carbon to supply the oxygen to the reactors. An XAD-2 resin column was used in the effluent of the batch reactor in order to filter the samplings. Rotameters (DWYER) with gas measurement limits varying between 0,012 and 100 L/h were used to measure the flow rate in the influent and effluent of the batch reactors. This study was performed in ambient conditions and at a temperature of 25 ^{o}C. A schematic configuration of the batch reactor is shown in Figure 5.1.

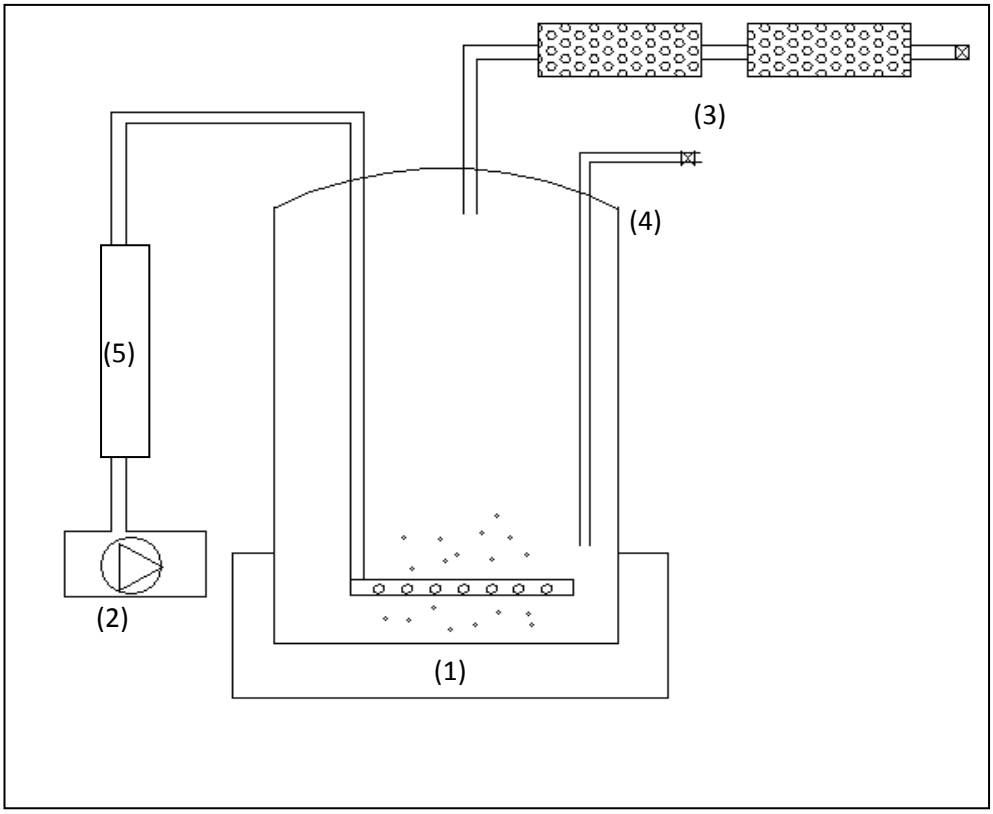

Figure 5.1 Configuration of batch reactors for biodegradation, volatilization and adsorption studies (1: magnetic stirred, 2: air pump, 3: resin colon, 4: sample port)

5.1.3 Configuration of the Batch Reactors for Specific Methanogenic Activity (SMA)

The specific methanogenic activity test (SMA) was conducted in 150 mL serum bottles at 35 ^{o}C under anaerobic conditions. Sodium thioglycolate and $NaHCO_3$ were added to reduce the redox potential and to keep the anaerobic conditions and the pH neutral throughout the study.

5.2 Experimental Set-Ups in Continuous Studies

5.2.1 Configuration of the Aerobic Continuously Stirred Tank Reactor (CSTR)

A stainless steel continuously stirred tank reactor (CSTR) was used in the continuous experiment. It consists of an aerobic and a settling compartment **(**Figure

5.2). The effluent wastewater from the aeration tank to the sedimentation tank passed through holes in a plate inclined at 45° to the horizontal axis. Effluent leaving the sedimentation tank was collected in an effluent tank. Effluent leaving the sedimentation tank collected in an effluent tank. The properties of the aeration tank are as follows: a length of 12 cm, a width of 20 cm, a height of 30 cm, and a volume of 9 L. The properties of sedimentation tank are as follows: a length of 20 cm, a width of 18 cm, a height of 15 cm and a volume of 1 L.

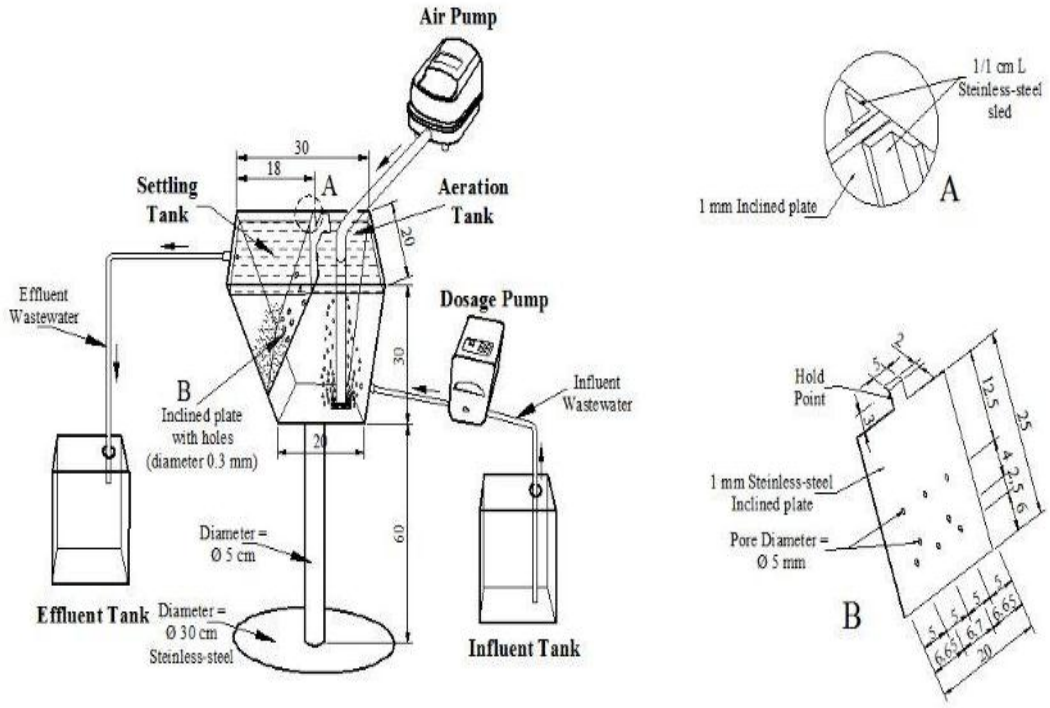

Figure 5.2 Configuration of the aerobic stirred tank reactor (CSTR)

5.2.2 Configuration of the Laboratory Scale Anaerobic Inverse Turbulent Bed Reactor (ITBR)

The anaerobic inverse turbulent bed reactor (ITBR) consisted of a stainless steel section with a 0.10 m internal diameter and with 1 m height containing a conic bottom with a total volume of 5.5 L (Figure 5.3). The system was equipped with a water jacket keeping the liquid temperature at 37±1 °C. A digital temperature probe

located in the middle part of reactor provided the constant operation temperature. This provided a homogenous temperature in whole anaerobic ITBR. Influent wastewater was pumped continuously into the anaerobic ITBR reactor from the top of the ITBR column by a peristaltic pump. Effluent wastewater was discharged through a port on the bottom of the column. The anaerobic ITBR system filled with 1.50 L of solid carrier corresponding to an apparent solid hold-up of 20%. The produced gas was collected via porthole in the top of reactor. The solid carrier with U-spheres is a mineral granular material composed mainly of silica and alumina.

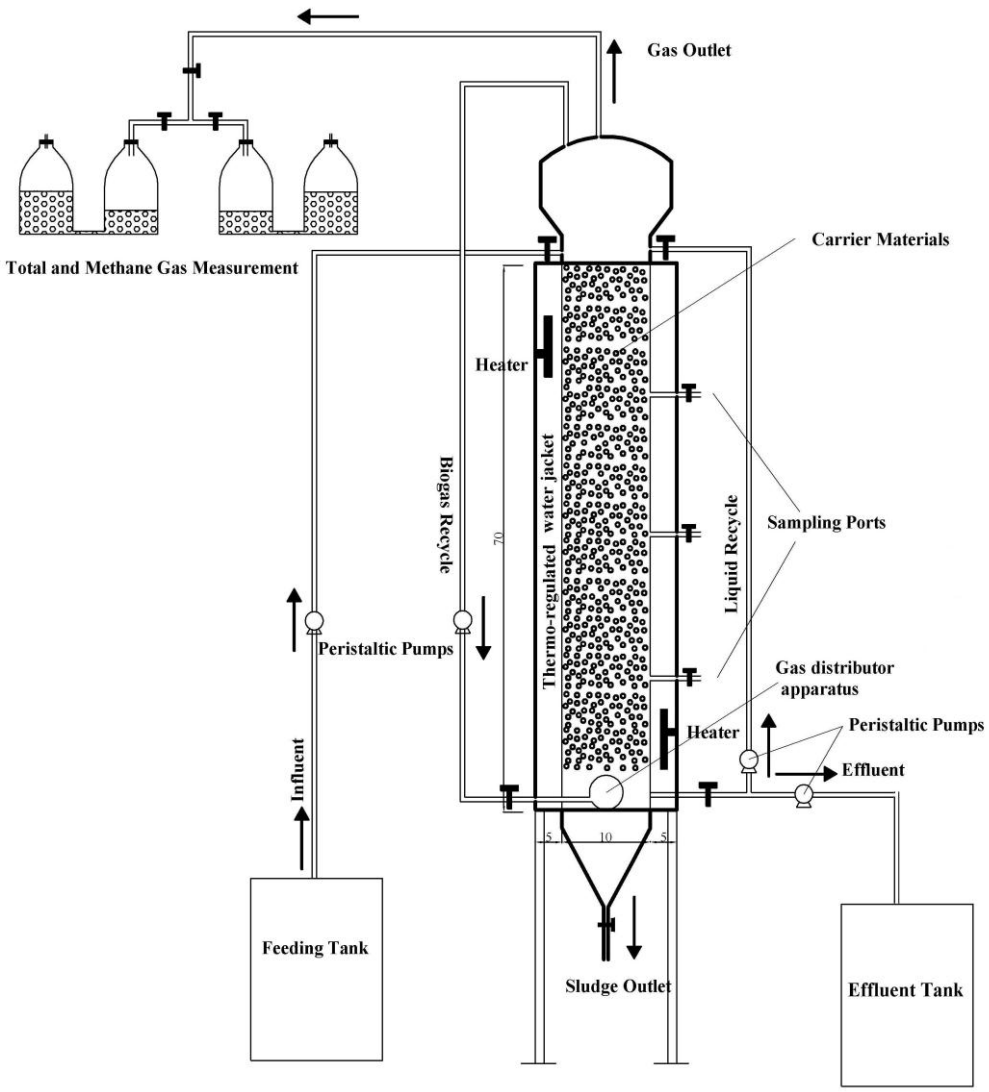

Figure 5.3 Configuration of the laboratory scale anaerobic inverse turbulent bed reactor (ITBR) system

5.2.2.1 Properties of Carrier Material in the Anaerobic ITBR System

In this study Omega U-Spheres W mineral was used as support material in the ITBR system. The physical properties of U-Spheres particles used in this study are given in Table 5.1 (Garcia-Calderon et al., 1998). The solid carrier U-spheres is a mineral granular material composed mainly of silica and alumina.

Table 5.1 Properties of support materials used in the anaerobic ITBR reactor

Commercial name	Omega U- Spheres W minerals
Size (equivalent particle diameter)	150-200 μm
Color	Light grey
Specific area	24.08 m^2/L
Apparent density	410 g/L
Specific dry density	0.7-0.8 g/cm^3
Specific density	696 g/L
Major compounds	Silica and Alumina

The picture of these Omega U- Spheres W minerals is given in Figure5.4 at beginning of the study before administered to the anaerobic ITBR. The diameters of these particles were measured between 169 and 200 μm with Olympus microscope.

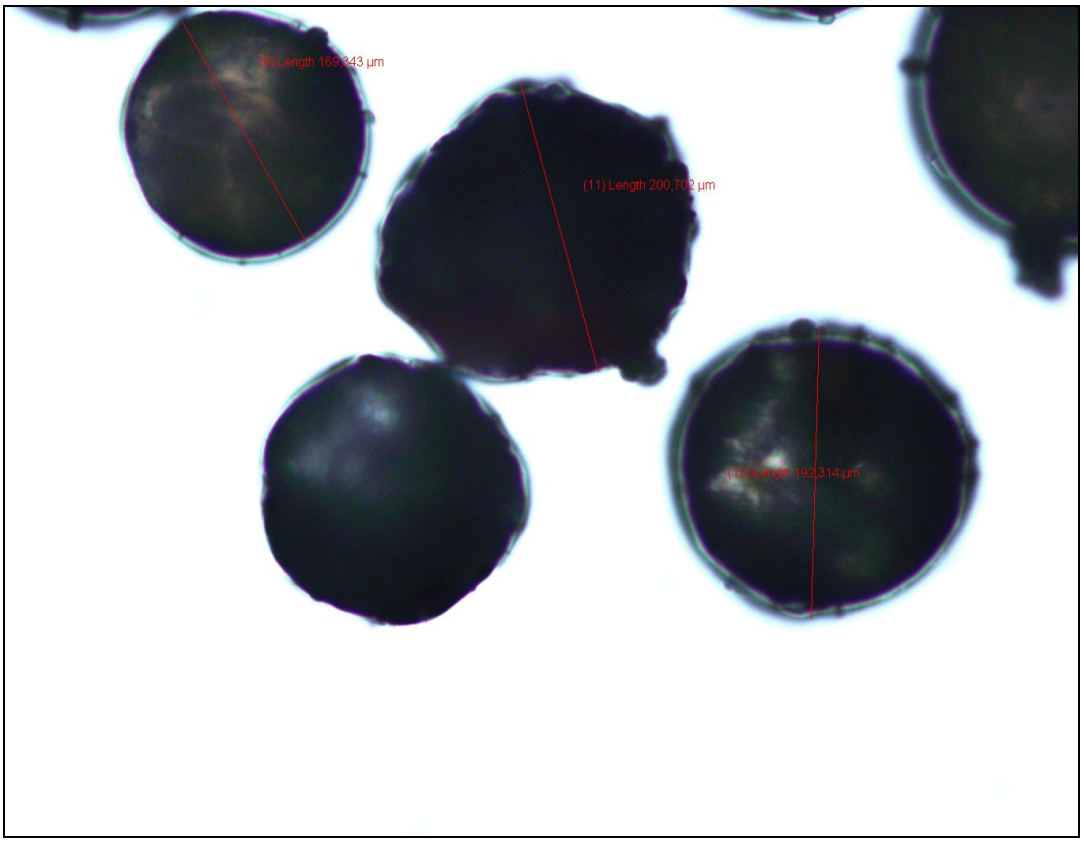

Figure 5.4 Microscopic observation of carrier material before operation the anaerobic ITBR system (magnification 20X10, average diameter: 195µm)

5.2.3 Configuration of Sequential Laboratory-Scale Anaerobic ITBR/Aerobic CSTR System

A schematic configuration of the lab-scale sequential anaerobic ITBR and aerobic CSTR used in this study is presented in Figure 5.5. A continuously fed stainless steel anaerobic ITBR and an aerobic CSTR reactor were used in sequence for the experimentation. The effluent of the anaerobic ITBR was used as the influent of aerobic CSTR. The configurations of the anaerobic and aerobic reactors were given in section of 5.2.1 and 5.2.2.

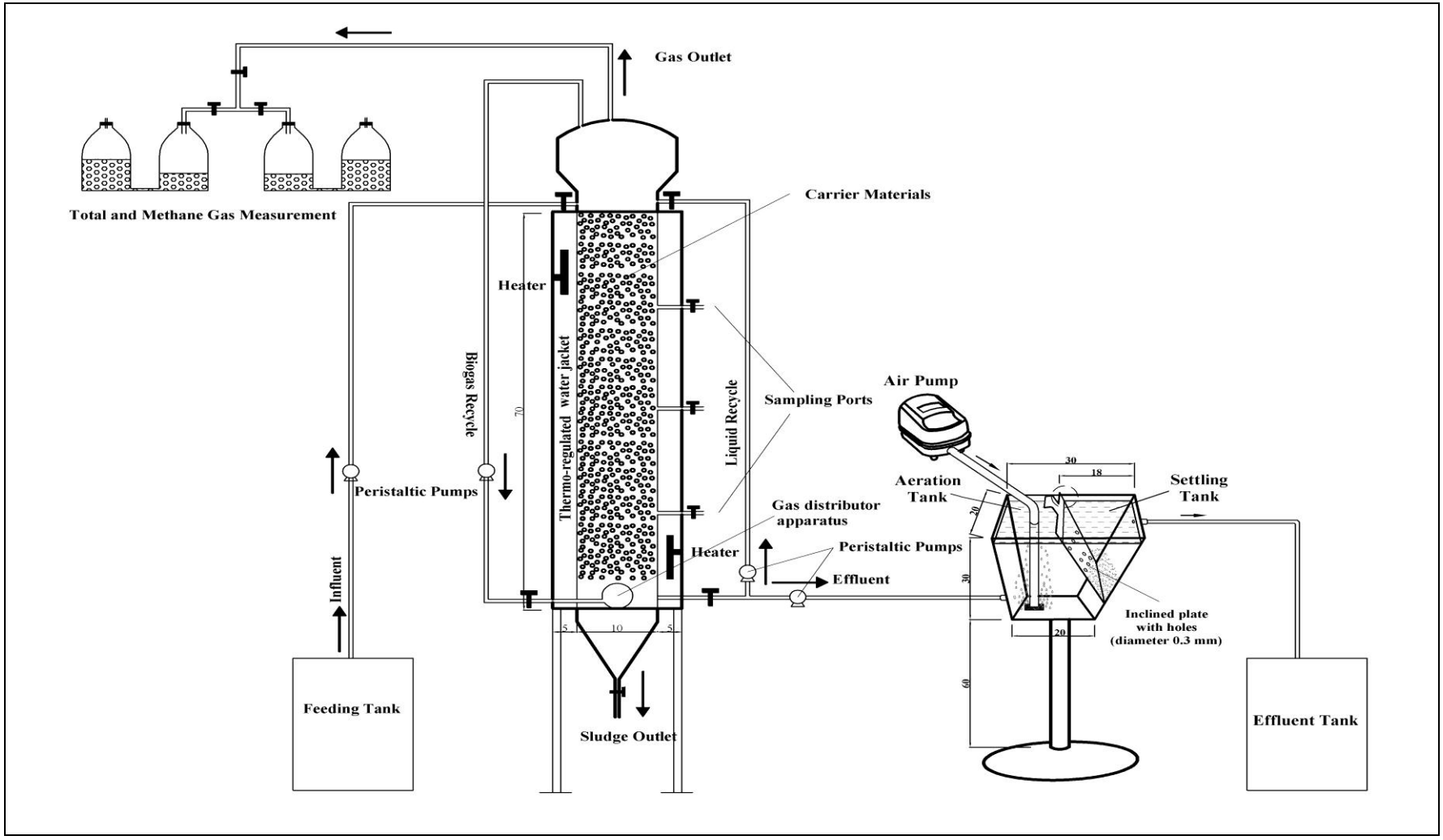

Figure 5.5 A schematic configuration of the lab-scale sequential anaerobic ITBR and aerobic CSTR

5.3 Operational conditions

5.3.1 Operational Conditions for Aerobic Batch Reactors

Study 1:

The first set of the batch study was performed to determine the biodegradability of PAHs using a batch scale aerobic continuously stirred tank reactor without rhamnolipid (RD) biosurfactant. The treatability studies of PAHs were conducted at room temperature (20-25 ^{o}C). 300 mL of inoculums and 400 mL real petrochemical wastewater was added into beakers. It was aerated through an air diffuser placed at the bottom of the reactors and air was given to the system during operation period during batch studies. The aeration was stopped and waited for a while during the settling of the sludge then the supernatant taken from the reactors used in the experimental analysis. The operational conditions of this batch study were given in Table 5.2. Flow rate, SRT, HRT were kept constant in aerobic batch reactors. These set of batch studies were performed to determine the total and individual 15 PAHs (acenaphthene (ACT), fluorene (FLN), phenanthrene (PHE), anthracene (ANT), carbazole (CRB), fluoranthene (FL), pyrene (PY), benz[a]anthracene (BaA), chrysene (CHR), benz[b]fluoranthene (BbF), benz[k]fluoranthene (BkF), benz[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (IcdP), dibenz[a,h]anthracene (DahA), and benzo[g,h,i]perylene (BghiP) removal efficiencies under aerobic conditions in batch scale aerobic reactors.

Table 5.2 The operational conditions of aerobic batch study

Operational conditions parameters	**Values**		
	Minimum	**Mean**	**Maximum**
Q (L/day)	0.30		
Batch reactor volume (L)	2.00		
COD_{total} (mg/L)	724	812	944
COD_{dis} (mg/L)	611	642	756
BOD_5 (mg/L)	112	128	144
HRT (days)	2.30		
OLR (gCOD/L.day)	0.092	0.096	0.011
F/M (gCOD/gMLVSS.days)	0.03	0.03	0.04
MLVSS (mg/L)	2800	2900	3000
SRT (days)	20		
Operational days (days)	30		
ACT (ng/mL)	22.74	23.30	24.74
FLN (ng/mL)	12.23	14.85	16.35
PHE (ng/mL)	10.71	12.18	14.11
ANT (ng/mL)	0.31	0.50	0.71
CRB (ng/mL)	0.21	0.42	0.68
FL (ng/mL)	0.45	0.97	1.20
PY (ng/mL)	0.71	0.94	1.16
BaA (ng/mL)	0.04	0.06	0.07
CHR (ng/mL)	0.01	0.14	0.16
BbF (ng/mL)	0.03	0.04	0.04
BkF (ng/mL)	0.01	0.02	0.02
BaP (ng/mL)	0.01	0.02	0.02
IcdP (ng/mL)	0.01	0.02	0.02
DahA (ng/mL)	0.03	0.04	0.04
BghiP (ng/mL)	0.01	0.01	0.01
Total PAHs (ng/mL)	47.64	53.52	59.39

Study 2:

These set of the batch studies were performed to determine the main degradation mechanisms (biodegradation, volatilization and adsorption) of the total and individual 15 PAHs (ACT, FLN, PHE, ANT, CRB, FL, PY, BaA, CHR, BbF, BkF, BaP, IcdP, DahA, and BghiP) under aerobic conditions in batch scale stirred aerobic reactors. Furthermore the effects of PAHs biosorption on biodegradation of PAHs was investigated with *Pseudomonas putida* and *Escherichia coli* cultures at 15 mg/L RD concentration in batch reactors. Flow rate, SRT and HRT were kept constant in this study. The operational conditions of this batch study were given in Table 5.3.

Table 5.3 Operational conditions for biodegradation, volatilization and adsorption studies of the PAHs in batch reactors

Operational conditions parameters	**For biodegradation study**			**For volatilization study**			**For adsorption study**		
	Minimum	**Mean**	**Maximum**	**Minimum**	**Mean**	**Maximum**	**Minimum**	**Mean**	**Maximum**
Q (L/day)	1.50			1.50			1.50		
Batch reactor volume (L)	2								
COD_{total} (mg/L)	1270	1475	1680	1270	1475	1680	1270	1475	1680
COD_{dis} (mg/L)	1100	1150	1200	1100	1150	1200	1100	1150	1200
BOD_5 (mg/L)	248	384	520	248	384	520	248	384	520
HRT (days)	2								
OLR (gCOD/L.day)	0.82	0.86	0.90	-	-	-	0.82	0.86	0.90
F/M (gCOD/g MLVSS.day)	0.44	0.49	0.53	-	-	-	0.44	0.49	0.53
MLVSS (mg/L)	1640	1750	1950	-	-	-	1640	1750	1950
SRT (days)	2								
RD concentration (mg/L)	15								
Operational days (days)	2								
ACT (ng/mL)	16.97	17.01	17.43	16.97	17.01	17.43	16.97	17.01	17.43
FLN (ng/mL)	16.32	16.95	17.41	16.32	16.95	17.41	16.32	16.95	17.41
PHE (ng/mL)	13.13	13.35	13.38	13.13	13.35	13.38	13.13	13.35	13.38
ANT (ng/mL)	3.12	3.13	3.14	3.12	3.13	3.14	3.12	3.13	3.14
CRB (ng/mL)	0.45	0.46	0.47	0.45	0.46	0.47	0.45	0.46	0.47
FL (ng/mL)	1.12	1.14	1.15	1.12	1.14	1.15	1.12	1.14	1.15
PY (ng/mL)	1.98	2.00	2.04	1.98	2.00	2.04	1.98	2.00	2.04
BaA (ng/mL)	0.57	0.59	0.60	0.57	0.59	0.60	0.57	0.59	0.6
CHR (ng/mL)	1.21	1.22	1.24	1.21	1.22	1.24	1.21	1.22	1.24
BbF (ng/mL)	0.14	0.16	0.17	0.14	0.16	0.17	0.14	0.16	0.17
BkF (ng/mL)	0.19	0.21	0.22	0.19	0.21	0.22	0.19	0.21	0.22
BaP (ng/mL)	0.25	0.26	0.27	0.25	0.26	0.27	0.25	0.26	0.27
IcdP (ng/mL)	0.25	0.26	0.27	0.25	0.26	0.27	0.25	0.26	0.27
DahA (ng/mL)	0.48	0.5	0.51	0.48	0.5	0.51	0.48	0.5	0.51
BghiP (ng/mL)	0.23	0.24	0.25	0.23	0.24	0.25	0.23	0.24	0.25
Total PAHs (ng/mL)	56.41	57.48	58.55	56.41	57.48	58.55	56.41	57.48	58.55

5.3.2 Operational Conditions for Continuous Reactors

Study 1:

In this run, the start-up period of the continuously with real petrochemical wastewater fed aerobic CSTR performance was investigated in the absence of the biosurfactants. The operational conditions of this study were given in Table 5.4.

Table 5.4 Operational conditions of the CSTR at the start-up period

Operational conditions parameters	**Values**		
	Minimum	**Mean**	**Maximum**
Q (L/day)	2		
Volume (L)	10		
COD_{total} (mg/L)	1425	1575	1725
COD_{dis} (mg/L)	858	1200	1356
BOD_5 (mg/L)	320	380	440
HRT (days)	5		
OLR (gCOD/L.day)	0.17	0.23	0.27
F/M (gCOD/g MLVSS.day)	0.06	0.08	0.10
MLVSS (g/L)	2650	2750	2800
SRT (days)	20		
Operation days (days)	40		
ACT (ng/mL)	28.47	29.43	30.33
FLN (ng/mL)	9.31	9.38	10.34
PHE (ng/mL)	14.47	15.01	16.24
ANT (ng/mL)	2.58	3.61	3.61
CRB (ng/mL)	0.81	0.90	1.01
FL (ng/mL)	2.65	2.98	3.04
PY (ng/mL)	2.11	2.19	2.24
BaA (ng/mL)	0.28	0.36	0.38
CHR (ng/mL)	0.64	0.72	0.75
BbF (ng/mL)	0.07	0.08	0.09
BkF (ng/mL)	0.08	0.09	0.10
BaP (ng/mL)	0.06	0.07	0.08
IcdP (ng/mL)	0.10	0.12	0.14
DahA (ng/mL)	0.24	0.27	0.29
BghiP (ng/mL)	0.08	0.09	0.10
Total PAHs (ng/mL)	61.95	65.32	68.74

Study 2:

In this step, the real petrochemical wastewater was used as feed in the aerobic CSTR reactor and it was continuously operated with 10-15-25 mg/L biosurtactant [Rhamnolipid, (RD)] concentrations and without RD. In this study, the effects of increasing RD concentrations on the removal efficiencies of the total and individual 15 PAHs and COD_{dis} were investigated. The operational conditions for this run were given in Table 5.5.

Table 5.5 Operational conditions of the CSTR reactor system with and without RD

Operational conditions parameters	**Values**		
	Minimum	**Mean**	**Maximum**
Q (L/day)	2		
Volume (L)	10		
COD_{total} (mg/L)	1955	2105	2255
COD_{dis} (mg/L)	1742	1816	1891
BOD_5 (mg/L)	320	380	440
HRT (days)	5		
OLR (g COD/L day)	0.35	0.36	0.38
F/M (g COD/g MLVSS day)	0.13	0.13	0.14
MLVSS (mg/L)	2650	2750	2800
SRT (days)	20		
Operational days (days)	45		
RD concentration (mg/L)	10-15-25		
ACT (ng/mL)	24.72	25.11	25.71
FLN (ng/mL)	22.87	23.12	24.07
PHE (ng/mL)	15.94	16.04	16.09
ANT (ng/mL)	1.07	1.11	1.12
CRB (ng/mL)	0.81	0.91	0.91
FL (ng/mL)	2.75	3.04	3.08
PY (ng/mL)	6.41	6.66	6.72
BaA (ng/mL)	1.87	2.02	2.08
CHR (ng/mL)	2.55	2.75	2.78
BbF (ng/mL)	0.48	0.51	0.51
BkF (ng/mL)	0.36	0.37	0.38
BaP (ng/mL)	0.90	0.91	0.92
IcdP (ng/mL)	0.26	0.27	0.28
DahA (ng/mL)	1.44	1.45	1.46
BghiP (ng/mL)	0.54	0.55	0.56
Total PAHs (ng/mL)	82.97	84.82	86.67

Study 3:

In this run of the study the effects of increasing SRTs on the performance of the aerobic CSTR, on the COD subcategories, the individual 15 PAHs (ACT, FLN, PHE, ANT, CRB, FL, PY, BaA, CHR, BbF, BkF, BaP, IcdP, DahA, and BghiP) yields and some environmental (DO, temperature, electron donors and pH) conditions on the PAH yield were investigated in rhamnolipid added (constant RD concentration of 15 mg/L) and non-added CSTR reactor in continuous mode. The effects of PAHs biosorption on biodegradation of PAHs was investigated with *Pseudomonas putida* and *Escherichia coli* cultures at 15 mg/L RD concentration. Furthermore, the effects of increasing SRTs on acute toxicity removals were investigated using *Daphnia magna* and *vibrio fischeri test*. The operational conditions for this study were given in Table 5.6.

Table 5.6 Operational conditions of the CSTR reactor at increasing SRTs

Operational conditions parameters	Values		
	Minimum	**Mean**	**Maximum**
Q (L/day)	2		
Volume (L)	10		
COD_{total} (mg/L)	1296	1475	2654
COD_{dis} (mg/L)	1050	1200	1950
BOD_5 (mg/L)	554	584	680
HRT (days)	5		
OLR (gCOD/L.day)	0.21	0.24	0.39
F/M (gCOD/g MLVSS.day)	0.09	0.10	0.14
MLVSS (mg/L)	2356	2420	2750
Operation days (days)	120		
SRT (days)	5-10-25-40		
RD concentration (mg/L)	15		
ACT (ng/mL)	28.47	29.43	30.33
FLN (ng/mL)	9.31	9.38	10.34
PHE (ng/mL)	14.47	15.01	16.24
ANT (ng/mL)	2.58	3.61	3.61
CRB (ng/mL)	0.81	0.90	1.01
FL (ng/mL)	2.65	2.98	3.04
PY (ng/mL)	2.11	2.19	2.24
BaA (ng/mL)	0.28	0.36	0.38
CHR (ng/mL)	0.64	0.72	0.75
BbF (ng/mL)	0.07	0.08	0.09
BkF (ng/mL)	0.08	0.09	0.10
BaP (ng/mL)	0.06	0.07	0.08
IcdP (ng/mL)	0.10	0.12	0.14
DahA (ng/mL)	0.24	0.27	0.29
BghiP (ng/mL)	0.08	0.09	0.10
Total PAHs (ng/mL)	61.95	65.32	68.74

Study 4:

This run was performed to detect the effects of increasing Rhamnolipid (RD), Emulsan (EM), Surfactin (SR) biosurfactants and increasing SRTs on the biodegradation of PAHs in a continuously fed CSTR reactor. The biodegradation and the inhibition kinetics of total PAHs were investigated. Therefore, Monod, first, zero and second order substrate removal kinetics were investigated to detect the effects of biosurfactant on the reaction kinetic. The inhibition kinetics of PAHs was investigated using competitive, non-competitive, uncompetitive and Haldane kinetics in the presence of excess biosurfactant concentrations. Furthermore, the effects of increasing RD concentrations on the metabolite productions of three less hydrophobic (ACT, FLN, PHE) and three more hydrophobic PAHs (BaP, IcdP, DahA) were investigated. The effects of increasing concentrations of RD, EM and SR on the growth of *Pseudomona aeruginosa* and *Zooglera ramigera* bacteria were investigated in the CSTR. The operational conditions for this study were given in Table 5.7.

Table 5.7 Operational conditions of the CSTR reactor system at increasing SRTs and biosurfactant (RD, EM and SR) concentrations

Operational conditions parameters	Values		
	Minimum	**Mean**	**Maximum**
Q (L/day)	2		
Volume (L)	10		
COD_{total} (mg/L)	1296	1475	1654
COD_{dis} (mg/L)	1050	1200	1350
BOD_5 (mg/L)	554	584	614
HRT (days)	5		
OLR (gCOD/L.day)	0.22	0.24	0.27
F/M (gCOD/g MLVSS.day)	0.09	0.10	0.11
MLVSS (mg/L)	2356	2500	2570
SRT (days)	5-10-25-40		
Operation days (days)	160		
RD concentration (mg/L)	10-15-25		
EM concentration (mg/L)	10-15-25		
SR concentration (mg/L)	10-15-25		
ACT (ng/mL)	28.47	29.43	30.33
FLN (ng/mL)	9.31	9.38	10.34
PHE (ng/mL)	14.47	15.01	16.24
ANT (ng/mL)	2.58	3.61	3.61
CRB (ng/mL)	0.81	0.9	1.01
FL (ng/mL)	2.65	2.98	3.04
PY (ng/mL)	2.11	2.19	2.24
BaA (ng/mL)	0.28	0.36	0.38
CHR (ng/mL)	0.64	0.72	0.75
BbF (ng/mL)	0.07	0.08	0.09
BkF (ng/mL)	0.08	0.09	0.10
BaP (ng/mL)	0.06	0.07	0.08
IcdP (ng/mL)	0.10	0.12	0.14
DahA (ng/mL)	0.24	0.27	0.29
BghiP (ng/mL)	0.08	0.09	0.10
Total PAHs (ng/mL)	61.95	65.32	68.74

Study 5:

In this run the effects of increasing hydraulic retention time (HRT) on the COD_{dis} and on the total and individual 15 PAHs (ACT, FLN, PHE, ANT, CRB, FL, PY, BaA, CHR, BbF, BkF, BaP, IcdP, DahA, and BghiP) yields were investigated in rhamnolipid added (15 mg/L) and non-added conditions throughout continuous operation of the CSTR reactor. Furthermore, the effects of increasing HRTs on acute toxicity removals were investigated using *Daphnia magna* and *vibrio fischeri test*. The operational conditions of continuously study were given in Table 5.8.

Table 5.8 Operational conditions of the CSTR reactor system at 25 days SRT at a constant RD concentration of 15 mg/L

Operational conditions parameters	Values		
	Minimum	**Mean**	**Maximum**
Q (L/day)	1-2-3-4		
Volume (L)	10		
COD_{total} (mg/L)	672	1915	3158
COD_{dis} (mg/L)	562	1753	2945
BOD_5 (mg/L)	104	318	532
HRT (days)	2.50-3.33-5-10		
OLR (gCOD/L.day)	0.06	0.53	1.18
F/M (gCOD/g MLVSS.day)	0.03	0.27	0.55
MLVSS (mg/L)	1750	1950	2150
SRT (days)	25		
Operation days (days)	90		
RD concentration (mg/L)	15		
ACT (ng/mL)	45.04	45.74	46.07
FLN (ng/mL)	47.97	48.18	48.78
PHE (ng/mL)	22.04	22.13	22.23
ANT (ng/mL)	1.01	1.07	1.10
CRB (ng/mL)	0.67	0.69	0.71
FL (ng/mL)	0.48	0.49	0.53
PY (ng/mL)	0.50	0.51	0.52
BaA (ng/mL)	0.07	0.08	0.09
CHR (ng/mL)	0.15	0.17	0.19
BbF (ng/mL)	0.03	0.04	0.05
BkF (ng/mL)	0.02	0.03	0.04
BaP (ng/mL)	0.02	0.03	0.04
IcdP (ng/mL)	0.17	0.18	0.19
DahA (ng/mL)	0.34	0.39	0.41
BghiP (ng/mL)	0.02	0.03	0.04
Total PAHs (ng/mL)	118.53	119.76	120.99

Study 6:

In this run the start-up period of the continuously fed anaerobic inverse turbulent bed reactor (ITBR) was investigated in the absence of the biosurfactants. The operational conditions of this study were given in Table 5.9.

Table 5.9 Operational conditions of the anaerobic ITBR at the start-up period

Operational conditions parameters	Values		
	Minimum	**Mean**	**Maximum**
Q (L/d)	2		
Volume (L)	5.50		
COD_{total} (mg/L)	3180	3378	3576
COD_{dis} (mg/L)	2735	2850	2975
BOD_5 (mg/L)	584	656	728
HRT (days)	2.75		
OLR (gCOD/L.day)	1.00	1.04	1.08
F/M (gCOD/g MLVSS.day)	0.04	0.05	0.06
MLVSS (g/L)	19.20	21.15	23.10
SRT (days)	55	63	67
Operation days (days)	300		
RD concentration (mg/L)	0		
ACT (ng/mL)	143.67	144.84	146.93
FLN (ng/mL)	39.74	40.54	41.71
PHE (ng/mL)	74.31	75.43	75.69
ANT (ng/mL)	4.12	4.16	4.21
CRB (ng/mL)	2.78	2.90	2.91
FL (ng/mL)	10.34	10.94	11.74
PY (ng/mL)	12.64	12.82	12.46
BaA (ng/mL)	0.04	0.05	0.06
CHR (ng/mL)	0.15	0.17	0.18
BbF (ng/mL)	0.02	0.04	0.05
BkF (ng/mL)	0.03	0.05	0.06
BaP (ng/mL)	0.01	0.02	0.03
IcdP (ng/mL)	0.01	0.02	0.03
DahA (ng/mL)	0.01	0.02	0.03
BghiP (ng/mL)	0.02	0.03	0.04
Total PAHs (ng/mL)	287.89	292.00	296.13

Study 7:

In this step, the effect of increasing RD concentrations on the removal efficiencies of the 15 PAHs and the COD_{dis} were investigated in continuous fed anaerobic ITBR. Furthermore, the effects of increasing SRTs on acute toxicity removals were investigated using *Daphnia magna* and *Vibrio fischeri* test. The operational conditions of study were given in Table 5.10.

Table 5.10 Operational conditions of the anaerobic ITBR at increasing RD concentrations

Operational condition parameters	Values		
	Minimum	**Mean**	**Maximum**
Q (L/day)	2		
Volume (L)	5.5		
COD_{total} (mg/L)	3180	3378	3576
COD_{dis} (mg/L)	2735	2850	2975
BOD_5 (mg/L)	584	656	728
HRT (days)	2.75		
OLR (gCOD/L.day)	1.00	1.04	1.08
F/M (gCOD/g MLVSS.day)	0.04	0.05	0.06
MLVSS (g/L)	19.20	21.15	23.10
SRT (days)	55	63	67
Operation days (days)	440		
RD concentration (mg/L)	0-15-50-75-100-150		
ACT (ng/mL)	143.67	144.84	146.93
FLN (ng/mL)	39.74	40.54	41.71
PHE (ng/mL)	74.31	75.43	75.69
ANT (ng/mL)	4.12	4.16	4.21
CRB (ng/mL)	2.78	2.90	2.91
FL (ng/mL)	10.34	10.94	11.74
PY (ng/mL)	12.64	12.82	12.46
BaA (ng/mL)	0.04	0.05	0.06
CHR (ng/mL)	0.15	0.17	0.18
BbF (ng/mL)	0.02	0.04	0.05
BkF (ng/mL)	0.03	0.05	0.06
BaP (ng/mL)	0.01	0.02	0.03
IcdP (ng/mL)	0.01	0.02	0.03
DahA (ng/mL)	0.01	0.02	0.03
BghiP (ng/mL)	0.02	0.03	0.04
Total PAHs (ng/mL)	287.89	292.00	296.13

Study 8:

In this step, the effect of HRTs on the removal efficiencies of the PAHs and the COD_{dis} were investigated in continuous fed anaerobic ITBR at an optimum RD concentration (75 mg/L). The operational conditions of study were given in Table 5.11.

Table 5.11 Operational conditions of the anaerobic ITBR at increasing HRTs

Operational condition parameters	Values		
	Minimum	**Mean**	**Maximum**
Q (L/day)	0.5-1-2-3-4		
Volume (L)	5.50		
COD_{total} (mg/L)	3180	3378	3576
COD_{dis} (mg/L)	2735	2850	2975
BOD_5 (mg/L)	584	656	728
HRT (days)	1.38-1.83-2.75-5.5-11		
OLR (gCOD/L.day)	0.26	1.04	2.07
F/M (gCOD/g MLVSS.day)	0.01	0.04	0.07
MLVSS (g/L)	23.15	26.78	30.17
SRT (days)	59	68	79
Operation days (days)	200		
RD concentration (mg/L)	75		
ACT (ng/mL)	143.67	144.84	146.93
FLN (ng/mL)	39.74	40.54	41.71
PHE (ng/mL)	74.31	75.43	75.69
ANT (ng/mL)	4.12	4.16	4.21
CRB (ng/mL)	2.78	2.90	2.91
FL (ng/mL)	10.34	10.94	11.74
PY (ng/mL)	12.64	12.82	12.46
BaA (ng/mL)	0.04	0.05	0.06
CHR (ng/mL)	0.15	0.17	0.18
BbF (ng/mL)	0.02	0.04	0.05
BkF (ng/mL)	0.03	0.05	0.06
BaP (ng/mL)	0.01	0.02	0.03
IcdP (ng/mL)	0.01	0.02	0.03
DahA (ng/mL)	0.01	0.02	0.03
BghiP (ng/mL)	0.02	0.03	0.04
Total PAHs (ng/mL)	287.89	292.00	296.13

Study 9:

In this step, the effect of RD on the removal efficiencies of the 15 PAHs and the COD_{dis} were investigated in continuous fed sequential anaerobic ITBR/aerobic CSTR. Furthermore, the effects of increasing SRTs on acute toxicity removals were investigated using *Daphnia magna* and *vibrio fischeri* test. The operational conditions of study were given in Table 5.12.

Table 5.12 Operational conditions of sequential anaerobic ITBR/aerobic at increasing HRTs

Operational condition parameters	Values		
	Minimum	**Mean**	**Maximum**
Q (L/day)	2		
Volume (L)	5.50		
COD_{total} (mg/L)	3180	3378	3576
COD_{dis} (mg/L)	2735	2850	2975
BOD_5 (mg/L)	584	656	728
HRT (days)	3.88-5.13-7.75-15.5-31		
OLR for ITBR (gCOD/L.day)	0.26	1.04	2.07
OLR for CSTR (gCOD/L.day)	0.030	0.034	0.040
F/M for ITBR (gCOD/g MLVSS.day)	0.01	0.04	0.07
F/M for CSTR (gCOD/g MLVSS.day)	0.010	0.012	0.014
MLVSS (g/L)	23.15	26.78	30.17
SRT (days)	88		
Operation days (days)	100		
RD concentration (mg/L)	90		
ACT (ng/mL)	143.67	144.84	146.93
FLN (ng/mL)	39.74	40.54	41.71
PHE (ng/mL)	74.31	75.43	75.69
ANT (ng/mL)	4.12	4.16	4.21
CRB (ng/mL)	2.78	2.90	2.91
FL (ng/mL)	10.34	10.94	11.74
PY (ng/mL)	12.64	12.82	12.46
BaA (ng/mL)	0.04	0.05	0.06
CHR (ng/mL)	0.15	0.17	0.18
BbF (ng/mL)	0.02	0.04	0.05
BkF (ng/mL)	0.03	0.05	0.06
BaP (ng/mL)	0.01	0.02	0.03
IcdP (ng/mL)	0.01	0.02	0.03
DahA (ng/mL)	0.01	0.02	0.03
BghiP (ng/mL)	0.02	0.03	0.04
Total PAHs (ng/mL)	287.89	292.00	296.13

5.4 Wastewater Composition

The wastewater used in this study was obtained from the influent of the activated sludge unit of a petrochemical wastewater treatment plant located in Izmir, Turkey. The properties of the real petrochemical wastewater used in this study are given in Table 5.13.

Table 5.13 Wastewater composition of petrochemical industry wastewater

Parameter	**Level. concentration**		
	Minimum	**Mean**	**Maximum**
pH	6.8	7.2	7.6
Sampling Temperature ($^{\circ}$C)	18.3	19.75	21.2
Dissolved oxygen (DO)	1.7	1.8	1.9
Total COD (COD_t)	1475	2462.5	3450
Dissolved COD (COD_{dis})	1050	1997.5	2945
BOD_5 (mg/L)	384	484	584
BOD_5/COD ratio	0.19	0.28	0.37
Total N (mg/L)	14.12	19.285	24.45
Total P (mg/L)	11.04	13.625	16.21
Ammonium (mg/L)	3.51	4.96	6.41
Nitrate (mg/L)	1.90	2.30	2.70
Nitrite (mg/L)	0.046	0.0665	0.087
Oil-grease (mg/L)	206	306	406
TSS (mg/L)	190	250	310
TVSS (mg/L)	158	204	250
Cd (mg/L)	0.003	0.004	0.005
Cr (mg/L)	0.004	0.006	0.008
Ni (mg/L)	0.020	0.030	0.040
Pb (mg/L)	0.001	0.0055	0.010
Zn (mg/L)	0.332	0.342	0.352
Fe (mg/L)	2.587	2.732	2.877
Cd (mg/L)	0.0032	0.00365	0.0041
Cr (mg/L)	0.0045	0.0054	0.0063
Ni (mg/L)	0.025	0.0305	0.036
Pb (mg/L)	0.009	0.0105	0.012
Mn (mg/L)	0.001	0.002	0.003
Co (mg/L)	0.001	0.0019	0.0028
Mg (mg/L)	0.045	0.0525	0.060
K (mg/L)	0.981	0.5475	0.114
ACT (ng/mL)	29.43	87.14	144.84
FLN (ng/mL)	9.38	24.96	40.54
PHE (ng/mL)	15.01	45.22	75.43
ANT (ng/mL)	3.61	3.89	4.16
CRB (ng/mL)	0.90	1.90	2.90
FL (ng/mL)	2.98	6.96	10.94
PY (ng/mL)	2.19	7.51	12.82
BaA (ng/mL)	0.36	0.20	0.05
CHR(ng/mL)	0.72	0.45	0.17
BbF (ng/mL)	0.08	0.06	0.04
BkF (ng/mL)	0.09	0.07	0.05
BaP (ng/mL)	0.07	0.04	0.02
IcdP (ng/mL)	0.12	0.07	0.02
DahA (ng/mL)	0.27	0.14	0.02
BghiP (ng/mL)	0.09	0.06	0.03
Total PAHs (ng/mL)	65.32	178.68	292.00

5.5 Seed Properties

Partially granulated anaerobic sludge was used as seed in the anaerobic ITBR. The seed sludge was obtained from an anaerobic reactor containing partially granulated biomass taken from the Pakmaya Yeast Beaker Factory in Izmir, Turkey. Aerobic activated sludge culture used as seed for the aerobic CSTR and it was taken from the recycle line of the final settling unit of the aeration tank of a petrochemical wastewater treatment plant in Izmir, Turkey. The mixed liquor solids concentration (MLSS) and the mixed liquor volatile suspended solid (MLVSS) concentration of anaerobic seed sludge were between 32-38 g/L and 24-32 g/L, respectively. MLSS and MLVSS concentration of the aerobic seed sludge were between 2.95-3.35 g/L and 2.5-2.8 g/L, respectively. In start-up period, 3 L and 4 L sludge were added to the anaerobic ITBR (volume of ITBR reactor= 5.5 L) and aerobic CSTR (volume of CSTR=10 L) reactors, respectively.

5.6 Analytical Procedures

5.6.1 Measurement of PAHs

Wastewater samples were filtered through a glass fiber filter (47 mm-diameter) to collect particle-phase in series with a resin column (~10 g XAD2) to collect dissolved-phase PAHs. Resin and wastewater filters were ultrasonically (Bandelin Electronic RK510 H ultrasonic bath, 35 kHz and 640 W) extracted for 60 min. with a mixture of 1:1 acetone:hexane. Prior to extraction, all samples were spiked with PAH surrogate standards to monitor analytical recovery efficiencies. The volume of extracts was reduced and was transferred into hexane using a rotary evaporator and a high-purity N_2 stream. After volume reduction to 2 mL by a gentle flow of N_2, the samples were cleaned up on an alumina-silicic acid column containing 3 g of silicic acid (3% water) and 2 g of alumina (6% water). The column was pre-washed with 20 mL of DCM (dichloromethane) followed by 20 mL of PE (petroleum ether). The sample in 2 mL of hexane was added to the top of the column and PAHs were eluted with 20 mL of DCM. After solvent exchange into hexane, the

final sample volume was adjusted to 1 mL by nitrogen blow-down (Odabaşı et al. 2001).

All extracts were analyzed for 15 PAHs (ACT, FLN, PHE, ANT, CRB, FL, PY, BaA, CHR, BbF, BkF, BaP, IcdP, DahA, and BghiP) with a gas chromatograph (GC) (Agilent 7890) equipped with a mass selective detector (Agilent 5975 inert MSD). A capillary column (HP5-MS, 30 m, 0.25 mm, 0.25 μm) was used. The initial oven temperature was held at 50°C for 1 min, was raised to 200°C at 25°C 1/min and from 200 to 300°C at 8°C 1/min, and was held for 5.5 min. The injector, ion source, and quadrupole temperatures were 295, 300, and 180°C, respectively. High purity helium was used as the carrier gas at constant flow mode (1.5 mL/ min, 45 cm/s linear velocity). The MSD was run in selected ion-monitoring mode. Compounds were identified on the basis of their retention times, target and qualifier ions and were quantified using the internal standard calibration procedure.

5.6.2 Measurement of PAHs Metabolites

Measurements were performed following the Separatory Funnel Liquid–Liquid Extraction Method (US EPA, 1992). Firstly, wastewater samples were extracted with liquid-liquid for 5 minutes with a mixture of 10:1(sample:hexane). Secondly, for the separation of the organic phase the wastewater samples were waited after mixed in a 5 min. This procedure was triplicated. Thirdly, the volume of extracts was reduced and was transferred into hexane using a rotary evaporator and a high-purity N_2 stream. After the volume was reduced to 1 mL by a gentle flow of N_2, 3 mL acetonitrile was added to the samples. After solvent exchange into hexane, the final samples volume was adjusted to 1 mL by N_2 blow-down. All extracts were analyzed with a high pressure liquid chromatography (HPLC) (Shimadzu CLASS–VP V6.14). Separations of intermediates PAH were performed in a Phenomenex EnviroSep-PP^{TM} with 5μm LC Column x 4.60 mm t at a temperature of 25 ^{o}C. A gradient was applied using distilled water and acetonitrile with a flow rate of 0.8/min. Initially 40 % of acetonitrile and 60% of water was applied to the samples, and then 100% acetonitrile with a flow rate 0.8 mL/min was added to the samples for 25 min. The

intermediates namely 5,8-dihyrox-1,4-naphthoquinone, 1,2-benzenedicarboxaldehyde, (phthaldialdehyde), Benz(a)anthracene–7,12dione, 9 hydroxyflourene and 9-phenanthrol were measured at 254 nm wave lengths with an UV detector.

5.6.3 Total COD (COD_{total})

Total COD was determined with Close Reflux Method following the Standard Methods 5220 D (APHA-AWWA-WEF, 2005). Firstly, digestion solution was prepared by adding 10.216 g K_2Cr2O_7, 167 mL concentrated H_2SO_4 and 33.3 g $HgSO_4$ into distilled water to be 1000 mL and the solution was cooled to room temperature. Secondly, 2.5 mL volume samples were treated with 1.5 mL $K_2Cr_2O_7$ with $HgSO_4$ and 3.5 mL H_2SO_4 which contains 0.55% (w/w) Ag_2SO_4. Thirdly the closed sample tubes were stored in a 148 °C heater (thermoreactor, CR 4200 WTW) for two hours. Finally, after cooling, the samples were measured at 600 nm with an Aquamate thermo electron corporation UV visible spectrophotometer. Potassium hydrogen phthalate (KPH) was used to prepare the standard solutions of COD_{dis} with 17 g KPH/L which is equivalent to 20 g COD_{dis}/L. The standard concentrations of COD_{dis} (mg/L) versus triplicate absorbance (Abs) and mean values are given Table 5.14 and Figure 5.6.

Table 5.14 Standard concentrations (mg/L) versus absorbance (Abs) values of COD (wave length = 600 nm) using an Aquamate UV visible spectrophotometer (2007).

Standard concentration of COD (mg/L)	Absorbance values			
	Analysis 1	Analysis 2	Analysis 3	Mean Abs
50	0.010	0.010	0.010	0.010
100	0.020	0.018	0.018	0.019
200	0.049	0.049	0.050	0.049
300	0.091	0.090	0.091	0.091
400	0.124	0.124	0.125	0.124
500	0.157	0.157	0.158	0.157
600	0.187	0.189	0.189	0.188
700	0.223	0.223	0.223	0.223
800	0.289	0.248	0.265	0.267
900	0.294	0.295	0.305	0.298

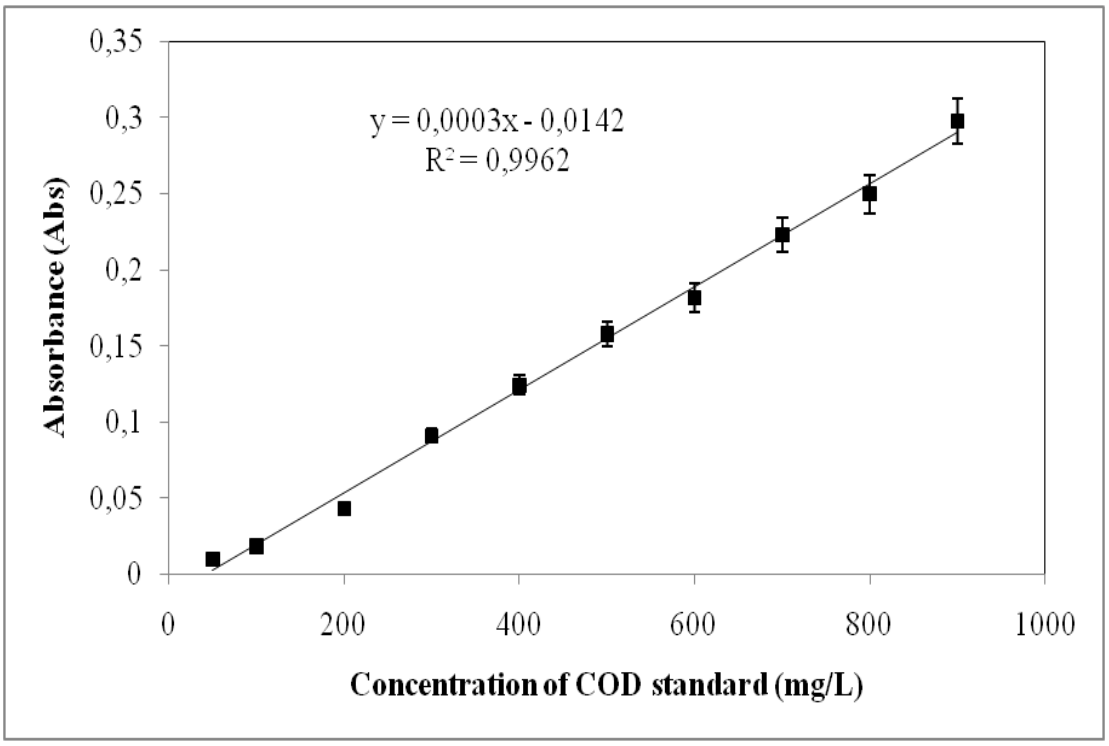

Figure 5.6 Calibration graphic of COD at 600 nm wave length

5.6.3.1 Dissolved Chemical Oxygen Demand (COD_{dis})

COD_{dis} was determined with Close Reflux Method following the Standard Methods 5220 D (APHA-AWWA-WEF, 2005). Samples were centrifuged at 8000 rpm for 20 minutes to remove the suspended solid from the liquid phase. Firstly, digestion solution was prepared by adding 10.216 g K_2Cr2O_7, 167 mL concentrated H_2SO_4 and 33.3 g $HgSO_4$ into distilled water to be 1000 mL and the solution was cooled to room temperature. Secondly, 2.5 mL volume samples were treated with 1.5 mL $K_2Cr_2O_7$ with $HgSO_4$ and 3.5 mL H_2SO_4 which contains 0.55% (w/w) Ag_2SO_4. Thirdly the closed sample tubes were stored in a 148 °C heater (thermoreactor, CR 4200 WTW) for two hours. Finally, after cooling, the samples were measured at 600 nm with an Aquamate thermo electron corporation UV visible spectrophotometer.

5.6.3.2 Determination of COD Subcategories [Dissolved COD (COD_{dis}), Inert COD (COD_i), Metabolic Product COD (COD_{mp}), the Readily Biodegradable COD (COD_r) and Slowly Biodegradable COD (COD_s)]

The total COD of a raw wastewater is usually made up of biodegradable and non-biodegradable fractions. On the other whole, the biodegradable fraction consists of dissolved, readily biodegradable, slowly biodegradable organics, while the non-biodegradable fraction consists of dissolved and particulate inert organics. The wastewater dissolved COD concentration, COD_{dis} is therefore, equal to the sum of the inert dissolved COD, (COD_i) and readily biodegradable dissolved COD, (COD_r) (Eq. 5.1) (Ince et al., 1998; Ekama et al., 1986; Orhon et al., 1994).

$$COD_{dis} = COD_r + COD_i \quad (5.1)$$

COD_{dis} was measured with Close Reflux Method following the Standard Methods 5220 D (APHA-AWWA-WEF, 2005). Firstly, the samples were filtered 0.45 µm membrane (Watman Membrane Filter, Germany). Secondly, 2.5 mL volume samples were treated with 1.5 mL $K_2Cr_2O_7$ with $HgSO_4$ and 3.5 mL H_2SO_4 which contains 0.55% (w/w) Ag_2SO_4. Thirdly the closed sample tubes were stored in a 148 °C heater

(thermoreactor, CR 4200 WTW) for two hours. Finally, after cooling, the samples were measured at 600 nm with an Aquamate thermo electron corporation UV visible spectrophotometer.

The COD_i was determined using the glucose comparison method in influent and effluent wastewater. In this method, two batch reactors were operated in parallel and started with the same initial COD, two being fed with the soluble part of the wastewater to be tested, and the other with glucose. The COD_{dis} in each reactor was measured periodically until a plateau value was reached, at which point the biodegradable substrate was almost entirely depleted (variations in COD within ± 5% were taken as an indication that the minimum COD was attained) (Figure 5.7).

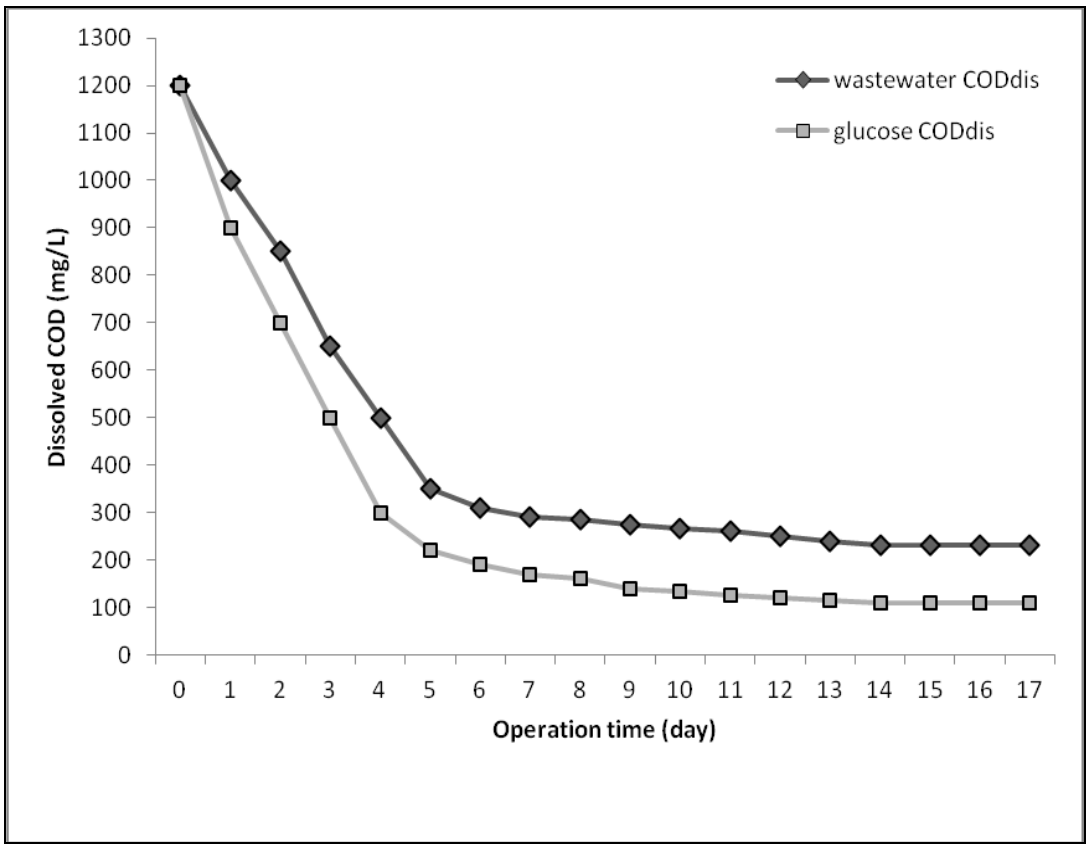

Figure 5.7 Evaluation of inert COD

Final residual COD_{dis} was assumed to be equal to sum of COD_i and soluble microbial product (COD_{mp}). The COD of the reactor fed on the glucose reached a lower value, which was taken to represent COD_{mp} alone, as glucose itself would have

contained no inert organic material. The COD_i content of the wastewater was calculated from the Eq. (5.2) with assumption that COD_{mp} (wastewater) ~ COD_{mp} (glucose);

$$COD_I = COD_I(wastewater) + COD_i\ (glucose) \quad (5.2)$$

COD associated with inert dissolved microbial products (COD_{mp}) was calculated using the Eq. (5.3);

$$COD_{mp} = [COD_{dis}\ (remaining\ COD) - COD_{dis}] \quad (5.3)$$

The readily biodegradable COD_r was calculated using the Eq. (5.4),

$$COD_r = \frac{dissolved\ O_2\ change}{1 - Y} \quad (5.4)$$

Y is the yield and it is equal to Eq. (5.5) which was obtained from the experimental data.

$$Y = \frac{produced\ biomass\ (VSS)}{1 - Y} \quad (5.5)$$

Slowly biodegradable COD (COD_s) is equivalent to Eq. (5.6)

$$COD_s = COD_{dis}[- [COD]_r + COD_i] \quad (5.6)$$

5.6.4 BOD_5 Measurement

BOD_5 measurements were carried out in Oxi Top IS 12 system manufactured by the WTW Merck Company. The range of expected BOD_5 values was then deduced and hence led to the volumes of sample and nitrification inhibitor (10 mg/L solution

of N-allylthiourea) which have to be added to the shake flask of the Oxitop apparatus.

5.6.5 Total Nitrogen (TN) and Total Phosphorus (TP) Measurements

The TN and TP were measured with 1.14537.0001 (0.50-15.00 mg/L), 1.14729.0001 (0.5-25.0 mg/L PO_4-P, 1.5-76.7 mg/L PO_4, 1.1-57.3 P_2O_5 mg/L) cell test spectroquant kit (Merck Chemical Company, Germany) using a spectroquant NOVA-60 (Merck) spectrophotometer (2003), respectively.

5.6.6 Ammonium-Nitrogen (NH_4-N) Nitrite-Nitrogen (NO_2-N) and Nitrate-Nitrogen (NO_3-N) Measurements

The NH_4-N, NO_2-N and NO_3-N were measured with 1.14752.0001 (0.010-3.00 mg/L NH_4-N, 0.013-3.86 NH_4 mg/L), 1.14547.0001. (0.01 - 0.70 mg/L), and 1.14773.0001 (0.20 - 20.0 mg/L NO_3-N, 0.90 - 88.50 NO_3 mg/L) cell test spectroquant kit (Merck Chemical Company, Germany) using a spectroquant NOVA-60 (Merck) spectrophotometer (2003), respectively.

5.6.7 Oil and Grease Analysis

Oil and Grease analysis were performed gravimetrically following Standard Methods 5520-B (APHA-AWWA-WEF, 2005).

5.6.8 Heavy Metals Analysis

Heavy metal measurements were performed following Standard Methods 3120-B using an inductively coupled plasma-optical emission spectrometry (ICP-OES) (APHA-AWWA-WEF, 2005).

5.6.9 pH, Dissolved Oxygen (DO), Oxidation Reduction Potential (ORP) and Temperature (T, ^oC) Measurements

pH and temperature measurements were carried out using A pH meter with an electronic probe WTW MultiLine P3 pH/Oxi-SET. ORP was measured using WTW SenTix ORP meter with $Ag/AgCl_2$ reference electrode in KCl solution and Pt electrode.

5.6.10 Mixed Liquor Suspended Solids (MLSS), Mixed Liquor Volatile Suspended Solids (MLVSS), Total Suspended Solids (TSS), Total Volatile Suspended Solids (TVSS), and Volatile Suspended Solids (VSS) Measurements

MLSS and TSS were measured following the Standard Methods 2540-B and 2540 D. MLVSS, TVSS and VSS measurements were performed according to Standard Methods 2540 E (APHA-AWWA-WEF, 2005).

5.6.11 Measurement of Carrier Material Diameter

The diameters of Omega U-sheper W these particles were measured between 169 and 200 μm with Olympus BX51-DP72 model microscope.

5.6.12 Total Volatile Fatty Acid (TVFA) and HCO_3 Alkalinity Measurements

HCO_3 alkalinity and TVFA concentrations were measured according to Anderson and Yang Method (1992). Samples were titrated by 0.1 N H_2SO_4 and then the bicarbonate and total volatile fatty acid concentration were calculated with a computer program. The samples were titrated with standard H_2SO_4 through two steps. The H_2SO_4 volumes titrate were recorded until the pH of the sample reduced to 5.1 to 3.5. The HCO_3 and the TVFA concentration were calculated with a computer program (Eq 5.7 and Eq. 5.8),

$$A_1 = \frac{[HCO_3^-].([H]_2 - [H]_1)}{[H]_1 + K_c} + \frac{[VA].([H]_2 - [H]_1)}{[H]_2 + K_{va}} \quad (5.7)$$

$$A_2 = \frac{[HCO_3^-].([H]_3 - [H]_1)}{[H]_3 + K_c} + \frac{[VA].([H]_3 - [H]_1)}{[H]_3 + K_{va}} \quad (5.8)$$

Where; A_1 and A_2 are the molar equivalent of the standard acid consumed to the first and second endpoints; $[HCO_3^-]$ is the bicarbonate concentration; [VA] is the volatile fatty acid ion concentration; $[H]_{1,2,3}$ are the hydrogen ion concentrations of the original sample and at the first and the second endpoints; K_C is a conditional dissociation constant of carbonic acid; and K_{VA} is a combined dissociation constant of the volatile fatty acids (C_2 to C_6), it was assumed that this pair of constants, being 6.6 X 10^{-7} for bicarbonate and 2.4 X 10^{-5} for volatile acids.

5.6.13 Total Gas and Methane Gas Measurements

They were measured using a liquid displacement system. The total gas production was monitored volumetrically with a glass syringe by passing it through a liquid displacement system containing 10% NaCl and 2% H_2SO_4 and methyl orange. The methane gas production was monitored with a liquid displacement system containing 3% NaOH and by passing the gas from the aforementioned device (Razo-Flores et al., 1997; Beydilli et al., 1998).

5.6.14 Specific Methanogenic Activity (SMA)

The specific methanogenic activity test was conducted in 150 mL serum bottles at 35 ^{0}C under anaerobic conditions. Sodium thioglycolate and $NaHCO_3$ were added to reduce the redox potential and to keep the anaerobic conditions and the pH neutral throughout the study. At 35 ^{o}C 395 mL of CH_4 production is equivalent to 1 g of COD reduction. The following formula was used to calculate the SMA (Eq. 5.9).

$$(SMA)\frac{gCH_4}{gVSS.d} = \frac{produced\ CH_4\ \ volume\ (mL).395\frac{mL}{g\ COD}}{sample\ volume\ (mL).incubation\ time\ (d).biomass\ concentration\ \left(\frac{g\ VSS}{L}\right)} \quad (5.9)$$

5.6.15 Rhamnolipid (RD) Measurements

Total rhamnolipid concentration in the sample was determined by measuring the concentration of hydrolysis-released rhamnose by the orcinol method after acid hydrolysis of the samples (Koch et al., 1991). Firstly, the samples were centrifuged for 10 min at 8000 rpm. Secondly, 0.19 g (0.19% w/v) orcinol was dissolved in 100 mL H_2SO_4 (53% v/v). Thirdly, for the centrifugation of the samples, 0.1 mL supernatant sample and 0.9 mL orcinol reagent were mixed in a test tube. After heating for 30 min at 80°C, the samples were cooled for 15 min at room temperature and the optical density was measured at a wave length of 421 nm with an Aquamate UV visible spectrophotometer. Standard concentrations of Rhamnolipid (mg/L) versus absorbance (Abs) values and calibration graphic at a wave length of 421 nm are shown in Table 5.15 and Figure 5.8, respectively.

Table 5.15 Standard concentrations of Rhamnolipid (mg/L) versus absorbance (Abs) values of rhamnolipid (wave length = 421 nm) using an Aquamate UV visible spectrophotometer.

Concentration of rhamnolipid standard (mg/L)	Analysis 1 (Abs)	Analysis 2 (Abs)	Analysis 3 (Abs)	Mean (Abs)
10	0.112	0.133	0.121	0.124
20	0.153	0.157	0.160	0.154
30	0.212	0.223	0.194	0.211
40	0.271	0.294	0.292	0.284
50	0.310	0.346	0.33	0.332
60	0.372	0.388	0.391	0.387
70	0.442	0.476	0.46	0.461
80	0.511	0.537	0.517	0.529
90	0.586	0.590	0.574	0.581
100	0.620	0.651	0.613	0.630

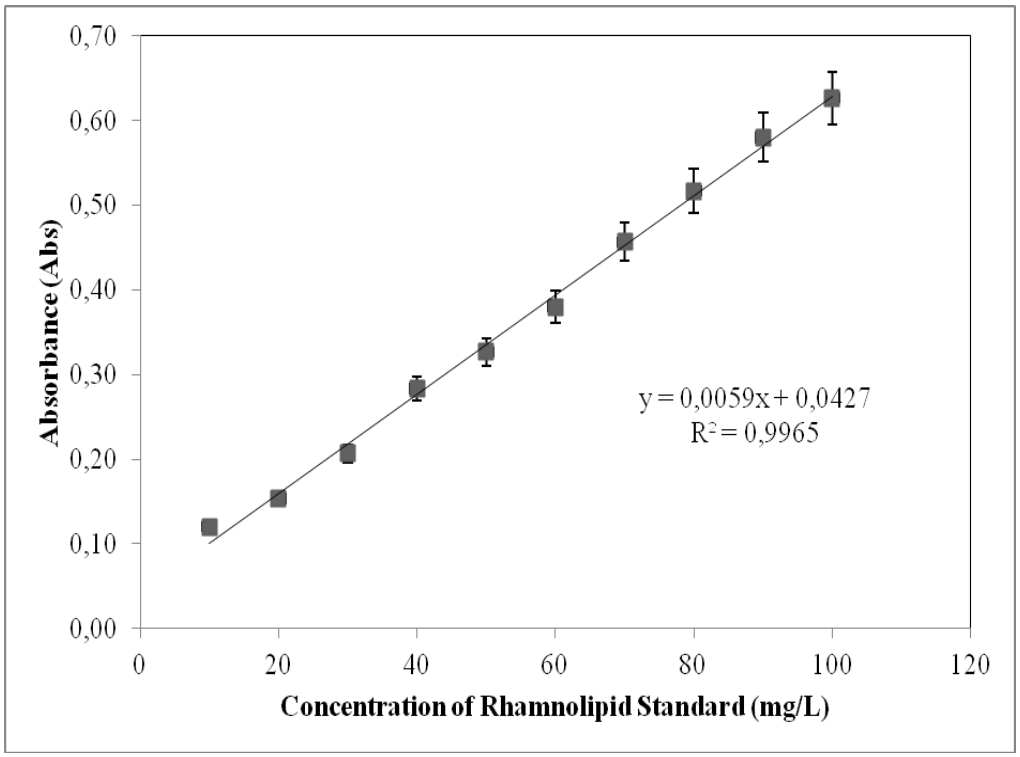

Figure 5.8 Rhamnolipid calibration graphic at a wave length of 421 nm

5.6.16 Surfactin (SR) Measurements

Wastewater samples were centrifuged in a Hettich Universal 320 model centrifuge at 8000 rpm for 15 min to remove the suspended solid. The samples were washed four times by filtration. Then methanol was added to the retentate until the concentration of methanol reached about 70% (v/v), and the solution was filtered through the 0.2 μm membrane. The final filtrate was concentrated by evaporation using a Heidolph VV2000 Rotaevaporator. The samples were measured with an AquaMED spectrophotometer at wavelengths of 302 nm (Shabtai et al., 2009). Standard concentrations of surfactin (mg/L) versus absorbance (Abs) values and calibration graphic at a wave length of 302 nm are shown in Table 5.16 and Figure 5.9, respectively.

Table 5.16 Standard concentrations of Surfactin (mg/L) versus absorbance (Abs) values of surfactin (wave length = 302 nm) using an Aquamate UV visible spectrophotometer.

Concentration of surfactin standard (mg/L)	Analysis 1 (Abs)	Analysis 2 (Abs)	Analysis 3 (Abs)	Mean (Abs)
10	0.061	0.054	0.055	0.051
20	0.128	0.130	0.126	0.124
30	0.184	0.195	0.203	0.194
40	0.256	0.250	0.253	0.251
50	0.326	0.319	0.341	0.322
60	0.416	0.425	0.434	0.428
70	0.510	0.526	0.532	0.529
80	0.626	0.583	0.62	0.612
90	0.717	0.706	0.722	0.710
100	0.814	0.827	0.844	0.823

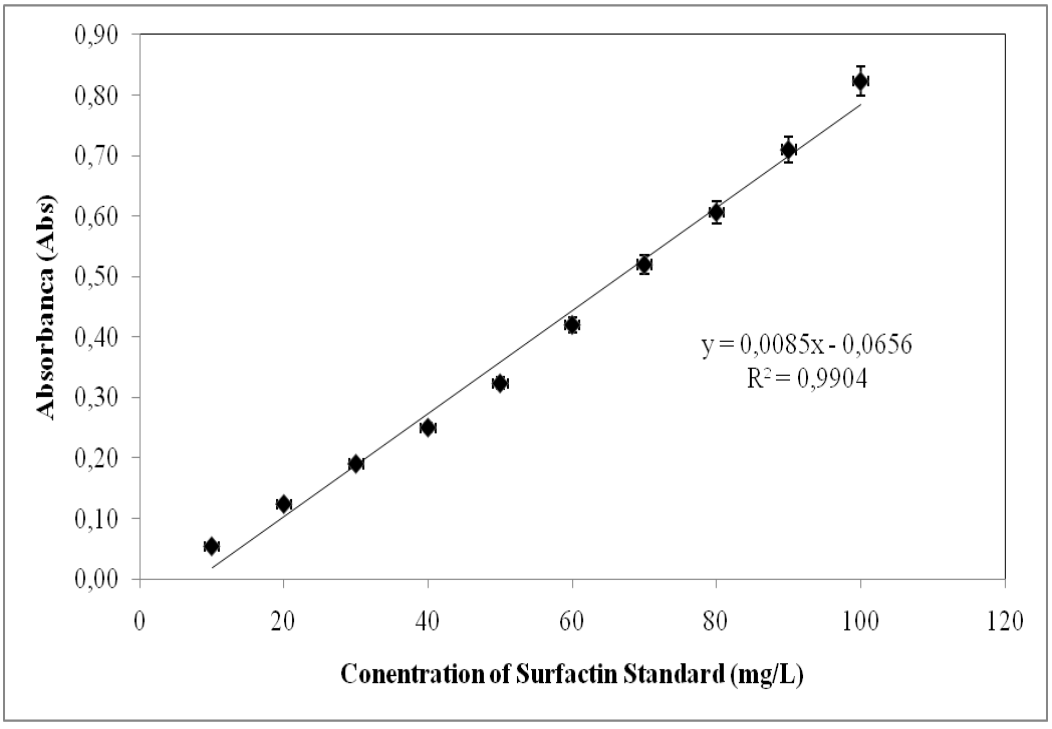

Figure 5.9 SR calibration graphic at a wave length of 302 nm

5.6.17 Emulsan (EM) Measurements

Wastewater samples were centrifuged in a Hettich Universal 320 model centrifuge at 8000 rpm for 15 min to remove the suspended solid. For quantification of EM, hexadecane and 2-methylnaphthalene, in a volumetric ratio of 1 : 1 was added to 20 mM tris-HCl buffer solution containing 10 mM $MgSO_4$, keeping a volumetric ratio of samples to water of 1 : 75. The assay mixtures were shaken at 30°C in baffled flasks for 1 h. The samples were measured with an AquaMED spectrophotometer at wavelengths of 298 nm (Coutte et al., 2010). The data were correlated with the standard curve for EM. Standard concentrations of Emulsan (mg/L) versus absorbance (Abs) values and calibration graphic at a wave length of 298 nm are shown in Table 5.17 and Figure 5.10, respectively.

Table 5.17 Standard concentrations of Emulsan (mg/L) versus absorbance (Abs) values of emulsan (wave length = 298 nm) using an Aquamate UV visible spectrophotometer.

Concentration of Emulsan standard (mg/L)	Analysis 1 (Abs)	Analysis 2 (Abs)	Analysis 3 (Abs)	Mean (Abs)
10	0.050	0.047	0.039	0.041
20	0.110	0.124	0.142	0.12
30	0.16	0.17	0.164	0.168
40	0.264	0.279	0.286	0.270
50	0.352	0.361	0.358	0.351
60	0.44	0.46	0.456	0.457
70	0.502	0.519	0.547	0.522
80	0.584	0.576	0.594	0.588
90	0.685	0.679	0.691	0.684
100	0.754	0.766	0.781	0.766

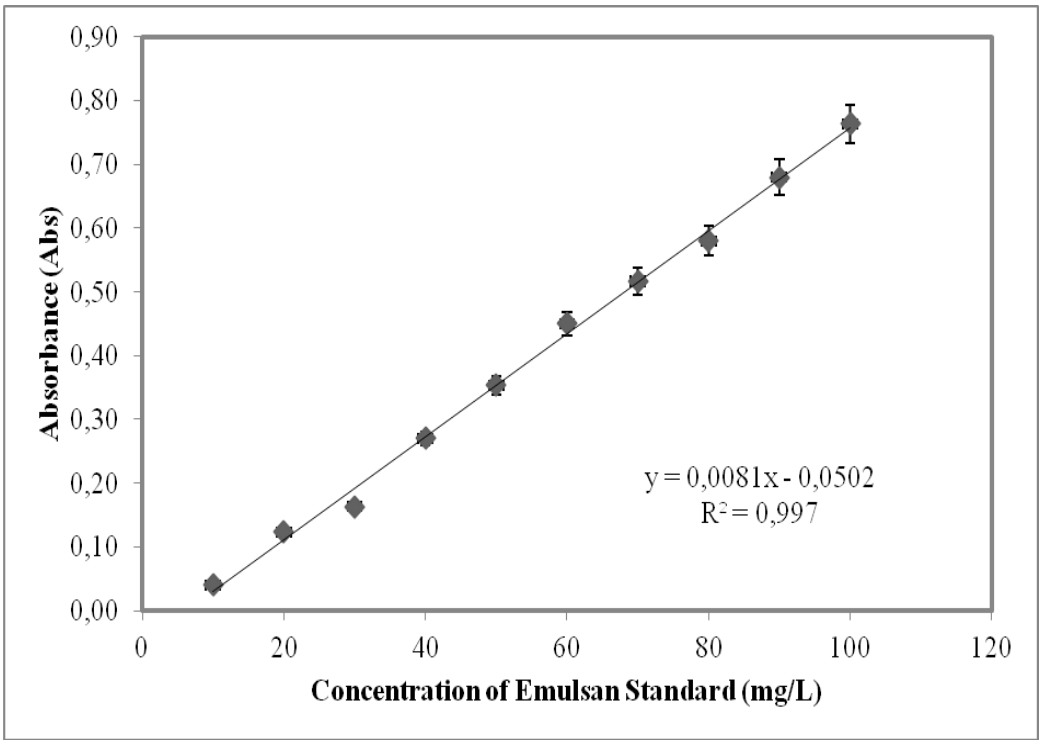

Figure 5.10 EM calibration graphic at a wave length of 298 nm

5.6.18 Adsorption Test

This test was used to determine the adsorption of PAHs on killed biomass. It was performed following the procedure given by Trably and Patureau, (2007) and Zheng et al., (2007). A 2 L reactor containing 1 L of raw wastewater was seeded with autoclaved aerobic biomass with a 1.2 g VSS/L and incubated for two days at ambient temperature. The control bottles contained only wastewater. The PAH concentrations in the samples taken from the test reactors were measured periodically and compared with a control.

5.6.19 Volatilization Test

In order to determine the transformation of PAHs by volatilization at ambient temperature their concentrations were monitored in a control and in a reactor (2 L) containing no aerobic sludge. After an incubation time of two days, the PAH concentrations in supernatant and in the headspace of the reactors were measured

following the procedure given by Trably and Patureau (2007) and Zheng et al. (2007).

5.6.20 Sorption of PAHs

This test was used to determine the equilibrium partition coefficient (K_p) for the sorption of PAHs on killed activated sludge biomass; it was performed following the procedure given by Dobbs et al. (1988). Harvested biomass samples were washed and re-suspended in distilled water for sorption experiments. The aerobic sludge was inactivated in an autoclave at 121 ^{o}C for 15 minutes under a pressure of 1.5 atm. The autoclaved sludge were inoculated on the glass plates containing suitable media and incubated to determine whether they were alive. It was found that all the sludge samples were death. Control and 2 L reactors containing 1 L of raw wastewater were seeded with autoclaved aerobic biomass which had a VSS concentration of 1.2 g/L. They were incubated for two days at an ambient temperature. The CSTR Reactors were sealed with Teflon lined caps and shaken at 21 ^{o}C for 3 days. The control bottles contained no wastewater. The PAH concentrations in the samples taken from the test reactors were measured periodically and compared with a control. An absorbance coefficient of 0.28 g dry weight biomass/L per absorbance unit was determined from the standard curves following the procedure reported by Kotch, (1994). After two days, the glass bottles containing sample and bacteria were centrifuged sufficiently to remove the bacteria, the supernatant was harvested and the PAHs in the supernatant were analyzed using GC-MS. The glass bottles containing control without biomass were compared to glass bottles with biomass to determine the amount of PAHs sorbed.

The partition coefficient (K_p), relates the sorption concentration of a solute, to the concentration in wastewater. As an equation, it is given as the biomass of solute on the solid phase per unit mass of solid phase divided by the concentration of solute in solution. Linear sorption isotherm of BaP for the biomass was obtained Figure 5.11.

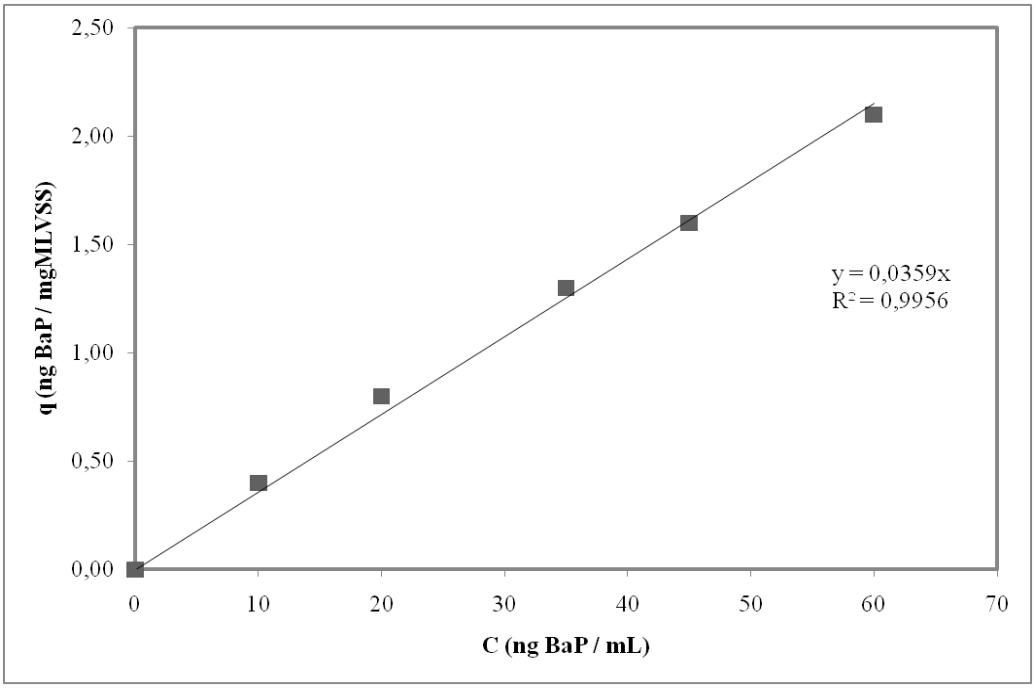

Figure 5.11 Sorption isotherm of BaP of aerobic biomass

A partition coefficient was determined from a linear sorption isotherm (Stringfellow et al. 1999), which was a plot that describes the amount of a species sorbed as a function of its concentration in solution, measured at a constant temperature. The distribution coefficient is related to the slope of the line created by this isotherm. Sorption of PAHs by biomass was fitted to the following linear sorption model:

$$K_p = \frac{P_x}{P_s} \qquad (5.10)$$

Where P_x is the mass of PAHs sorbed per gram of bacterial dry weight and P_s is the mass of dissolved PAH per liter solution, yielding a K_p in L/g biomass.

In order to determine the influence of biosorption on biodegradation, cultures of *Pseudomonas putida* (a PAH-degrader bacteria) and *Escherichia coli* (non- PAH degrader bacteria with high K_p) were grown separately on 10 g/L peptone, 10 g/L glucose harvested and re-suspended in mineral salts (2 mg/L $CaCI_2$, 2 mg/L NaCI) media. Three test were performed i) PAH-degraders alone, ii) non-PAH-degraders

and a killed control. The reactors contained 30 mg/L *Pseudomonas putida* and 30 mg/L *Escherichia coli*. The control also contained 30 mg/L autoclaved *Escherichia coli*. At the beginning, 67 ng/mL benzo(a)pyrene was added to each vial prior to placement in a 23 ^{o}C shaker. Samples from each vial were taken at appropriate intervals and the total benzo(a)pyrene was measured for each time-point using GC-MS. Triplicate sampling was carried out for each concentration and control. The operational conditions of this batch study were given in Table 5.3.

5.7 Isolation and Identification of the Bacteria

5.7.1 Isolation and Identification of Pseudomonas aeruginosa

The isolation and identification of Pseudomonas aeruginosa were performed in M-PA media following the Standard Methods (APHA-AWWA-WEF, 2005). The ingredients of the M-PA agar was as follows for 1 L distilled water: L-lysine HCI (5 g), NaCl (5 g), yeast extract (2 g), xylose (2.5 g), sucrose (1.25 g), lactose (1.25 g), phenol red (0.08 g), ferric ammonium citrate (0.8 g), sodium thiosulfate, ($Na_2S_2O_3$ (6.8 g) and agar (15 g).

Initially, the basal media were dissolved by boiling and then the pH was adjusted to 7.2, after autoclaving at 121°C for 15 min. The media was kept at 55°C before the antibiotics [(sulfapyridine (176 mg), kanamycin (8.5 mg), nalidixic acid (37.0 mg) and cycloheximide (150 mg)] were added and then poured with filtering from the diameter 50x12 mm petri plates. 200 mL wastewater samples were filtered through sterile membrane filters (pore size 0.45 μm, diameter 47 mm, Sartorius stedim Biotech GmbH, Germany. Each membrane was placed on a poured plate of M-PA agar so that there was no air space between the membrane and the agar surface. The plates were inverted and incubated at 41.5 ^{o} C for 72 h.

P. aeruginosa colonies were 0.8 to 2.2 mm in diameter and flat in appearance with light outer rims and brownish to greenish-black center. A number of typical and atypical colonies were confirmed using milk agar. An isolated colony was used on a

milk agar (Instant nonfat milk 100 g/L, nutrient broth 12.5 g/L, NaCl 2.5 g/L and agar 15 g/L) plate and incubated at 35^{o}C for 24 h. *P.aeruginosa* hydrolyzes casein and produces a yellowish to green diffusible pigment. The result is given as the colony number of *P. aeruginosa*/ 100 mL.

5.7.2 Isolation and Identification of Escherichia coli

The isolation and identification of Escherichia coli was performed in M-EC media according to the Standard Methods (APHA-AWWA-WEF, 2005). The culture medium consisted of triptose (10 g/L), proteose peptone (5 g/L), yeast extract (3 g/L), NaCl (5 g/L) lactose (12.5 g/L), bile salts (1.5 g/L), aniline blue (0.1 g/L) in 1 L distilled water. 100 mL wastewater samples were filtered through sterile membrane filters (pore size 0.45 µm, diameter 47 mm, Sartorius stedim Biotech GmbH, Germany). Each membrane was placed on a poured plate of M-EC agar so that there was no air space between the membrane and the agar surface. After the incubation was performed throughout 24 h at 44.5 ^{o}C colonies produced by fecal coliform bacteria on basal medium were various shades of blue. Typical blue colonies must be verified and confirmed. *E.coli* colonies inoculated in EC-MUG broth medium (tryptose 20 g/L, lactose 5 g/L, bile salt mixture 1.5 g/L, K_2HPO_4 4 g/L, KH_2PO_4 1.5 g/L NaCl 5 g/L, 4-methylumbelliferyl-B- D-glucuronide (MUG), 0.05 g/L in 1 L distilled water) and brilliant green lactose broth (peptone 10 g/L, lactose 10 g/L, oxgall 20 g/L brilliant green 0.0133 g/L in 1 L distilled water) tubes (APHA-AWWA-WEF, 2005). These tubes were incubated at 48 ^{o}C for 24-48 h. The production of an acidic reaction or gas in the durham tubes within the incubation time constitutes a positive presumptive reaction. The tubes were submitted with a positive presumptive reaction to the confirmed phase. The confirmation test of *E.coli* was used GAD reagent (L-glutamic acid 1 g/L, NaCl 90 g/L, bromocresol green 0.05 g/L, polyethylene glycol actylphenyl ether 3.0 mL in 1.0 L distilled water). 5 mL broth was added from the fermentation tube to 15 mL centrifuge tube with a graduated pipet. Concentrate the bacterial cells from the broth was centrifuged at 2500 rpm for 10 min. Supernatant and resuspend cells were discarded in 5 mL phosphate buffer. Reconcentrate cell was centrifuged at 2500 rpm for 10 min. The

supernatant was added into 1 mL GAD reagent. The presence of a blue color is considered a positive response for *E.coli*. Then the purified isolate was used to inoculate on several media for biochemical testing. The biochemical tests namely Indol, Methyl Red, Voges Proskauer, Oxidase, and Catalase tests were performed on the positive tubes. The blue broth was inoculated into tryptone broth (Tryptone, 10 g/L and NaCI 5 g/L) for the Indole test, Methyl-Red-Voges-Proskauer broth (peptone from meat 7 g/L, D (+) glucose 5 g/L, phosphate buffer 5 g/L) for the Methyl Red and Voges Proskauer tests and a Simmons Citrate slant ($NH_4H_2PO_4$ 1 g/L, K_2HPO_4 1 g/L, NaCI 5 g/L, sodium citrate 2 g/L, $MgSO_4$ 0.2 g/L, agar 15 g/L, bromthymol blue 0.1 g/L) for the Citrate test. *E. coli* (green sheen colonies) produces a + + - - IMVIC test result. Thus it was positive in the Indole and Methyl Red tests and negative in the Voges Proskauer and Citrate tests.

5.7.3 Isolation and Identification of Zoogloea ramigera

Zoogloea ramigera bacteria analyses were performed in M-ZR media as reported by Paterson-Beedle et al. (2000). The culture M-ZR medium consisted of glucose (20 g/L), yeast extract (5 g/L), peptone (3 g/L), agar (15 g/L), K_2HPO_4 (1 g/L) KH_2PO_4 (1 g/L) and $MgSO_4.7H_2O$ (0.5 g/L), in 1 L distilled water and the pH was adjusted to 6.8 before sterilization at 121 ^oC, for 15 min. in an autoclave. The basal media were dissolved by boiling and then the pH was adjusted to 7.1 after autoclaving. 200 mL samples were filtered through sterile membrane filters with a pore size of 0.2 mm (Sartorius stedim Biotech GmbH, Germany) and with a diameter of 47 mm. The filters were placed on M-ZA agar and incubated at 30 ^oC for 150 h. The bacteria numbers were determined with a colony counter. Some biochemical tests (flagella, pigment formations and hydrolysis of gelatin tests) applied to the colonies in M-ZR agar to identify the *Zoogloea ramigera* bacteria.

5.7.4 Isolation and Identification of Pseudomonas putida

The isolation and identification of Pseudomonas putida was performed in M-PP media according to Yang et al. (2011). 50 mL wastewater sample was used as inoculum into 50 ml of sterile base mineral medium (BMM) in flasks. One liter of BMM contained 5.17 g K_2HPO_4, 1.7 g KH_2PO_4, 1.63 g NH_4Cl, and 10 mL of a salt solution. One liter of the salt solution contained 8.5 g $MgSO_4$, 5 g $MnSO_4$, 5 g $FeSO_4$, 0.3 g $CaCl_2$. The initial pH value of media was 7.2 (Garg, et al., 2012). Wastewater sample (10 mL) as inoculums were added into 50 mL of sterile modified base mineral medium in flasks. The inoculated flasks were agitated on an shaker, at 200 rpm at 30°C for 72 h. 1 mL of the culture medium was transferred to another 50 mL of fresh culture medium and cultivation was carried out under the same condition. Enriched sample was plated onto solid agar and then every specific colony was inoculated separately onto nutrient broth and nutrient agar. In this manner pure cultures were isolated; also, a number of colonies were determined with a colony counter. The bacteria numbers were record as the colony number of *P. putita*/100 mL.

5.7.5 Isolation and Identification of Flavobacterium

Flavobacterium bacteria analyses were performed in media were modification by Cepeda et al. (2004). The culture medium consisted of bacto peptone, 5 g/L; yeast extract, 0.5 g/L; sodium acetate, 0.01 g/L; agar, 15 g/L and broth bacto peptone, 5 g/L; yeast extract, 0.5 g/L; sodium acetate, 0.01 g/L and skimmed milk. The media were adjusted to pH 7.2–7.4 and sterilized by autoclaving (121 ^{o}C, 15 min). For the preparation skimmed milk, 10% (w/v) solutions of carbohydrates and skimmed milk were separately sterilized by filtration (0.22 μm pore size). 200 mL samples were filtered through sterile membrane filters (pore size 0.2 mm, diameter 47 mm). Place each membrane on a poured plate of the agar so that there is no air space between the membrane and the agar surface. Colonies were counted after incubation (18 ^{o}C, 96 h). Bacterial colonies displaying a characteristic orange-yellow color and rhizoid

morphology were selected. Some biochemical tests (flagella, pigment formations and hydrolysis of gelatin tests) applied to the colonies in the agar to identify the bacteria.

5.7.6 Isolation and Identification of Comamonas

The mineral medium MMN (mineral medium without nitrogen and carbon) is derived from mineral medium by eliminating all nitrogen. The MMN medium contained 1.42 mg of Na_2HPO_4, 1.36 mg of KH_2PO_4, 98.5 mg $MgSO_4$, 5.88 mg of $CaCl_2x2H_2O$, 1.16 mg of H_3BO_4, 2.78 mg of $FeSO_4x7H_2O$, 1.15 mg of $ZnSO_4x7H_2O$, 1.69 mg of $MnSO_4xH_2O$, 0.38 mg of $CuSO_4x5H_2O$, 0.24 mg of $CoCl_2x6H_2O$, 0.10 mg of MoO_3, and 3.2 mg of EDTA in 1 liter of distilled water. The liquid mineral media were supplemented with 150 to 250 mg aniline/L, while for the solidified media; aniline and 3-CA (3-chloroaniline) were added at a concentration of 500 mg/L. Luria broth (LB) medium containing 10 g of bacto peptone, 5 g of bacto yeast extract, and 5 g of NaCl in 1 L of distilled water was used as a rich medium. These media were solidified with 2% agar or plate growth (Boon et al., 2000). Cultures were incubated on a rotary shaker under aerobic conditions at 28°C. Growth was monitored by measuring the turbidity at 600 nm. 200 µL of an overnight-grown LB culture of strain, washed twice in saline (0.85% NaCl), was inoculated in 200 mL of MMN medium with 3-CA (150 mg/L) (0.1% inoculation) to monitor the transformation of 3-CA. *Comamonas* was isolated from an enrichment culture obtained from the CSTR after repeated supplementation with 3-CA. During 3 days a 1 liter Erlenmeyer flask containing 200 mL of the activated sludge (4 g dw/L) was supplemented everyday with 200 mg of 3-CA/L. After 3 days, 0.5 L Erlenmeyer flask containing 200 mL of MMN with 3-CA (200 mg/L) was inoculated with 2 mL of the activated sludge. After 3 days, 2 mL of this enrichment culture was transferred to a new 0.5 L Erlenmeyer flask with 200 mL of MMN with 3-CA (200 mg/L). After another 3 days, this culture was spread onto MMN–3-CA agar plates (500 mg of 3-CA/L) and incubated at 28°C for 2 days. The grown colonies from solid were exposed under UV illuminator at the wavelength 280-360 nm. The orange fluorescence colors observed from positive colonies were different in brightness and

it depends on the types of microorganisms able to store compounds (Zakaria et al., 2008).

5.8 Toxicity Measurements

5.8.1 Daphnia Magna Acute Toxicity Test

Toxicity was tested using 24 h born *Daphnia magna* as described in Standard Methods (APHA-AWWA-WEF, 2005). After preparing the test solution, experiments were carried out using 5 or 10 *daphnids* introduced into test vessel. These vessels were controlled with 100 mL of effective volume at 7-8 pH, providing minimum dissolved oxygen concentration of 6 mg/L at a ambient temperature of 20-25°C. *Daphnia magna* are used in the test (in first start ≤24 h old). A 24 h exposure is generally accepted for a Daphnia acute toxicity test. Results were expressed as mortality percentage of the *Daphnids*. The immobile animals which were not able to move were determined as the death of *Daphnids* (APHA-AWWA-WEF, 2005).

5.8.2 Lumistox Toxicity Measurements

A specific strain of the marine bacterium, *Vibrio fischeri* (LCK 491), was used in this test to determine the toxicity of PAHs. Reductions in light intensity at 5th, 15th and 30th minute were chosen to measure the toxicity (Lange, 1994). The standard culture, *Vibrio fischeri* (LCK 491), was obtained from Dr.Bruno Lange GmnH & Co. KG industrial measurement technique in Germany. Microtox testing was performed according to the standard procedure recommended by the manufacturer. The bioluminescence of the sample was measured in a luminometer (LUMISmini LPG 297, Germany). Before toxicity assay, the pH of the sample was adjusted between 5.5 and 8.5 using 0.1 N NaOH or HCI. The room temperature was maintained at between 15 and 24 ^{o}C. Samples were serially diluted with 2% NaCl (w/v). Sodium chloride (2%) was used as the control. Samples containing bacterial luminescence were measured for 5^{th}, 15^{th} and 30^{th} min. incubation times in a luminometer. The decrease in bioluminescence indicated the toxic effect of the samples. Toxicity

evaluation criteria for luminescent bacteria are explained by the percent inhibition effect (H). If the percent inhibitory effect (H) changed between 0% and 5%, the effect is non-toxic. When it is between 5% and 20%, the effect is possibly toxic, and when the inhibitor effect is between 20% and 90%, the effect is toxic (Lange, 1994). The standard culture, *Vibrio fischeri* (LCK 491), was obtained from Dr.Bruno Lange GmnH & Co. KG, Germany. Calculation of % inhibition was calculated using the Eqs (5.11-5.13),

$$f_K = \frac{I_t}{I_0} \tag{5.11}$$

Where,

f_K :temporary correction factor determined from the control measurements,

l_t= bioluminescence values measured at 5^{th}, 15^{th} and 30^{th} min after 0,5 mL of control and test sample was added to 0,5 mL bacterial suspension .

l_o= initial values of luminescence of the control and test samples in 0,5 mL bacterial suspension

$$I_{ct} = avg f_K .\ I_0 \tag{5.12}$$

I_{ct} : I_0 value adjusted by the correction factor

avg f_K: The average values of f_K ratio,

$$\%\ Inhibition = \frac{(I_{ct} - I_t).100}{I_{ct}} \tag{5.13}$$

Detail information for calculation of the acute toxicity in Microtox tests is given in Table 5.18.

Table 5.18 A sample calculation for Microtox acute toxicity test after 5 minutes incubation time

Dilution ratio of samples	Bioluminescence values $T=0^{th}$ min.(Io)	Bioluminescence values $T=5^{th}$ min.(It)	Average values	avg.fk	I_{ct}	% Inhibition
D 1/1	148	58	-	0.92	136.16	57.40
D 1/2	158	120	-	0.92	145.36	17.45
D 1/4	161	133	-	0.92	148.12	10.21
Control	169	155	0.92	0.92	155.48	0.00
Dilution ratio of samples	Bioluminescence values $T=0^{th}$ min.(Io)	Bioluminescence values $T=15^{th}$ min.(It)	Average values	avg.fk	I_{ct}	% Inhibition
D 1/1	148	45	-	0.90	133.20	66.22
D 1/2	158	114	-	0.90	142.20	19.83
D 1/4	161	128	-	0.90	144.90	11.66
Control	169	152	0.90	0.90	152.10	0.00
Dilution ratio of samples	Bioluminescence values $T=0^{th}$ min.(Io)	Bioluminescence values $T=30^{th}$ min.(It)	Average values	avg.fk	I_{ct}	% Inhibition
D 1/1	148	36	-	0.88	130.24	72.36
D 1/2	158	110	-	0.88	139.04	20.89
D 1/4	161	126	-	0.88	141.68	11.07
Control	169	148	0.88	0.88	148.72	0.00

5.9 Calculation of SRT in the Aerobic CSTR and in the Anaerobic ITBR

Sludge retention time or mean sludge retention time (SRT), defined as the mass of organisms in the reactor divided by the mass of organisms removed from the system each day, is given by the Eq. (5.14). The use of SRT is simply based on the fact that, to control of the growth rate of microorganisms and hence their degree of waste stabilization.

$$SRT = \frac{V_{r\ (CSTR)} \cdot X_{(CSTR)}}{Q_{e(CSTR)} \cdot X_{e\ (CSTR)} + Q_{w\ (CSTR)} \cdot X_{w(CSTR)}} \quad (5.14)$$

$V_{r\ (CSTR)}$ and $X_{\ (CSTR)}$ are effective volume of CSTR reactor and microorganism concentration in the aeration tank, respectively. $Q_{e\ (CSTR)}$ and $X_{e\ (CSTR)}$ were defined as flow rate and microorganism concentration measured in the settling tank. $Q_{w\ (CSTR)}$ and $X_{w\ (CSTR)}$ are the flow rate and microorganism concentration wasted from settling tank of the CSTR reactor, respectively. The CSTR system used in this study are recycled reactor. In other words, the sludge was recycled 100% from the settling tank to the aeration tank. If wasting is directly from the reactor and the solids in the effluent $X_{e\ (CSTR)}$ are negligible. Since the activated sludge was withdrawn from the inside of the aeration stage, the microorganism concentration in the reactor ($X_{\ (CSTR)}$) was equal to the wasted microorganism concentration ($X_{w\ (CSTR)}$). Therefore, SRT in this reactor was calculated by using equation (5.14) with rearranged equation (5.15).

$$SRT = \frac{V_{r\ (CSTR)}}{Q_{w\ (CSTR)}} \quad (5.15)$$

Since no sludge wasting was applied for granule formation in the ITBR reactors, SRT in these reactors were determined using equations (5.16) and (5.17).

$$SRT = \frac{V_{r\ (CSTR)}}{Q_{w\ (CSTR)}} \quad (5.16)$$

$V_{r\ (IBTR)}$ (L) and $X_{\ (ITBR)}$ (mgVSS/L) were defined as volume of the ITBR reactor and microorganism concentration in the reactor, respectively. $Q_{w\ (ITBR)}$ (L/day) and $X_{w\ (ITBR)}$ (mgVSS/L) were defined as flow of liquid rate and microorganism concentrations in wasted sludge stream, respectively. $Q_{e\ (ITBR)}$ (L/day) and $X_{e\ (IBTR)}$ (mgVSS/L) were defined as flow rate of liquid and microorganism concentrations in the waste sludge stream, respectively. The term $Q_{w\ (ITBR)}.X_{w(ITBR)}$ only makes sense if

there is a waste sludge stream. Since no sludge wasting was applied in the ITBR, SRT can be expressed as follows:

$$SRT = \frac{V_{r(ITBR)} \cdot X_{(ITBR)}}{Q_{e(ITBR)} \cdot X_{e(ITBR)}} \quad (5.17)$$

The number of days that the materials stay in a tank is called as the Hydraulic Retention Time (HRT). HRT is the volume of the reactor divided by the influent flow rate. HRT in aerobic and anaerobic reactor were calculated using equation (5.18).

$$HRT = \frac{V_r}{Q} \quad (5.18)$$

Where, V_r (L) and Q (L/day) were defined as reactor volume and influent flow rate, respectively.

5.10 Chemicals Used in This Study

5.10.1 Standard Chemicals Used for PAHs Analysis

PAHs Internal/Calibration Standards

The PAHs internal standard (4 mg/mL in CH_2Cl_2, lot number: 210031186) solution contained carbazole five deuterated PAHs (naphthalene–d8 (CAS no:1146-65-2), acenaphthene–d10 (CAS no:15067-26-2), phenanthrene–d10 (CAS no:1517-22-2), chrysene–d12 (CAS no:1719-03-05) and perylene–d12 (CAS no:1520-96-3) and it was purchased from AccuStandard Company (New Haven, USA) for PAHs measurements in GC-MS.

XAD-2 Amberlit Resin

XAD-2 (Amberlit XAD2), (CAS number: 9060-05-3, 20-60 mesh, surface area ~300 m^2/g, density 1.02 g/mL at 25 °C, Sigma-Aldrich Chemie GmbH) was used for the preparation of the samples before PAHs extraction.

n–Hexane ($CH_3(CH_2)_4CH_3$)

n–Hexane ($CH_3(CH_2)_4CH_3$) for gas chromatography with a purity of > 98.00 (CAS number: 110-54-3, EC number: 203-777-6, Molar mass: 86.18 g/mol, Merck Chemical Company) was used for the preparation of the PAHs extraction before GC-MS analysis. Product information and chemical-physical properties of n-hexane are given in Table 5.19.

Table 5.19 Product information and chemical-physical properties of n-hexane

Hill Formula	C_6H_{14}
Chemical formula	$CH_3(CH_2)_4CH_3$
HS Code	2901 10 00
EC number	203-777-6
Molar mass	86.18 g/mol
EC index number	601-037-00-0
CAS number	110-54-3
Ignition temperature	240 °C
Solubility	0.0095 g/l (20 °C)
Melting point	-94.3 °C
Molar mass	86.18 g/mol
Density	0.66 g/cm^3 (20 °C)
pH value	(H_2O) not applicable
Boiling point	69 °C (1013 hPa)
Vapor pressure	160 hPa (20 °C)
Explosion limit	1.0 - 8.1 %(V)
Flash point	-22 °C
Refractive index	1.375
Viscosity kinematic	0.50 mm^2/s (20 °C)

Acetone (CH_3COCH_3)

Acetone (CH_3COCH_3) for gas chromatography with a purity of >99.80% (CAS number: 67-64-1, Molar mass: 58.08 g/mol, Merck Chemical Company) was used for preparation of the PAHs extraction before GC-MS analysis. Product information and chemical-physical properties of acetone are given in Table 5.20.

Table 5.20 Product information and chemical-physical properties of acetone

Synonyms	Dimethyl ketone, Propanone
Hill Formula	C_3H_6O
Chemical formula	CH_3COCH_3
HS Code	2914 11 00
EC number	200-662-2
Molar mass	58.08 g/mol
EC index number	606-001-00-8
CAS number	67-64-1
Ignition temperature	465 °C DIN 51794
Solubility	(20 °C) soluble
Melting point	-95.4 °C
Molar mass	58.08 g/mol
Density	0.79 g/cm^3 (20 °C)
pH value	5 - 6 (395 g/L, H_2O, 20 °C)
Boiling point	56.2 °C (1013 hPa)
Vapor pressure	233 hPa (20 °C)
Explosion limit	2.6 - 12.8 % (V)
Flash point	-18 °C
Refractive index	1.35868 (20 °C)
Water absorption	1000 g/kg

Silicic Acid (H_2O_3Si)

Silicic acid (H_2O_3Si), (SILA–200 500 G; CAS number: 7699-41-4, Molar mass: 78.11 g/mol, 60–200 mesh, Sigma-Aldrich) was used for the preparation of the PAHs extraction before GC-MS analysis. H_2O_3Si was dried at 103^oC, 24 h before of the PAHs analysis.

Dichloromethane (CH_2Cl_2)

Dichloromethane (CH_2Cl_2) for gas chromatography with a purity of > 99.80 (CAS number: 75-09-2, Molar mass: 84.93 g/mol, Merck Chemical Company) was used for the preparation of the PAHs extraction before GC-MS analysis. Product information and chemical-physical properties of dichloromethane are given in Table 5.21.

Table 5.21 Product information and chemical-physical properties of Dichloromethane

Hill Formula	CH_2Cl_2
Chemical formula	CH_2Cl_2
HS Code	2903 12 00
EC number	200-838-9
Molar mass	84.93 g/mol
EC index number	602-004-00-3
CAS number	75-09-2
Ignition temperature	605 °C DIN 51794
Solubility	20 g/l (20 °C)
Melting point	-95 °C
Molar mass	84.93 g/mol
Density	1.33 g/cm^3 (20 °C)
pH value	(H_2O, 20 °C) neutral
Boiling point	40 °C (1013 hPa)
Vapor pressure	475 hPa (20 °C)
Explosion limit	13 - 22 %(V)
Refractive index	1.42
Evaporation number	1.9

Petroleum Ether (Petroleum Benzine)

Petroleum Ether (Petroleum Benzine) for gas chromatography with a purity of > 99.99 (CAS number: 64742-49-0, EC number: 265-151-9, Merck Chemical Company) was used for the preparation of the PAHs extraction before GC-MS analysis. Product information and chemical-physical properties of Petroleum Ether are given in Table 5.22.

Table 5.22 Product information and chemical-physical properties of Petroleum Ether

HS Code	2710 12 25
EC number	265-151-9
EC index number	649-328-00-1
CAS number	64742-49-0
Ignition temperature	250 °C DIN 51794
Solubility	0.01 g/L (20 °C)
Density	0.645 - 0.665 g/cm^3 (15 °C)
Boiling point	36 - 83 °C
Vapor pressure	350 h Pa (20 °C)
Explosion limit	0.8 - 7.4 %(V)
Viscosity kinematic	0.45 mm^2/s (20 °C)

Sodium Sulfate Anhydrous (Na_2SO_4)

Sodium Sulfate Anhydrous (Na_2SO_4) for analysis with a purity of > 99.00 (CAS number: 7757-82-6, Molar mass: 142.04 g/mol, Merck Chemical Company) was used for the preparation of the PAHs extraction. Product information and chemical-physical properties of Sodium Sulfate Anhydrous are given in Table 5.23.

Table 5.23 Product information and chemical-physical properties of Sodium Sulfate Anhydrous

Grade	ACS,ISO,Reag. Ph Eur
Hill Formula	Na_2O_4S
Chemical formula	Na_2SO_4
HS Code	2833 11 00
EC number	231-820-9
Molar mass	142.04 g/mol
CAS number	7757-82-6
Solubility	200 g/l (20 °C)
Melting point	888 °C
Molar mass	142.04 g/mol
Density	2.70 g/cm^3 (20 °C)
Bulk density	1400 - 1600 kg/m^3
pH value	4 - 7 (200 g/L, H_2O, 20 °C)

Aluminium Oxide 90 Active Neutral (Al_2O_3)

Aluminium oxide 90 active neutral (activity stage I) (Al_2O_3), (TA1575477 909, CAS number: 1344-28-1, Molar mass: 101.96 g/mol, Merck Chemical Company), for column chromatography 0.063–0.200 mm (70–230 mesh ASTM) was used for the preparation of the PAHs extraction before GC-MS analysis. Al_2O_3 was dried at 450^{o}C, 3 h before the PAHs analysis.

Nitrogen Gas (N_2)

Pure N2 gas tube (CAS no: 7727-37-9, EC: 231-783-9, Impurities (ppm): HiQ 5, H_2O <=5; O_2<=5, gas amount 50 L, gas pressure 200 bar, Linde Group), with a purity of 99.999% was used for the extraction of the PAHs from the wastewater before PAHs analysis.

Helium Gas (He)

Pure He gas tube (CAS no: 7440-59-7, EC: 231-168-5, Impurities (ppm): $H_2O \leq 3$, $O_2 \leq 2$, CnHm ≤ 0.5, $N_2 \leq 5$, gas amount 50 L, gas pressure 200 bar, Linde Group) with a purity of 99.999% was used as the mobile phase for PAHs measurements.

5.10.2 Standard Chemicals Used for Biosurfactants (RD, EM, SR) Analysis

Rhamnolipid

The technical grade active ingredient (TGAI) is rhamnolipid biosurfactant, a transparent liquid with a light to dark amber tint and a mild, sweet soapy odor. The TGAI is a mixture of two rhamnolipid molecules (R1 and R2). The technical grade active ingredient (TGAI) mixture of R1 and R2 is decanoic acid, 3-[[6-deoxy-2-O-(6-deoxy-%-L-mannopyranosyl)-%-Lmannopyranosyl]oxy]-,1-(carboxymethyl) octyl ester, mixture with 1-(carboxymethyl)octyl 3[(6-deoxy-%-L mannopyranosyl)oxy]decanoate, [CAS no: 147858-26-2, PC code 110029, R1 and R2 mix rhamnolipid ($C_{32}H_{58}O_{1}3$-$C_{26}H_{48}O_9$)]. A mixture of R1 and R2 rhamnolipid biosurfactants (purchased from Jeneil Biosurfactant Company 400 N Dekora Woods Blvd. Saukville, WI 53080) were used as standard for RD measurements.

Acetonitrile (CH_3CN)

Acetonitrile (CH_3CN) for high pressure liquid chromatography (HPLC) with a purity of ≥ 99.90 (CAS number: 75-05-8, Molar mass: 41.05 g/mol, Merck Chemical Company) was used as the mobile phase for surfactin measurements. Product information and chemical-physical properties of acetonitrile are given in Table 5.24.

Table 5.24 Product information and chemical-physical properties of Acetonitrile

Grade	Reag. Ph Eur
Hill Formula	C_2H_3N
Chemical formula	CH_3CN
HS Code	2926 90 95
EC number	200-835-2
Molar mass	41.05 g/mol
EC index number	608-001-00-3
CAS number	75-05-8
Ignition temperature	524 °C
Solubility	(20 °C) soluble
Melting point	-45.7 °C
Molar mass	41.05 g/mol
Density	0.786 g/cm^3 (20 °C)
pH value	(H_2O) no data available
Boiling point	81.6 °C (1013 hPa)
Vapor pressure	97 hPa (20 °C)
Explosion limit	3.0 - 17 %(V)
Flash point	2 °C

Trifluoroacetic acid ($C_2HF_3O_2$)

Trifluoroacetic acid ($C_2HF_3O_2$) for high pressure liquid chromatography (HPLC) with a purity of ≥ 99.00 (CAS number: 76-05-1, Molar mass: 114.02 g/mol, Sigma-Aldrich Chemie GmbH) was used as the mobile phase for surfactin measurements. Product information and chemical-physical properties of trifluoroacetic acid are given in Table 5.25.

Table 5.25 Product information and chemical-physical properties of Trifluoroacetic acid

Product Number	302031
CAS Number	76-05-1
MDL	MFCD00004169
Formula	$C_2HF_3O_2$
Formula Weight	114.02 g/mol

Orcinol ($CH_3C_6H_3$-1,3-$(OH)_2$)

Orcinol ($CH_3C_6H_3$-1,3-$(OH)_2$) with a purity of ≥ 97.00 (CAS number: 504-15-4, Molar mass: 124.14 g/mol, Sigma-Aldrich Chemie GmbH) was used as a solution for RD measurements.

Surfactin ($C_{53}H_{93}N_7O_{13}$)

Surfactin ($C_{53}H_{93}N_7O_{13}$) with a purity ≥ 98 % (CAS no: 24730-31-2, Molar mass: 1036.34 g/mol, Sigma-Aldrich Chemie GmbH) was used as a solution for SR measurements.

Emulsan ($C_{205}H_{366}N_3O_{11}7P_5$)

Emulsan biosurfactant (purchased from Sigma-Aldrich Chemie GmbH) was used as standard for SR measurements.

5.10.3 Standard Chemicals Used in Anaerobic and Aerobic Reactors

Sulfuric Acid (H_2SO_4)

Sulfuric Acid (H_2SO_4) for analysis with a purity of > 95-97 (CAS number: 7664-93-9, Molar mass: 98.08 g/mol, Merck) was used to adjust the pH in aerobic and

anaerobic reactors, throughout gas measurement and COD_{dis} analysis. Product information and chemical-physical properties of sulfuric acid are given in Table 5.26.

Table 5.26 Product information and chemical-physical properties of Sulfuric Acid

Grade	ISO
Hill Formula	H_2O_4S
Chemical formula	H_2SO_4
HS Code	2807 00 00
EC number	231-639-5
Molar mass	98.08 g/mol
EC index number	016-020-00-8
CAS number	7664-93-9
Solubility	(20 °C) soluble
Melting point	-20 °C
Molar mass	98.08 g/mol
Density	1.84 g/cm^3 (20 °C)
pH value	0.3 (49 g/L, H_2O, 25 °C)
Boiling point	335 °C
Vapor pressure	0.0001 hPa (20 °C)

Sodium Hydroxide (NaOH)

Sodium Hydroxide (NaOH) with a purity of > 98 (CAS number: 1310-73-2, Molar mass: 44 g/mol, Merck) was used for adjust the pH in aerobic and anaerobic reactors and toxicity samples. Product information and chemical-physical properties of sodium hydroxide are given in Table 5.27.

Table 5.27 Product information and chemical-physical properties of Sodium Hydroxide

Synonyms	Soda caustic
Hill Formula	HNaO
Chemical formula	NaOH
HS Code	2815 11 00
EC number	215-185-5
Molar mass	40.00 g/mol
EC index number	011-002-00-6
CAS number	1310-73-2
Solubility	1090 g/l (20 °C)
Melting point	323 °C
Molar mass	40.00 g/mol
Density	2.13 g/cm^3 (20 °C)
pH value	14 (50 g/l, H_2O, 20 °C)
Boiling point	1390 °C (1013 hPa)
Vapor pressure	(20 °C)

Rhamnolipid

The technical grade active ingredient (TGAI) is rhamnolipid biosurfactant, a transparent liquid with a light to dark amber tint and a mild, sweet soapy odor. The TGAI is a mixture of two rhamnolipid molecules (R1 and R2). The technical grade active ingredient (TGAI) mixture of R1 and R2 is decanoic acid, 3-[[6-deoxy-2-O-(6-deoxy-%-L-mannopyranosyl)-%-Lmannopyranosyl]oxy]-,1-(carboxymethyl)octyl ester, mixture with 1-(carboxymethyl)octyl 3[(6-deoxy-%-L mannopyranosyl)oxy]decanoate, [CAS no: 147858-26-2, PC code 110029, R1 and R2 mix rhamnolipid ($C_{32}H_{58}O_13$-$C_{26}H_{48}O_9$)]. A mixture of R1 and R2 rhamnolipid biosurfactants (purchased from Jeneil Biosurfactant Company 400 N Dekora Woods Blvd. Saukville, WI 53080) was used in this study.

Surfactin ($C_{53}H_{93}N_7O_{13}$)

Surfactin ($C_{53}H_{93}N_7O_{13}$) was used as a biosurfactant (CAS no: 24730-31-2, Molar mass: 1036.34 g/mol, Sigma-Aldrich Chemie GmbH) with a purity ≥ 98% in the aerobic reactor systems.

Emulsan ($C_{205}H_{366}N_3O_{11}7P_5$)

Emulsan ($C_{205}H_{366}N_3O_{11}7P_5$) was used as a biosurfactant (purchased from Sigma-Aldrich Chemie GmbH) with a purity ≥ 98 % in the aerobic reactor systems.

5.11 Procedural Recoveries

Influent and effluent wastewater samples were spiked with PAHs surrogate standards prior to extraction. Recoveries of surrogate standards were 56± 12% for naphthalene–d_8, 61± 16% for acenaphthene–d_{10}, 83 ± 14% for phenanthrene-d_{10}, 87 ± 12% for chrysene-d_{12}, and 89 ± 10% for perlylene-d_{12}. Six levels of calibration standards (40, 400, 1000, 4000, 6000, 10000 ng/mL for PAHs and internal standard at a concentration of 8000 ng/mL) were used to calibrate the GC/MS system. The rations signal to noise (S/N) was taken into consideration for every PAH compound at their lowest standard concentration in calibration curve using the area of a peak having a signal to noise (S/N) ratio of 3. The limit of detection (LOD) and limit of quantification (LOQ) data varied at between 0.019 ng/mL and 0.084 ng/mL and at between 0.057 and 0.252 ng/mL, respectively.

5.12 Statistical Analysis

The regression analysis between *y* (dependent) and *x* (independent) variables was carried out using Windows Excel, 2007. An ANOVA test was performed in order to determine the statistical significance between *x* and *y* variables. Each data are the mean of triplicate sampling with standard deviation (SD) values. A Windows Excel

statistical program was used for the 95% confidence intervals with a significance level of α =0.05.

5.13 Theoretical Background

5.13.1 Kinetic Model Based on Monod Equation

Traditionally a Monod type rate model (Eq.5.19) has been widely used for describing the rate of degradation of a substrate by living cells. In other words, the substrate removal rate is commonly expressed by a deterministic model developed by Monod (1949);

$$-\frac{dS}{dt} = -R = -\frac{R_{max}S}{K_S+S} \qquad (5.19)$$

R_{max} is maximum substrate (COD_{dis} or PAH) utilization rate (mg/L.day), S is residual substrate concentration at selected time through substrate removal (mg/L) and K_s is half saturation concentration (mg/mL).

In order to obtain the specific kinetic parameters for this model, R_{max} and K_s or a ratio between them, which constitutes the objective of the present kinetic study, Eq. (5.20) can be linearized in the form:

$$\frac{1}{R} = \left(\frac{1}{R_{max}}\right) + \left(\frac{K_S}{R_{max}}\right)\left(\frac{1}{S}\right) \qquad (5.20)$$

According to Eq. (5.20), a Lineweaver-Burk's plot of 1/R versus 1/S for each experiment should give a straight line whose intercept and slope will be $1/R_{max}$ and K_s/R_{max}, respectively.

5.13.2 Kinetic models in the CSTR system

Knowledge of biokinetics is essential for biological wastewater treatment system design and optimization of operational conditions (Mohan et al., 2006). In the case of pure cultures and limited substrate growth, it has been experimentally found that the kinetics of substrate or nutrient biodegradation can be defined by Monod model (Monod, 1949). Kinetics modeling of petrochemical wastewater by the Monod model has been reported in the literature. Neufeld and Valiknac (1979) and Hsu (1986) used the Monod model for analysis of petrochemical wastewater treatment data.

5.13.2.1 Application of Conventional Monod Kinetic Model in the CSTR System

The relationship between the bacteria growth rate and concentration of the growth-limiting substrate is generally expected by Monod's equation. This study is to determine the maximum utilization rate (μ_{max}) (g/L.day), the half saturation value constant (K_s) (g/L.day), death rate constant (K_d) and growth yield coefficient (Y) (g/g) values.

For a CSTR reactor with biomass recycle, microbial mass balance can be expressed using Eqs. (5.21) and (5.32).

A mass balance for the microorganisms in the entire system for CSTR can be as follows:

$$(Accumulation)^a = (Inflow)^b - (Outflow)^c + (Net\ growth)^d \quad (5.21)$$

Where

[a] is the rate of accumulation of microorganism within the system boundary,

[b] is the rate of flow of microorganism into the system boundary,

[c] is the rate of flow of microorganism out of the system boundary,

^{d} is the net growth of microorganism within the system boundary,

For a CSTR reactor, the rate of change of microorganism mass balance in the system can be expressed as Eq. (5.22).

$$\frac{dX}{dt}V_r = QX_0 - [Q_W X + Q_e X_e] + V_r r_g \quad (5.22)$$

Where, dX/dt is rate of change of biomass concentration in reactor measured as mgVSS/L.day, Q is influent flow rate (L/day), X_0 is concentration of biomass in influent (mgVSS/L), Q_w is flow rate of liquid containing the biological cells wasted from the system (L/day), X is concentration of biomass in reactor (mgVSS/L), X_e is concentration of biomass in effluent (mgVSS/L), V_r is reactor volume (L) and r_g is net rate of bacterial growth (mg/L.day)

$$r_g = -Yr_{su} - k_d X \quad (5.23)$$

Where, Y is defined the growth yield coefficient (mgVSS/mgCOD_{dis}), r_{su} is substrate utilization rate (mg/L.day) and k_d is endogenous decay coefficient (1/day).

The bacterial growth rate given in Eq. (5.23) is substituted into Eq. (5.22). The new equation [Eq. (5.24)] could be written as fallows;

$$\frac{Q_w X_w + Q_e X_e}{V_r X} = Y\frac{r_{su}}{X} - k_d \quad (5.24)$$

The left hand side of Eq. (5.24) represents the inverse of the SRT as defined in Eq. (5.14). By taken into consideration Eqs (5.14) and (5.24) the inverse of SRT could be defined in Eq. (5.25) (Metcalf Eddy, 1991).

$$\frac{1}{SRT} = -Y\frac{r_{su}}{X} - k_d \quad (5.25)$$

The term r_{su} is determined using the following expression:

$$r_{su} = -\frac{Q}{V_r}(S_0 - S) = -\frac{S_0 - S}{SRT} \quad (5.26)$$

Where, S_0 and S are defined as influent and effluent substrate concentration (mg/L), respectively.

The substrate utilization rate could be also defined by Eq. (5.27) (Metcalf Eddy, 1991).

$$r_{su} = -\frac{k\,X\,S}{K_s + S} \quad (5.27)$$

Where, k, K_s, X and S are defined as maximum rate of substrate utilization (mg/mg.day), half-velocity constant (mg/L), concentration of microorganism (mg/L) and substrate concentration (mg/L), respectively.

If Eq. (5.26) is equalized to Eq. (5.27) the r_{su} could be defined with Eq. (5.28)

$$r_{su} = -\frac{k\,X\,S}{K_s + S} = -\frac{S_0 - S}{SRT} \quad (5.28)$$

Dividing by X yields,

$$\frac{k\,S}{K_s + S} = \frac{S_0 - S}{X\,SRT} \quad (5.29)$$

The linearization of Eq. (5.29) could be given by Eq. (5.30).

$$\frac{X\,SRT}{S_0 - S} = \frac{K_s}{k}\frac{1}{S} + \frac{1}{k} \quad (5.30)$$

Where, K_s is half velocity constant (mg/L) and k is maximum rate of substrate utilization (mg/mg day). The values of Ks and *k* can be determined by plotting the term [X.SRT/(So-S)] versus (1/S). If the value of r_{su} given in Eq. (5.26) is substituted into Eq. (5.28) Eq. (5.31) could be obtained:

$$\frac{1}{SRT} = Y\frac{S_0 - S}{X\,SRT} - k_d \qquad (5.31)$$

The values of Y and k_d may be determined using Eq. (5.31), by plotting (1/SRT) versus (S_0-S/X.SRT) (Metcalf eddy, 1991).The slope of line in the graph given in Eq. (5.28) give the Y value and the intercept is equal to k. The value of μ_{max} was determined using Eq. (5.32) (Metcalf eddy, 1991).

$$k = \frac{\mu_{max}}{Y} \qquad (5.32)$$

Where, μ_{max} is maximum specific growth rate, (1/day).

5.13.2.2 Zero-Order Substrate Removal Model

The rate of change in substrate concentration in the system with assuming zero-order removal model for substrate removal can be expressed in Eq. (5.33)

$$r = -\frac{dS}{dt} = k \qquad (5.33)$$

Where r is the reaction rate and k is the reaction rate coefficient (mg/day). If this differential equation is integrated it gives an Eq. (5.34) often called the integrated zero-order rate law.

$$S = S_0 - k_0.t \qquad (5.34)$$

Where, S_0 and S are influent and effluent COD_{dis} (mg/L) and PAHs (ng/mL) concentrations, respectively. t is incubation time through substrate removal (day). k_0 is zero-order kinetic constant (mg COD_{dis}/L.day; ng PAH/mL.day) and can be obtained from the slope of the line plotting the Eq. (5.34) (Sponza and Işık, 2005).

5.13.2.3 Half-Order Substrate Removal Model

The rate of change in substrate concentration in the system with assuming the half-order model for substrate removal could be expressed as follows:

$$-\frac{dS}{dt} = k.\sqrt{S} \qquad (5.35)$$

The integrated half-order rate law is

$$\sqrt{S} = \sqrt{S_0} - \frac{1}{2}k_{1/2}.t \qquad (5.36)$$

Where, S_0 and S are influent and effluent COD_{dis} (mg/L) and PAHs (ng/mL) concentrations, respectively. t is incubation time through substrate removal (day). $k_{1/2}$ is the half-order kinetic constant (L/mgCOD_{dis}.day; mL/ngPAH.day) and can be obtained by plotting the $\sqrt{S}$ versus time using Eq. (5.36). The slope of the line gives the $k_{1/2}$.

5.13.2.4 First-Order Substrate Removal Model

The rate of change in substrate concentration in the system with assuming the first-order removal model for substrate removal could be expressed as follows:

$$r = -\frac{dS}{dt} = k.S \qquad (5.37)$$

The integrated first-order rate law is

$$lnS = lnS_0 - k_1.t \quad (5.38)$$

$$S = S_0.e^{-k1.t} \quad (5.39)$$

Where, S_0 and S are influent and effluent COD_{dis} (mg/L) and PAHs (ng/mL) concentrations, respectively. t is incubation time through substrate removal (day). k_1 is the first-order kinetic constant (1/day). k_1 can be obtained by plotting the lnS versus time using in Eq.(5.39). The slope of the line gives the k_1 (Sponza and Işık, 2005).

5.13.2.5 Second-order Substrate Removal Model

The rate of change in substrate concentration in the system with assuming the second order model for substrate removal could be expressed as follows:

$$-\frac{dS}{dt} = k.S^2 \quad (5.40)$$

The integrated second-order rate law is

$$\frac{1}{S} = \frac{1}{S_0} + k_2 t \quad (5.41)$$

Where, S_0 and S are influent and effluent COD_{dis} (mg/L) and PAHs (ng/mL) concentrations, respectively. t is incubation time through substrate removal (day). k_2 is the second order kinetic constant (L/mg COD_{dis}.day; mL/ng PAH.day) and can be obtained by plotting the 1/S versus time using Eq (5.41). The slope of the line gives the k_2 value (Sponza and Isık, 2005).

5.13.3 Kinetic Models in the Anaerobic ITBR System

Process modeling is a useful tool for the evaluation of the persistence of organic pollutants as well as to predict a bioreactor performance with respect to the degradation of organic compounds. Kinetic analyses of anaerobic treatment of industrial wastewaters have been reported in the literature. Kinetic models that have been successfully tested include the Modified Stover-Kincannon and the Contois model for determine the kinetic constants relevant to COD_{dis} and PAHs in the anaerobic ITBR system. Of these, the Monod model seems to be employed most frequently to describe the anaerobic treatment kinetics.

5.13.3.1 Modified Stover-Kincannon Model

In this model the substrate utilization rate is expressed as function of the organic loading rate by monomolecular kinetic in the anaerobic ITBR reactor Equation of the modified Stover-Kincannon model is as follows:

$$\frac{dS}{dt} = \frac{R_{max}\,(\frac{QS_i}{V})}{K_B + (\frac{QS_i}{V})} \tag{5.42}$$

Where; dS/dt is defined in Eq. (5.43):

$$\frac{dS}{dt} = \frac{Q(S_i - S_e)}{V} \tag{5.43}$$

Eq. (5.44) obtained from the linearization of Eq. (5.43) as follows:

$$(\frac{dS}{dt})^{-1} = \frac{V}{Q\,(S_i - S_e)} = \frac{K_B}{R_{max}}\,\frac{V}{Q\,S_i} + \frac{1}{R_{max}} \tag{5.44}$$

If the maximum utilization rate (R_{max}) (g/L.day) and the saturation value constant (K_B) (g/L.day) values obtained for COD_{dis} and PAHs were substituted in Eq. (5.43).

($Q.S_i/V$) explain the OLR applied to the reactor. Q and V are the inflow rate (L/day) and the volume of the anaerobic reactor (L), respectively. S_i and S_e are influent and effluent COD_{dis} ($gCOD_{dis}/L$) and PAH (ng PAH/mL) concentrations, respectively.

5.13.3.2 Contois Kinetic Model

The relationship between specific growth rate and limiting substrate concentrations was given as follows (Contois, 1959).

$$\mu = \frac{\mu_{max}}{\beta\, X+S} \tag{5.45}$$

Where, β is the kinetic parameter (g COD_{dis}/g biomass). By substituting Eq. (5.45) instead of the Monod equation into Eq. (5.19), Eq. (5.46) can be obtained:

$$\frac{\mu_{max}\, S_i}{\beta\, X+S_i} = \frac{1}{SRT} + k_d \tag{5.46}$$

The values of μ_{max}, and β can be obtained by plotting Eq. (5.47), which is obtained by rearranging Eq. (5.46). The value of μ_{max} can be calculated from the intercept of the straight line and finally, β could be obtained from the slope of the line.

$$\frac{SRT}{1+SRT\, k_d} = \frac{\beta}{\mu_{max}}\frac{X}{S_i} + \frac{1}{\mu_{max}} \tag{5.47}$$

5.13.3.3 Monod Model

The Monod model was used to describe the biodegradation of COD_{dis}, and PAHs. Eqs. (5.48) to (5.49) represent growth and substrate removals. For an anaerobic ITBR reactor with no biomass recycle, microbial and substrate mass balance can be expressed using Eq. (5.48) and Eq. (5.49).

Microorganism mass balance. A mass balance for the microorganisms in the entire system for anaerobic ITBR system can be as follows:

$$(Accumulation)^a = (Inflow)^b - (Outflow)^c + (Net\ growth)^d \qquad (5.48)$$

Where

[a] is the rate of accumulation of microorganism within the system boundary,

[b] is the rate of flow of microorganism into the system boundary,

[c] is the rate of flow of microorganism out of the system boundary,

[d] is the net growth of microorganism within the system boundary,

Mathematically, Eq. (5.42) can be written as Eq. (5.43).

$$\frac{dX}{dt} = \frac{Q}{V} \times X_i - \frac{Q}{V} X_e + \mu \times X - k_d \times X \qquad (5.49)$$

Where; V is reactor volume (L); Q the flow rate (L/day); X_i and X_e are the influent and effluent biomass concentration (g/L); X is concentration of biomass in the reactor (g/L); μ is specific growth rate (1/day) and k_d is endogenous decay coefficient (1/day).

If it is assumed that the concentration of biomass in the influent can be neglected, at steady-state dX/dt=0, and the HRT (day) is defined as the volume of the reactor divided by the flow rate of the influent, since the relationship between the specific growth rate and the rate limiting substrate concentration can be expressed by the Monod equation (5.50) (Monod, 1949);

$$\mu = \frac{\mu_{max}\ S}{K_s + S} \qquad (5.50)$$

Where; K_S is the half-saturation concentration (mg/L). It determines how rapidly μ approaches μ_{max} and is defined as the substrate concentration at which μ is equal to

half of μ_{max}. The smaller K_S is the lower the substrate concentration at which μ approaches μ_m. S is substrate concentration, mg/L.

Mathematically, Eq. (5.49) can be written as Eq. (5.51).

$$\frac{Q}{V} X_e = X \left(\mu - k_d\right) \tag{5.51}$$

Both sides of the Eq. 4.45 are divided by the value of X and the other side of the equation is constant with a term of k_d. This mathematical change gives the Eq. (5.52) or Eq. (5.47).

$$\frac{Q X_e}{V X} + k_d = \mu \tag{5.52}$$

or

$$\mu = \frac{1}{SRT} + k_d \tag{5.53}$$

Mathematically, Eq. (5.50) and Eq. (5.53) can be written as Eq. (5.48):

$$\frac{\mu_{max} S}{K_s + S} = \frac{1}{SRT} + k_d \tag{5.54}$$

$$\frac{SRT}{1+(SRTk_d)} = \frac{K_s}{\mu_{max}} \frac{1}{S} + \frac{1}{\mu_{max}} \tag{5.55}$$

The value of maximum specific growth rate (μ_{max}) (1/day) and half saturation concentration (K_s) (mg/L) can be obtained by plotting SRT/ (1+SRT.k_d) versus 1/S in Eq. (5.55). The slope of the line gives the K_s. The intercept point of the line gives the μ_{max}.

Substrate Mass Balance. A substrate mass balance for the reactor can be described as Eq. (5.56):

$$\begin{pmatrix}\text{Substrate}\\ \text{change}\\ \text{rate}\end{pmatrix}=\begin{pmatrix}\text{Substrate}\\ \text{input}\\ \text{rate}\end{pmatrix}-\begin{pmatrix}\text{Substrate}\\ \text{utilization}\\ \text{rate}\end{pmatrix}-\begin{pmatrix}\text{Substrate}\\ \text{output}\\ \text{rate}\end{pmatrix} \tag{5.56}$$

This equation can be rearranged to estimate the effluent substrate concentration at the steady state condition as follows:

$$S=\frac{K_S\left(k_d+\frac{1}{SRT}\right)}{\mu_{\max}-k_d-\frac{1}{SRT}} \tag{5.57}$$

Where; S is substrate concentration (mg/L). The rate of change in substrate concentration in the system could be expressed with Eq. (5.58):

$$-\frac{dS}{dt}=\frac{Q}{V}S_i-\frac{Q}{V}S_e-\frac{\mu X}{Y} \tag{5.58}$$

Where; dS/dt is defined as the rate of substrate removal (g/L.day). S_i and S_e are influent and effluent substrate concentration (g/L). Y is defined the growth yield coefficient (gVSS/gCOD_{dis}). k_d is the endogenous decay coefficient (1/day).

Under steady-state conditions, the rate of change in substrate concentration dS/dt is negligible and by a similar technique to that used for the substrate concentration, the above equation with substituting Eq. (5.58) can be reduced to Eq. (5.59);

$$\frac{(S_i-S_e)}{HRT}=\frac{X}{Y}\left(\frac{1}{SRT}+k_d\right) \tag{5.59}$$

The above equation can then be rearranged to estimate the effluent biomass concentration under steady-state condition as follows:

$$X = \frac{SRT\ \ Y\ \left(S_i - S_e\right)}{HRT\ \ \left(1 + k_d\ SRT\right)} \tag{5.60}$$

The kinetic parameters Y, k_d can be obtained by rearranging Eq. (5.59) as shown below:

$$\frac{\left(S_i - S_e\right)}{HRT\ \ X} = \frac{1}{Y}\frac{1}{SRT} + \frac{1}{Y}k_d \tag{5.61}$$

The values of Y and k_d can be obtained by plotting (S_i-S_e/HRT.X) versus (1/SRT) in Eq. (5.59), which is obtained by rearranging Eq. (5.61). k_d can be obtained by plotting the (SRT/(1+(SRT.k_d)) versus 1/S in Eq.(5.61). The value of Y can then be calculated from intercept of the straight line while k_d can be obtained from the slope of the line.

5.13.4 Biogas Production Kinetics

5.13.4.1. Modified Stover-Kincannon Model

Methane production is an important parameter for anaerobic treatment systems; therefore, the methane production kinetics should also be determined. Methane production kinetic models were applied to overall of the model reactor. The biogas and methane gas production rates can also be mathematically modeled in terms of substrate removal. The biogas and methane gas productions and quality are dependent on the substrate removal and substrate loading rate. The model developed by Stover Eq. (5.62) and Eq. (5.53) can be used to determine the total gas and the specific methane gas production rates.

The total gas production rate could also be explained with Eq. (5.62).

$$G = \frac{G_{max}(Q\frac{S_i}{V})}{G_B + (Q\frac{S_i}{V})} \tag{5.62}$$

($Q\ S_i/V$) explain the organic loading rate (OLR) applied to the reactor. Q and V are the inflow rate (L/day) and the volume of the anaerobic reactor (L), respectively. Eq. (5.62) can be written as Eq. (5.63).

$$G = \frac{G_{max}\ OLR}{G_B + OLR} \tag{5.63}$$

Eq (5.63) gives the total specific gas production rate. Where, G, G_{max} and G_B can be explained as specific methane gas production rate (mL/L.day), maximum specific methane gas production rate (mL/L.day) and proportionality constant (mg/L.day), respectively.

The methane production rate can be expressed as follows:

$$M = \frac{M_{max}(Q\frac{S_i}{V})}{M_B + (Q\frac{S_i}{V})} \tag{5.64}$$

Where, Q, V, M, M_{max}, M_B and $Q.S_i/V$ are defined as the flow rate (L/day) and reactor volume (L), specific methane production rate (mL/L.day), maximum specific methane production rate (mL/L.day), proportionality constant and organic loading rate (g/L.day), respectively. Eq (5.64) can be written as Eq (5.65).

$$M = \frac{M_{max}\ OLR}{M_B + OLR} \tag{5.65}$$

The inverse of the methane production rate is plotted against the inverse of the OLR, a straight line portion of intercept and slope of line gives $1/M_{max}$ and M_B/M_{max}, respectively.

Linearization of Eqs. (5.63) and (5.65) gives Eqs. (5.66) and (5.67) which these equations could be used to determine the kinetic constants for specific total gas and methane gas productions:

$$\frac{1}{G} = \frac{G_B}{G_{max}} \frac{1}{OLR} + \frac{1}{G_{max}} \quad (5.66)$$

$$\frac{1}{M} = \frac{M_B}{M_{max}} \frac{1}{OLR} + \frac{1}{M_{max}} \quad (5.67)$$

5.13.4.2. Van der Meer and Heertjes Model

To describe the kinetic of methane gas production, the following empirical Eq. (5.68) was used Van der Meer & Heertjes model (Wang et al., 2009).

$$G_{CH4} = k_{sg} Q\ (S_i - S_e) \quad (5.68)$$

Where k_{sg} is the Van der Meer and Heertjes kinetic constant (mL/mg), and G_{CH4} is the methane gas production (L/day), S_i is influent COD_{dis} concentration (mg/L) and S_e is effluent COD_{dis} concentration (mg/L).

5.13.5 Inhibition Kinetics of PAHs

From the study of enzyme inhibitors valuable information has been obtained on the mechanism and pathway of enzyme catalysis, the substrate specificity of enzymes, and the nature of the functional groups in maintaining the active conformation of the enzyme molecule. The four major types of reversible enzyme inhibition, Competitive, un-competitive, non-competitive and Haldane, can be experimentally distinguished by the effects of the inhibitor on the reaction kinetics of the enzyme, which may be analyzed in terms of the basic Monod rate equation. Monod kinetics is one of the simplest and best-known models of enzyme kinetics.

It involves an enzyme (E) binding to a substrate (S) to form a complex (ES), which in turn is converted into a product (P) and the enzyme. This may be represented schematically as

$$E+S \longrightarrow ES \longrightarrow E+P$$

Where, the double arrows between S and ES represent the fact that enzyme-substrate binding is a reversible process.

Under certain assumptions such as the enzyme concentration being much less than the substrate concentration the rate of product formation is given by

$$-R = -\frac{dS}{dt} = \frac{R_{max}\, S}{K_S + S} \tag{5.69}$$

Where R is substrate utilization rate (mg/L.d), Rmax is maximum substrate removal rate (mg/L.day), K_s is half-saturation concentration (mg/L), S is PAH concentration (ng/mL).

The inverse of Eq. (5.69) could be given by Eq. (5.70).

$$\frac{1}{R} = \frac{K_S + S}{R_{max}\, S} \tag{5.70}$$

The linearization of Eq. (5.70) could be given by Eq. (5.71).

$$\frac{1}{R} = \left(\frac{K_S}{R_{max}}\right)\left(\frac{1}{S}\right) + \left(\frac{1}{R_{max}}\right) \tag{5.71}$$

Of these transformations, by far the most popular has been Eq. (5.71), which corresponds to the Lineweaver-Burk, or "double reciprocal" method of plotting kinetic data. When the inverse of substrate (PAH) utilization rate 1/R is plotted against the reciprocal of substrate (PAH) 1/S, a straight line is obtained (Lineweaver-Burk plot) in Eq. (5.71). This line will have a slope of [K_s /R_{max}], an intercept of 1/R_{max} on the 1/R axis, and an intercept of −1/K_s on the 1/S axis. Such a double reciprocal plot has the advantage of allowing much more accurate determination of R_{max} and K_s (Dowd and Riggs, 1965).

Competitive Inhibition

Competitive inhibition is easily recognized experimentally because the percent inhibition at a fixed inhibitor concentration is decreased by increasing the substrate concentration. For quantitative kinetic analysis, the effect of varying the substrate concentration (S) on the initial velocity (R) is determined at affixed concentration of inhibitor. The competitive inhibition could be expressed as follows:

$$\frac{dS}{dt} = -R = -\frac{R_{max}\,S}{K_S\,(1+\frac{I_D}{K_{ID}})+S} \qquad (5.72)$$

Where, R is substrate utilization rate, (mg/L.day), R_{max} is maximum substrate utilization rate (mg/L.day), S is PAH concentration (ng/mL), K_s is half saturation constant for PAH (ng/mL), I_D is inhibitor concentration (mg/L) and K_{ID} is inhibition constant (ng/mL). Plot of 1/R versus 1/S are then prepared, one for each concentration of inhibitor. The plots characteristically give a family of straight lines intersecting at a common intercept on the $1/R_{max}$ axis. The apparent K_s for the substrate will be greater than the true K_s by the increase in the intercept on the 1/S axis. Since the slope of the plot of the uninhibited reaction is K_s/R_{max} and the slope for the inhibited reaction is K_s/R_{max} (1+ I/K_{ID}), the slope is increased by a factor of (1+I/K_{ID}).

Noncompetitive Inhibition

Noncompetitive inhibition is recognized from plots of 1/R versus 1/S in the presence of different fixed concentrations of inhibitor (Table 5.28). The noncompetitive inhibition could be expressed as follows:

$$\frac{dS}{dt} = -R = -\frac{R_{max}}{(1+\frac{K_S}{S})(1+\frac{I_D}{K_{ID}})} \qquad (5.73)$$

The plots differ in slope but do not share common intercept on the $1/R_{max}$ axis. The intercept on the $1/R_{max}$ axis greater for inhibited that uninhibited enzyme, indicating that R_{max} is decreased by the inhibitor.

Uncompetitive Inhibition

Uncompetitive inhibition is most easily recognized from plots of 1/R versus 1/S at fixed inhibitor concentrations. The uncompetitive inhibition could be expressed as follows:

$$-\frac{dS}{dt} = -R = -\frac{\frac{R_{max}\,S}{1+\frac{I_D}{K_{ID}}}}{[\frac{K_S}{1+\frac{I_D}{K_{ID}}}]+S} \qquad (5.74)$$

As the Table 5.28 show, it is typical uncompetitive inhibition that the slope of the plots remains constant at increasing concentrations of the inhibitor, but R_{max} decreases. Uncompetitive inhibition is rare in one substrate reactions but common in two substrate reactions.

Haldane inhibition

This model describes relatively well the microbial growth in the presence of a substrate which is at the same time an inhibitor of the metabolism of this microbial population that is pure or mixed. Even at low concentrations, phenol had a substantial inhibitory effect on the specific growth rate (μ_{max}). The specific growth rate tends to increase with the substrate (Monod type relationship), but μ rises to a peak and finally decreases due to the inhibitory effect of S as its concentration is increased. The Haldane model that has frequently been used to describe this inhibition is:

$$\frac{dS}{dt} = -R = -\frac{\mu_{max}\,S}{[K_S + S + \left(\frac{S^2}{K_{ID}}\right)]\,Y} \qquad (5.75)$$

Where, μ_{max} is the maximum specific growth rate (1/day), S is the concentration of substrate (mg/L), K_s is the half-saturation constant for PAH (ng/mL) and Y is yield (unitless). The inhibition functions, the slope, the intercepts and the type of inhibitions are given in Table 5.28.

Table 5.28 Summary of the effects of inhibitors on Lineweaver-Burk plots 1/R versus 1/S

	Inhibition functions	Slope	Intercept on ordinate	Eqs.
Competitive inhibition	$-\frac{dS}{dt} = -R = -\frac{R_{max}\, S}{K_S\,(1+\frac{I_D}{K_{ID}})+S}$	$\frac{K_s}{R_{max}}\,(1+\frac{I_D}{K_{ID}})$	$\frac{1}{R_{max}}$	(5.76)
Non-competitive inhibition	$-\frac{dS}{dt} = -R = -\frac{R_{max}}{(1+\frac{K_s}{S})(1+\frac{I_D}{K_{ID}})}$	$\frac{K_s}{R_{max}}\,(1+\frac{I_D}{K_{ID}})$	$\frac{1}{R_{max}}\,(1+\frac{I_D}{K_{ID}})$	(5.77)
Uncompetitive inhibition	$-\frac{dS}{dt} = -R = -\frac{\frac{R_{max}\, S}{1+\frac{I_D}{K_{ID}}}}{[\frac{K_s}{1+\frac{I_D}{K_{ID}}}]+\, S}$	$\frac{K_s}{R_{max}}$	$\frac{1}{R_{max}}\,(1+\frac{I_D}{K_{ID}})$	(5.78)
Haldane inhibition	$-\frac{dS}{dt} = -R = -\frac{\mu_{max}\, S}{[K_s\, + S + \left(\frac{S^2}{K_{ID}}\right)]\, Y}$	$\frac{K_s}{\mu_{max}}$	$\frac{1}{\mu_{max}}\,(1+\frac{I_D}{K_{ID}})$	(5.79)

S, PAH concentration (ng/mL), I_D, inhibitor concentration (mg/L), K_{ID}, inhibition constant (ng/mL), K_s, half saturation constant for PAH (ng/mL), R, substrate utilization rate (ng/mL.day), R_{max}, maximum substrate utilization rate (ng/mL.day). X, biomass concentration (mg/L)

CHAPTER SIX
RESULTS AND DISCUSSIONS

6.1 Composition of Real Petrochemical Wastewater Used in the Study

Wastewater treatment plants, especially those serving both urban and industrial areas, consistently receive complex mixtures and a wide variety of organic pollutants. Groups of compounds present in these mixtures include PAHs, which are listed by the US-EPA and by the EU as priority pollutants (Busetti et al., 2006). Their concentrations, therefore, need to be controlled in treated wastewater effluents due to their toxic, mutagenic and carcinogenic properties (Busetti et al., 2006).

Petrochemical wastewater has characteristics of large water volume and poor biodegradability (Zhao et al., 2006; Zhang et al., 2011). Its influent was a mixed waste stream from a petrochemical industry producing dyestuff, chemical fertilizers, calcium carbide, glycol, oxirene, acrylon, synthetic resin and pesticides (Zhang et al., 2011). Moreover, petrochemical wastewater contains oil, metal salt, sulfide, aromatics, PAHs, volatile phenol and other substances apart from nitrogen and phosphorus (Ma et al., 2009).

All wastewater used in this study was obtained from a petrochemical wastewater treatment plant in Izmir-Turkey. The wastewater treatment plant has a treatment capacity of 18000 m^3/day. The primarily treatment processes of the wastewater treatment plant are oil-water separator, neutralization, flocculation, and coagulation, dissolved air flotation followed by an aeration (activated sludge system) and sedimentation tank as its secondary treatment The real wastewater samples were taken from the influent of the activated sludge process after primary treatment. The activated sludge was taken from the recycle line of the sedimentation unit of the aeration tank. The properties of the real wastewater used in the present study are given in Table 6.1

Table 6.1 Characteristics of petrochemical industry wastewater

Parameters	**Values**		
	Minimum	**Mean**	**Maximum**
pH	7.05	7.12	7.52
Temperature (^{o}C)	19.4	20.4	22.8
DO (mg/L)	2.1	2.2	2.8
COD_{total} (mg/L)	724	812	944
COD_{dis} (mg/L)	611	642	756
BOD_5 (mg/L)	112	128	144
NH_4-N (mg/L)	1.90	2.20	4.36
NO_3-N (mg/L)	1.45	1.80	2.45
NO_2-N (mg/L)	0.02	0.04	0.80
Oil-grease (mg/L)	187	206	406
MLVSS (mg/L)	2800	2900	3000
TSS (mg/L)	351	485	564
Cd (µg/ L)	2.95	3.18	4.57
Cr (µg/ L)	1.96	4.55	6.75
Ni (µg/ L)	24.10	25.08	36.78
Pb (µg/ L)	10.78	12.76	16.78
Zn (µg/ L)	103.30	352.90	455.30
Fe (µg/ L)	1085	2587	2895
ACT (ng/mL)	22.74	23.30	24.74
FLN (ng/mL)	12.23	14.85	16.35
PHE (ng/mL)	10.71	12.18	14.11
ANT (ng/mL)	0.31	0.50	0.71
CRB (ng/mL)	0.21	0.42	0.68
FL (ng/mL)	0.45	0.97	1.20
PY (ng/mL)	0.71	0.94	1.16
BaA (ng/mL)	0.04	0.06	0.07
CHR (ng/mL)	0.01	0.14	0.16
BbF (ng/mL)	0.03	0.04	0.04
BkF (ng/mL)	0.01	0.02	0.02
BaP (ng/mL)	0.01	0.02	0.02
IcdP (ng/mL)	0.01	0.02	0.02
DahA (ng/mL)	0.03	0.04	0.04
BghiP (ng/mL)	0.01	0.01	0.01
Total PAHs	47.64	53.52	59.39

The influent COD_{dis}, TN, TP concentrations in wastewater used in batch reactors were 642, 26 and 12 mg/L, respectively. The NH_4-N, NO_2-N and NO_3-N concentrations in wastewater used in batch reactors were 3.18, 2.00 and 0.30 mg/L, respectively. The oil-grease MLVSS and TSS concentrations were 342, 2900 and 485 mg/L, respectively. The influent total PAHs concentration was 53.52 ng/mL.

The COD_{dis}, total PAH, BOD_5 and the concentrations for other parameters given in this table are characteristic for petrochemical industry wastewater and exhibits similarities with the petrochemical industry wastewaters used by Manoli and Samara (2008) and Busetti et al. (2006) in their studies.

The composition of the wastewater generated from a refinery is very much dependent on the complexity of the process. Sarathy et al., (2002) investigated the performance of the aerobic batch biological reactor treating petrochemical wastewater. In their study, the refinery wastewater contained 510 mg/L of COD_{dis} and 234 mg/L of BOD_5. Dold (1989) showed that the petrochemical wastewater contained BOD_5 and COD_{dis} levels in the range of 150–350 and 300–800 mg/L, respectively. The oil-grease concentrations were 3000 mg/L while the, suspended solid (SS) concentration were more than 100 mg/L. The BaP, heavy-metal a chrome levels were between 1 and 100 mg/L, between 0.10 and 100 mg/L and between 0.2 and 10 mg/L.

6.2 Removals of COD_{dis} and PAHs from Real Petrochemical Wastewater in Batch Reactor

A couple of batch studies were performed to determine the COD_{dis} and the total-individual 15 PAHs removal efficiencies under aerobic conditions in batch scale reactors. The aerobic batch reactors were filled with 400 mL real petrochemical wastewater and 300 mL inoculums (See Table 5.3 in Chapter Five for operational conditions). Influent, effluent values of the wastewater and removal efficiencies after 30 days of operation time in aerobic batch reactor system were given in Table 6.2.

Table 6.2 Influent, effluent values of wastewater and removal efficiencies after 30 days of operation time in aerobic batch reactor system

	Values		
Parameters	**Influent wastewater**	**Effluent wastewater**	**Removal Efficiency (%)**
COD_{dis} (mg/L)	642	206	68
BOD_5 (mg/L)	128	48	63
NH_4-N (mg/L)	3.10	0.97	69
NO_3-N (mg/L)	2.00	0.58	71
NO_2-N (mg/L)	0.30	0.10	67
Oil-grease (mg/L)	342	98	71
TSS (mg/L)	485	105	78
Cd (µg/ L)	3.18	1.15	64
Cr (µg/ L)	4.55	1.96	57
Ni (µg/ L)	25.08	9.21	63
Pb (µg/ L)	12.76	5.78	55
Zn (µg/ L)	352.90	103.3	71
Fe (µg/ L)	2587	985	62
ACT (ng/mL)	23.30	6.56	72
FLN (ng/mL)	14.85	7.87	47
PHE (ng/mL)	12.18	3.94	68
ANT (ng/mL)	0.50	0.38	24
CRB (ng/mL)	0.42	0.34	19
FL (ng/mL)	0.97	0.79	19
PY (ng/mL)	0.94	0.85	10
BaA (ng/mL)	0.06	0.05	11
CHR (ng/mL)	0.14	0.12	14
BbF (ng/mL)	0.04	0.04	12
BkF (ng/mL)	0.02	0.02	11
BaP (ng/mL)	0.02	0.02	14
IcdP (ng/mL)	0.02	0.02	11
DahA (ng/mL)	0.04	0.03	17
BghiP (ng/mL)	0.02	0.01	13
Total PAHs (ng/mL)	53.52	21.04	61

The flow rate and HRT were constant as 0.30 L/d, and 2.3 days, respectively. The F/M ratio and OLR in the aerobic batch reactor system were measured as 0.03 $gCOD_{dis}$/gMLVSS.day and 0.096 $gCOD_{dis}$/L.day, respectively (See Table 5.2 in

Materials and Methods). MLVSS concentration in the aerobic batch system was 2.9 g/L.

To verify the ability of the aerobic batch reactor to degrade the organic contaminants of the petrochemical industry wastewater, some key parameters including the COD_{dis} and 15 individual PAHs were analyzed in the influent and effluent wastewater of the aerobic batch reactor. COD_{dis} and PAHs were selected as the indicator for biodegradability of the petrochemical industry wastewater.

The adaptation period is very important since the bacterial population used as seed is going to be exposed to the petrochemical industry wastewater in the aerobic batch reactors. To acclimate the aerobic biomass to the petrochemical wastewater, the aerobic batch reactors were operated through 30 days to start-up period at a SRT 20 days. Aerobic batch reactor reached steady-state conditions after an operation period of 14 days.

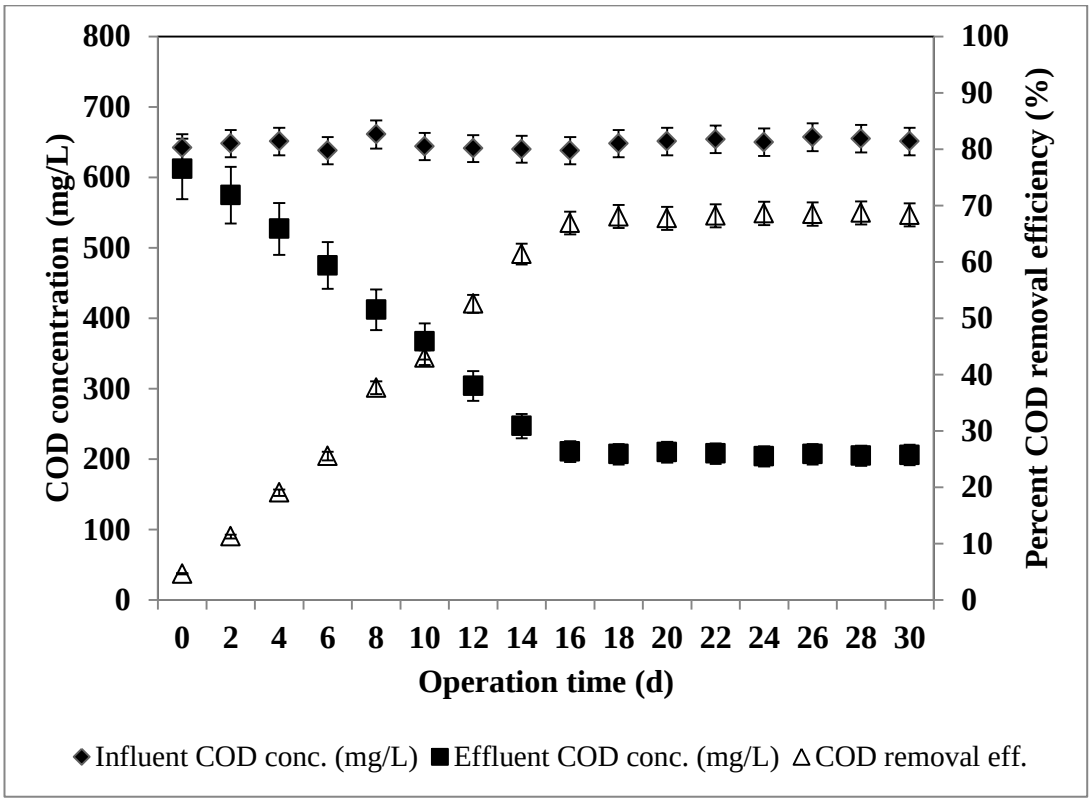

Figure 6.1 COD_{dis} variations through operation period in aerobic batch reactor, SRT= 20 days (all the COD concentration in figure are COD_{dis})

After 30 days of operation period the COD_{dis}, BOD_5, total PAHs concentrations decreased from 642 mg/L, 128 mg/L, 53.52 ng/mL to 206 mg/L, 48 mg/L, 21.04 ng/mL in the effluent of the aerobic batch reactor system (Table 6.2). The COD_{dis}, BOD_5 and total PAHs removed with treatment efficiencies of 68%, 63% and 61%, respectively, after this period (Table 6.2). The NH_4-N, NO_2-N and NO_3-N concentrations decreased from 3.10, 2.00, 0.30 mg/L to 0.97, 0.58, 0.10 mg/L in aerobic batch reactor system. The NH_4-N, NO_2-N and NO_3-N removal efficiencies were obtained as 69%, 71% and 67%, respectively in aerobic batch reactor system (Table 6.2). Total PAHs removal efficiency was obtained as 61% at the end of operation time in system (Fig. 6.2). The studies performed with aerobic batch reactors showed that the total PAH removal efficiencies were between 19% and 72%, between 10% and 19% between 11% and 14%, between 11% and 17% for three, four, five and six ring PAHs, respectively (Figure 6.2).

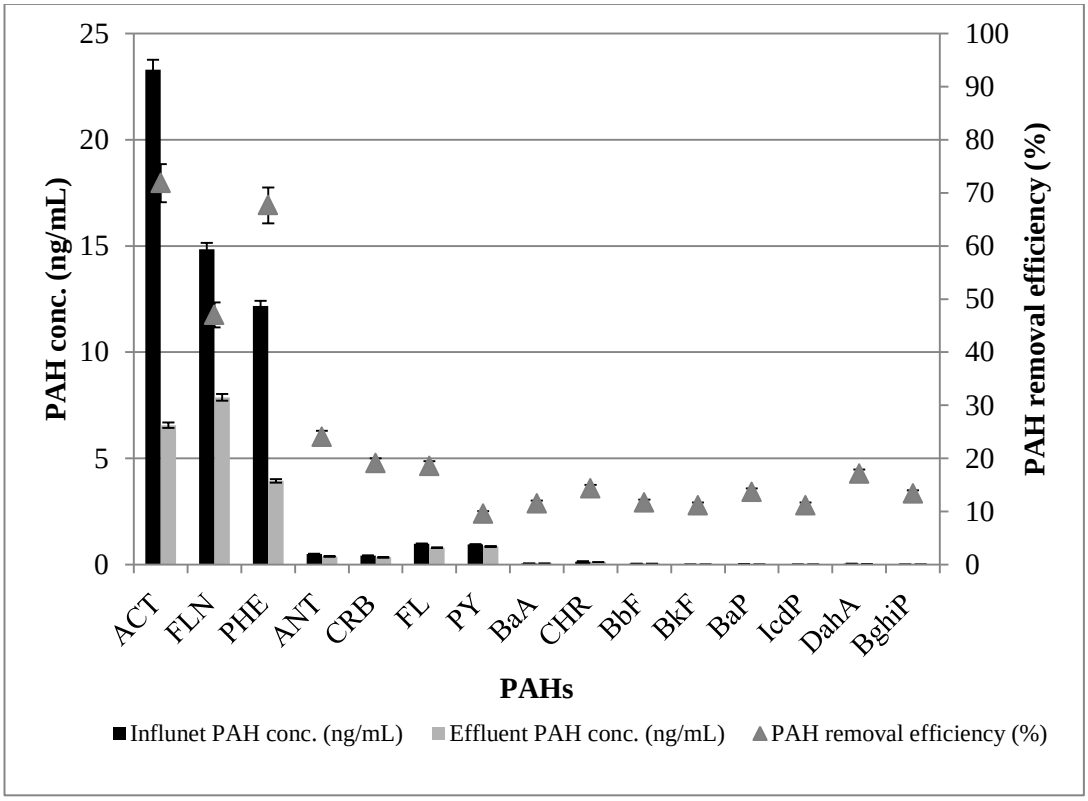

Figure 6.2 Individual PAHs variations through start-up period (30 days) in the aerobic batch reactor

It was observed that the PAHs such as ACN, FLN and PHE with low molecular weight were removed with total treatment efficiencies of 72%, 47%, and 68%, respectively. The total removal efficiencies in PY, BbF, BaP and BghiP were found to be lower, for example, 10%, 12%, 14%, % and 13% (See Fig. 6.2). This could be attributed to the differences in molecular weight of the PAHs and to the benzene ring numbers of the PAHs.

Zheng et al. (2007) found that the lower weight PAHs with three and four rings were biodegraded more rapidly than the higher weight (≥5 ring) PAH compounds. The transfer of PAHs from the bulk liquid to the biomass is essential for aerobic removal of hydrophobic PAHs. Lower molecular weight PAHs with three rings also tend to be more volatile (i.e., have a higher vapor pressure) and more readily partition into air from pure water (i.e., have a higher Henry's law constant). In this study, significant difference in PAHs yields was observed between some PAHs with four (FL, PY, BaP and CHR), five (BbF, BkF and BaP) and six rings (DahA, BghiP and BghiP) although the PAHs with high benzene rings became increasingly less soluble in water with an increasing molecular weight and lower Henrry's low constant and less volatility which were biodegraded effectively in the aerobic batch system (Anova; R^2=0.92, F=3.58, P=0.01). The results obtained in this study are lower than the data obtained by Augulyte et al. (2009). Augulyte et al. (2009) found 99% PAHs removal efficiency in the treatment of a synthetic petrochemical wastewater in a biological aerobic reactor system.

6.3 The Performance of Aerobic CSTR System Treating the Real Petrochemical Industry Wastewater

6.3.1 Optimization of SRT for the Aerobic CSTR Reactor Performance

In activated sludge systems the SRT theoretically determines the mean microbial life-time, and hence microbial population and activity. SRT is an important operational parameter for the removal of hydrophobic organic compounds like PAHs. Initially, the aerobic CSTR systems were inoculated with activated sludge from the petrochemical wastewater treatment plant in Izmir. The aerobic CSTR systems were operated at four different SRTs (5-10-25-40 days) to determine the optimum SRT for maximum PAH and COD_{dis} removals under steady state conditions. During the start-up, the reactors were operated at a constant HRT of 5 days since the recent literatures investigating the aerobic biodegradation of petrochemical wastewaters recommend short HRTs in the start-up of the reactors (Guo et al., 2009; Lin et al., 2001; Ma et al., 2008).

The results of this study showed that as the SRTs were increased from 5 to 25 days both COD_{dis} and total PAH removals increased from 70% to 79% and from 60 to 69% in the CSTR, respectively (Table 6.3). The COD_{dis} and total PAH yields decreased from 79% to 77% and from 74% to 50% as the SRTs were increased from 25 days up to 40 days. The maximum PAH and COD_{dis} yields were obtained at a SRT of 25 days. Although a part of COD_{dis} oxidation occurred even at a SRT of 5 days, average COD_{dis} removal efficiency increased with increasing SRT.

In this study high SRT such as 25 days provided enough contact time between microorganisms and PAHs to degrade these organics containing PAHs. The reason for the decrease in COD_{dis} and PAHs yields at SRTs > 25 days could be attributed to the inhibitory effect of the long contact time on the bacteria through degradation of PAHs with high benzene rings and to the bacteria aged which result a loss of enzymatic activities in the CSTR reactor.

Table 6.3 Concentrations of COD_{dis} and PAHs and removal efficiencies of influent/effluent wastewater in CSTR system at increasing SRTs

	SRT 5 days		SRT 10 days		SRT 25 days		SRT 40 days	
	COD_{dis} (mg/L)	PAHs (ng/mL)	COD_{dis} (mg/L)	PAHs (ng/mL)	COD_{dis} (mg/L)	PAHs (ng/mL)	COD_{dis} (mg/L)	PAHs (ng/mL)
Influent	1200±3.51	65.32±0.09	1200±3.51	65.32±0.09	1200±3.51	65.32±0.09	1200±3.51	65.32±0.09
Effluent	360±2.42	32.51±0.02	324±1.54	29.39±0.04	252±1.10	16.98±0.02	276±1.21	32.66±0.05
Removal Eff. (%)	70±0.74	50±0.33	73±0.87	55±0.39	79±0.48	74±0.54	77±0.58	50±0.61

6.3.2 Start-up Period of Aerobic CSTR Reactor in Continuous Mode under Constant SRT and HRT without Biosurfactant

Generally, the wastewaters from the petrochemical industry are treated with conventional activated sludge systems. Since such systems are unable to completely remove the main 15 PAHs (ACT, FLN, PHE, ANT, CRB, FL, PY, BaA, CHR, BbF, BkF, BaP, IcdP, DahA, and BghiP) these are released into receiving bodies. Therefore an adaptation period is needed to acclimate the bacteria to the petrochemical wastewater.

The adaptation (start-up) period is very important since the bacterial population used as seed is going to be exposed to the petrochemical industry wastewater in the aerobic CSTR reactor. To acclimate the aerobic biomass to the petrochemical wastewater, four lab-scale CSTR reactors were operated through 45 days to reach steady-state conditions at a SRT of 25 days and a HRT of 5 days since the recent literatures investigating the aerobic biodegradation of petrochemical wastewaters recommend short HRTs in the start-up of the reactors (Shah et al., 1998; Zhang et al., 2011; Chakraborty and Veeramani, 2006)

The steady-state conditions were defined with COD_{dis} and total 15 PAHs removal efficiencies higher than 75% and 60%, respectively, for consecutive 12 days. After this operation time, the PAHs and the total COD_{dis} removal efficiencies remained constant approximately at 79% and at 69%, respectively, through continuous operation in CSTR without biosurfactant on day 36. As shown in Figures 6.4 and 6.5, aerobic CSTR reactor reached steady-state conditions after an operation period of 36 days under constant SRT and HRT. During the start-up period the dissolved oxygen concentration and the redox potential were around 3 mg/L and + 90 mV.

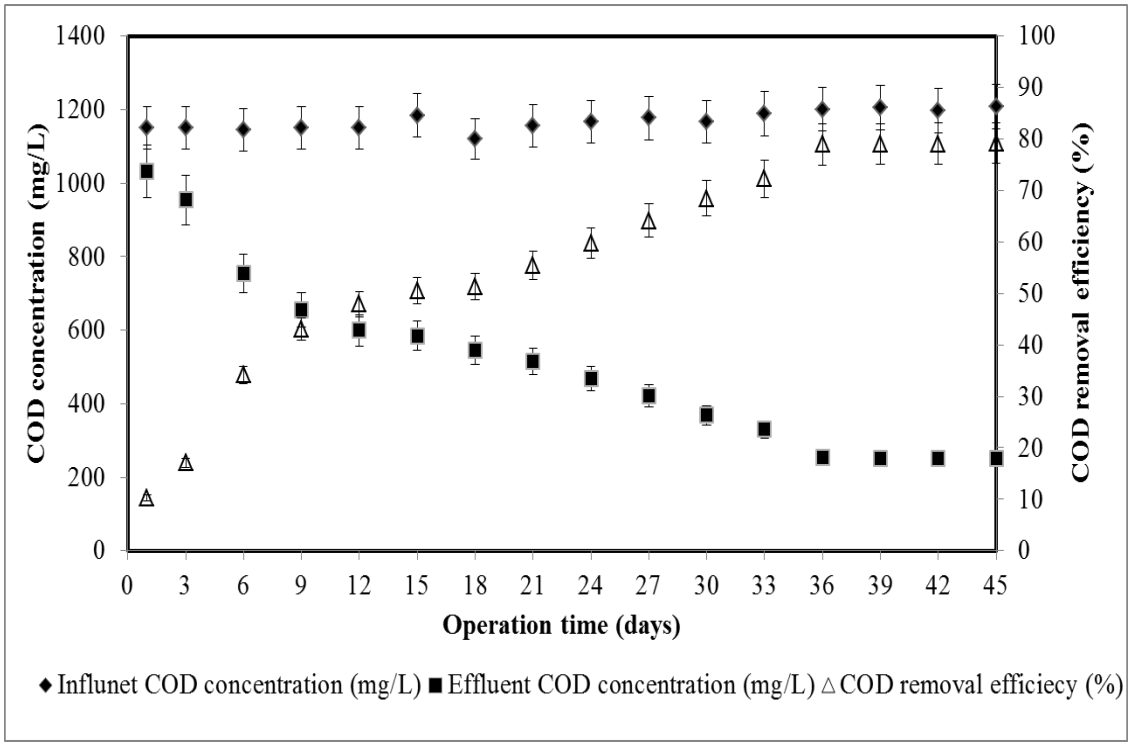

Figure 6.4 COD_{dis} variations through start-up period at constant HRT and SRT in the CSTR (n=3, mean±SD)

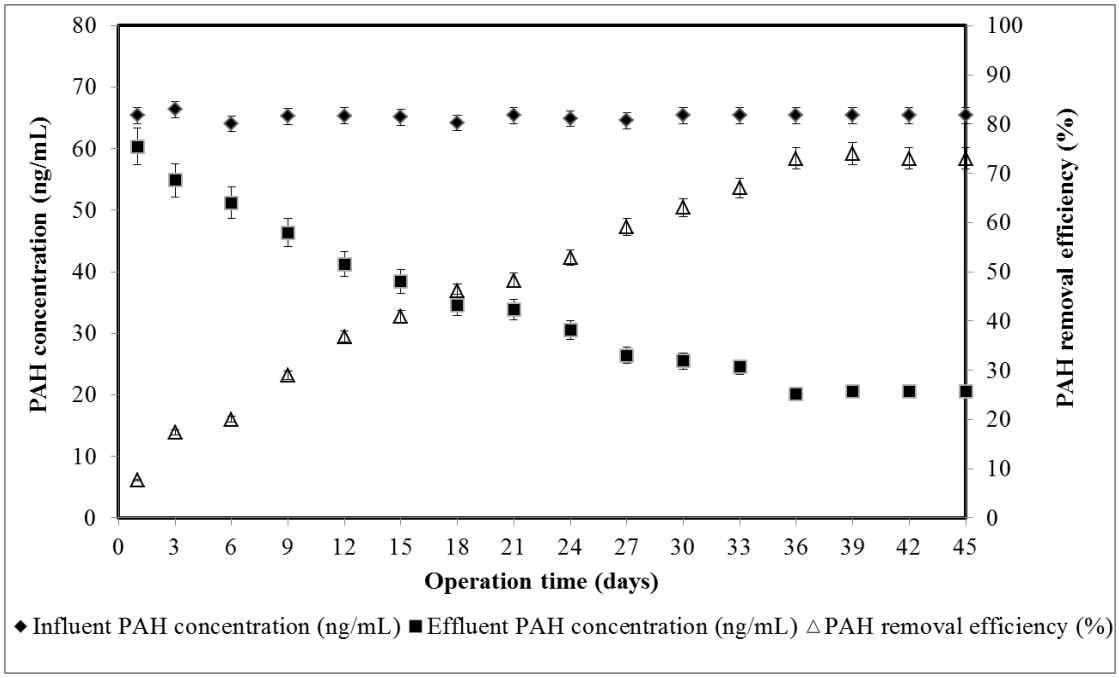

Figure 6.5 PAH variations through start-up period at constant HRT and SRT in the CSTR (n=3, mean±SD)

6.3.3 Removal of Total and Individual PAHs without Biosurfactant at Increasing SRTs

In activated sludge systems the SRT should be long enough to provide sufficient retention time for contact of biomass with toxic organics like PAHs and inhibitory substances. The SRT values typically used in full-scale aerobic wastewater treatment plants are in the range of 4–10 days for carbon oxidation (Tchobanoglous et al., 2003). For the treatment of wastewaters containing inert and toxic compounds the activated sludge systems should be operated as long as necessary in order to maintain enough contact time between the microorganism and organic substrates in question. Four different SRTs were used as 5, 10, 25 and 40 days in an aerobic CSTR. As the SRTs were increased from 5 to 25 days total PAH removals increased from 49 to 69% in the CSTR (Table 6.4). The total PAH yields decreased from 69% to 56% as the SRT were increased from 25 days up to 40 days.

Different results were reported concerning the removal of PAHs in aerobic biodegradation: Thangaraj et al., (2008) and Manoli and Samara (2008) showed that PAHs are biodegraded. Wang et al. (2008) reported that the concentration levels of PAHs and related catabolic genes including upper-pathway dioxygenase genes and down-pathway catechol dioxygenase genes provide 93% of the total PAHs removals in the aerobic and anoxic units of the reactor system. Trably and Patureau (2007) investigated the ability of aerobic microorganisms to degrade light and heavy PAHs in continuous bioreactors treating trace-level PAH-contaminated wastewaters. Habe and Omori (2003) showed that both enzymologic and genetic properties are important for effective PAHs biodegradation with aerobic bacteria. It was found that the lower molecular weight (2-, 3- and 4-ring) PAHs were found to be biodegraded more rapidly than the heavier (5–6 ring) compounds (Chauhan et al., 2008).

Table 6.4 Individual PAH removal efficiencies in the CSTR at increasing SRTs without biosurfactant (n=3, mean±SD)

PAHs	Number of rings	Influent PAH concentration (ng/mL)	PAH removal efficiency (%) SRT 5 days	PAH removal efficiency (%) SRT 10 days	PAH removal efficiency (%) SRT 25 days	PAH removal efficiency (%) SRT 40 days
ACT	3	29.43±0.09	56±1.40	60±1.55	80±1.92	57±1.33
FLN	3	9.38±0.06	34±1.60	40±1.20	59±2.42	47±2.71
PHE	3	15.01±0.02	50±2.80	54±2.56	74±3.06	53±2.40
ANT	3	3.61±0.04	45±2.52	56±1.81	78±2.05	60±1.80
CRB	3	0.90±0.06	40±1.10	55±2.80	80±1.50	63±2.10
FL	4	2.98±0.07	45±0.90	60±1.20	71±0.75	58±2.10
PY	4	2.19±0.05	34±1.10	50±2.15	60±1.10	54±3.20
BaA	4	0.36±0.03	13±1.84	15±0.95	37±0.88	28±1.94
CHR	4	0.72±0.02	34±0.64	56±0.91	79±0.65	53±1.32
BbF	5	0.08±0.08	45±2.40	53±3.30	74±2.82	56±2.27
BkF	5	0.09±0.04	24±1.20	32±0.94	54±1.50	42±1.72
BaP	5	0.07±0.06	50±1.50	56±0.80	73±0.75	57±1.25
IcdP	6	0.13±0.02	55±1.40	64±1.25	87±0.76	60±0.83
DahA	6	0.28±0.07	45±0.60	54±1.65	86±1.10	57±1.31
BghiP	6	0.09±0.03	50±1.05	58±0.95	84±0.67	67±0.98
Total PAHs eff. %)		65.32±0.05	49±2.12	55±2.56	74±1.45	56±2.14

In this study the PAH yields with high rings were biodegraded as high the PAH with low rings. Some times higher PAH yields were observed in PAHs with 6 rings (84% and 87% for BghiP and IcdP PAHs, respectively) compared to the PAHs with 4 rings (37% and 71% for BaA and FL PAHs, respectively) (Table 6.4). This could be attributed to the CSTR reactor configuration, to the type of PAH degradading bacteria, to the sludge age and to the composition of the petrochemical industry wastewater.

6.3.4 Effects of Increasing RD, EM, SR Biosurfactants on the Total PAH Removals in the CSTR System at Increasing SRTs

In order to determine the optimum biosurfactant dose for the maximum removals of PAHs with three, five and six benzene rings; 10 mg/L, 15 mg/L, 25 mg/L RD, EM and SR were administered to the feed of the CSTR system at four increasing SRTs. The presence of RD supported and stimulated the total PAH biodegradation yields with respect to the control whereas EM and SR biosurfactants did not affect the total PAH yields significantly as RD at all SRTs (Table 6.5). As the RD concentrations and the SRTs increased from 10 mg/L to 15 mg/L and from 5 to 25 days, respectively, in the CSTR system resulted in an enhancement in total PAH biodegradation (Table 6.5). The total PAH biodegradation yields in the samples containing 10-15 mg/L SR exhibited similarities to EM biosurfactant for a SRT of 5 days and decreased at a SRT of 10 days. As the SRT was increased to 25 days the total PAH degradation yields increased at all biosurfactants (Table 6.5). The highest PAH biodegradation yields (96%) were achieved at a SRT of 25 days and at a RD concentration of 15 mg/L (Table 6.5).

Table 6.5 Effects of increasing biosurfactant concentrations on the total PAH removals in CSTR system at increasing SRTs (n= 3, mean values)

	SRT 5 days										SRT 10 days									
BSRF		RD			EM			SR				RD			EM			SR		
SD	C	10	15	25	10	15	25	10	15	25	C	10	15	25	10	15	25	10	15	25
ITPC	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61
EPC	32±0.12	7±0.24	12±0.38	14±0.58	17±0.64	14±0.50	21±0.60	18±0.54	14±0.68	23±0.95	30±0.67	12±0.60	10±0.58	13±0.35	16±0.28	15±0.64	19±0.60	16±0.50	15±0.40	20±0.38
TPRE	51±0.50	78±1.10	81±1.28	79±1.90	73±1.60	78±1.54	68±0.90	73±0.64	79±1.20	65±1.38	53±1.64	82±1.40	85±1.65	80±0.90	74±1.68	76±1.50	70±2.10	75±1.00	77±0.60	68±1.60
	SRT 25 days										SRT 40 days									
BSRF		RD			EM			SR				RD			EM			SR		
SD	C	10	15	25	10	15	25	10	15	25	C	10	15	25	10	15	25	10	15	25
ITPC	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61	65±0.61
EPC	16±0.20	7±0.35	3±0.45	16±0.64	12±0.72	9±0.61	26±0.81	11±0.67	9±0.70	26±0.20	32±0.10	22±0.38	14±0.80	26±0.91	27±1.00	20±0.94	25±0.64	24±0.80	26±0.30	32±0.60
TPRE	74±1.67	89±1.80	96±0.90	76±0.80	81±0.50	86±1.30	60±1.67	82±1.50	85±1.38	60±2.10	50±2.50	65±1.90	78±1.00	60±0.60	59±1.30	69±1.40	62±1.45	54±1.20	60±0.70	51±0.90

SRT: Sludge retention time (day); BSRF : Biosurfactants used in this study ; RD : Rhamnolipid concentration (mg/L); EM Emulsan concentration (mg/L); SR: Surfactin concentration (mg/L); SD : Biosurfactant concentration (mg/L); ITPC: Influent total PAH concentration in CSTR system (ng/mL); EPC : Effluent total PAH concentration in CSTR system (ng/mL); TPRE : Total PAH removal efficiency (%); C: Control, without biosurfactant.

As the SRT and the EM concentrations were increased from 5 to 25 days and from 10 to 15 mg/L the total PAH removals increased from 51% to 78%, 76% and 86%, respectively (Table 4.6). The yields of PAH increased as the SRT was increased from 5 to 25 days, but decreased as the SRT was further increased to 40 days. In the presence of 15 mg/L EM a longer SRT (40 days) remarkably increased from 86% to 69% the total PAH removals in the CSTR system. As the SRT and the SR concentrations were increased from 5 to 25 days and from 10 to 15 mg/L the total PAH removals increased from 51% to 79%, 77% and 85% respectively (Table 6.5). The total PAH biodegradation yields in the samples containing 15 mg/L SR exhibited similarities to EM biosurfactant for a SRT of 5 days and decreased at a SRT of 40 days. The yields of PAH increased as the SRT was increased from 5 to 25 days, but decreased as the SRT was further increased to 40 days. The PAH yields remained lower for the 15 mg/L EM administered samples (85%) at a SRT of 25 days. The PAH yields decreased to 69% as the SRT was increased to 40 days for EM biosurfactant of 15 mg/L, respectively. The high PAH removals with 15 mg/L EM and SR could be attributed to the decrease in the surface tension of wastewater, which subsequently increased the substrate, flux from aqueous medium to bacterial cells (Yu et al., 2007). At low and high EM and SR concentrations (10, 15, 25 mg/L) the PAH removals were low in the CSTR systems for all studied SRTs compared to the 15 mg/L EM and SR (Table 6.5). Addition of EM and SR to the CSTR reactors improved PAH removals (86% and 85% for EM and SR respectively).

Among the biosurfactants used in the study, the maximum PAH yield was obtained as 96% with 15 mg/L RD, followed by15 mg/L EM (as 86%) and 15 mg/L SR (as 85%) at a SRT of 25 days in the CSTR system.

The RD, EM and SR biosurfactant concentrations of 10 mg/L and 15 mg/L at SRTs of 5, 10 and 25 days, are utilized by the bacteria probably as an additional carbon and energy sources in the CSTR system. The differences between biosurfactants to the PAH yields could be attributed to their specificity, biodegradability, low-high toxicity effects and their availabilities (Yu et al., 2007;

Sartoros et al., 2005). These biosurfactants improve the PAH bioconversion process by increasing the PAH bioavailability and mass transfer rates to cells.

Biosurfactants like RD, EM and SR are capable of lowering the surface tension of PAHs with high benzene rings (Mulligan et al., 2001). Furthermore, it was reported that the biosurfactants mentioned above are able to shorten the extended lag phase of bacteria for PAH biotransformation by the uptake of dissolved COD to the bacterial cell together with fast PAH diffusion (Bautista et al., 2009).

The administration of 15 mg/L RD supported the maximum total PAHs yields with respect to the control whereas EM and SR did not affect the total PAHs yields significantly as RD at all SRTs. In this study it was found that biosurfactants enhance significantly the PAH biodegradation yields at a 15 mg/L optimum doses (R^2= 0.93, F= 3.45, P = 0.05; R^2= 0.98, F=3.67, P = 0.05; R^2= 0.89, F=2.98, P = 0.05) for RD, SR and EM, respectively. It was reported that the biosurfactants are used effectively to obtain high PAH biodegradation yields in the petrochemical industry wastewaters (Bautista et al., 2009). Sufficient SRT of substrate, biosurfactant and biomass are necessary for uptake and biodegradation of the PAHs by the bacteria in CSTR (Clara et al., 2005).

More hydrophobic PAHs were uptaken with biosurfactants to the bacterial cell at optimum SRTs since they had enough contact time to acclimate to the reactor conditions together and metabolize the biosurfactants by reducing the interfacial tension of wastewater. In this study, most probably a significant uptake of PAHs by bacteria together with biosurfactant occurred since a preferential adsorption of surfactants on the bacterial membrane to prevent the PAHs uptake was observed at RD, EM and SR concentrations > 15 mg/L. The reason for the decrease in PAH yields at SRTs > 25 days could be attributed to the inhibitory effect of the non-degraded PAH accumulation on the bacterial biomass at long contact times, to the increase of inert residual microbial soluble products and to the low enzymatic activities of the aged biomass in the CSTR system as reported by Sartoros et al. (2005). Furthermore, the aged aerobic microorganisms at SRT > 25 days were not

active enough to metabolize and uptake the RD and PAH mixtures. Similar results also were reported by Zhou and Zhu (2007) and Yin et al. (2009).

6.3.5 Effects of Increasing RD, EM, SR Biosurfactants on the Individual PAH Removals in the CSTR System at Increasing SRTs

6.3.5.1 Effects of Increasing RD Biosurfactant on the Individual PAH Removals in the CSTR System at Increasing SRTs

As the RD concentrations were increased from 10 to 15 mg/L at a SRT of 5 days ACT, FLN, PHE, ANT and CRB PAHs yields increased from 70%, 66%, 69%, 56%, 54% to 80%, 74%, 78%, 70%, 69%, respectively (Table 6.6). The removal efficiencies of FL, PY, BaA and CHR PAHs also increased from 57%, 52%, 54%, 51% to 70%, 68%, 60%, 51%, respectively. The yields of BbF, BkF, BaP, IcdP, DahA and BghiP were obtained as 70%, 70%, 70%, 68%, 65% and 66%, respectively, at a RD concentration of 15 mg/L at a SRT of 5 days (Table 6.6). As the RD concentrations were increased from 15 to 25 mg/L at SRT of 5 days, the yields of ACT, FLN, PHE, ANT and CRB PAHs decreased from 80%, 74%, 78%, 70%, 69% to 79%, 70%, 70%, 67%, 60%, respectively. Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs also decreased from 68%, 70%, 68%, 60% to 62%, 65%, 62%, 57%, respectively, when 25 mg/L RD was added to the CSTR at a SRT of 5 days. The BbF, BkF, BaP, IcdP, DahA and BghiP removals were found as 68%, 68%, 68%, 60%, 60% and 58%, respectively, for the aforementioned RD concentration at a SRT of 5 days (Table 6.6).

Table 6.6 Effect of increasing RD concentrations on individual PAH removals at increasing SRTs in the CSTR system (n=3, mean values)

	PAH removal efficiencies at SRT 5 days				PAH removal efficiencies at SRT 10 days				PAH removal efficiencies at SRT 25 days				PAH removal efficiencies at SRT 40 days			
PAHs	C[a]	RD[b] 10	RD[c] 15	RD[d] 25	C[a]	RD[b] 10	RD[c] 15	RD[d] 25	C[a]	RD[b] 10	RD[c] 15	RD[d] 25	C[a]	RD[b] 10	RD[c] 15	RD[d] 25
ACT	56±1.50	70±1.13	80±2.34	79±2.05	57±1.67	69±1.88	87±0.99	74±0.86	80±2.11	90±1.93	96±2.41	76±1.86	62±1.37	83±1.73	93±2.35	70±1.05
FLN	59±1.25	66±1.85	74±2.85	70±2.13	60±1.56	68±1.45	86±1.35	70±0.77	59±1.01	89±2.35	97±1.85	63±1.60	62±0.87	70±2.06	78±1.87	74±1.11
PHE	60±0.85	69±1.64	78±2.11	70±1.54	63±1.03	70±1.62	85±1.27	65±1.09	74±0.80	90±1.60	96±1.54	65±1.30	60±0.59	60±0.46	67±2.11	60±0.66
ANT	53±2.31	56±1.87	70±2.65	67±2.11	60±0.88	60±0.97	83±1.65	73±1.18	78±0.54	88±2.16	94±1.87	70±0.85	64±1.92	62±1.07	69±0.77	60±0.97
CRB	47±1.90	54±0.95	69±0.98	60±1.10	55±0.67	60±1.11	85±1.71	79±1.34	80±1.68	87±1.87	94±1.92	69±0.98	68±1.64	62±0.49	68±1.65	59±1.35
FL	34±1.45	57±0.88	68±1.05	62±1.65	44±1.42	63±1.28	80±1.09	73±0.62	71±0.91	86±0.56	93±0.97	70±0.24	57±1.34	58±0.87	60±0.84	55±1.87
PY	44±1.35	52±1.46	70±1.30	65±0.67	52±1.64	60±1.34	79±0.61	72±0.60	60±1.68	88±0.81	95±0.54	69±0.69	53±2.81	57±1.37	59±2.11	50±1.44
BaA	54±1.61	54±1.63	68±1.65	62±0.93	60±0.74	63±1.86	76±0.81	70±1.10	33±1.97	80±1.06	94±0.89	80±1.38	40±0.21	51±1.72	61±0.68	52±0.76
CHR	34±0.84	51±1.10	60±2.45	57±1.13	45±0.64	62±0.60	82±1.09	72±1.28	65±0.53	82±1.86	94±1.45	70±0.12	58±0.37	60±0.81	60±1.69	53±0.44
BbF	44±0.95	62±1.30	70±2.33	68±1.45	45±0.55	69±1.25	82±0.38	65±0.87	74±1.35	79±1.39	95±1.37	74±0.29	50±0.94	60±1.50	69±1.58	65±0.63
BkF	50±1.02	60±0.63	70±2.84	68±1.36	52±0.81	69±1.11	80±0.51	65±1.57	45±1.29	78±1.45	96±1.06	68±1.64	50±1.26	32±1.67	43±1.94	59±0.80
BaP	51±1.47	62±1.54	70±2.62	68±1.78	53±1.07	67±0.66	77±1.24	68±1.84	67±1.67	73±1.58	95±0.52	70±1.37	47±1.04	60±1.94	65±1.37	60±1.19
IcdP	43±0.84	59±2.23	68±1.98	60±1.64	53±1.54	65±0.48	85±1.16	46±1.67	73±1.60	70±0.64	96±0.34	67±1.68	43±1.29	74±0.38	89±1.54	60±1.38
DahA	34±0.55	56±0.99	65±1.54	60±0.67	47±0.61	66±0.36	84±1.28	34±1.58	57±0.37	80±0.94	96±1.99	74±1.91	42±0.76	78±1.48	83±1.29	62±1.53
BghiP	38±0.98	54±1.36	66±0.54	58±1.09	43±0.39	65±1.09	84±1.34	35±1.62	76±0.79	78±1.29	95±0.67	74±0.68	40±0.96	74±1.22	87±1.84	64±1.37

a: Control without biosurfactant; b, c and d: RD 10, RD 15 and RD 25: RD concentrations (mg/L)

As the SRT were increased from 5 to 10 days, the yields of ACT, FLN, PHE, ANT and CRB PAHs increased from 57%, 60%, 63%, 60% and 55% to 69%, 68%, 70%, 60% and 60%, respectively, at a RD concentration of 10 mg/L and a SRT of 10 days (Table 6.6). Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs also increased from 44%, 52%, 60%, 45% to 63%, 60%, 63%, 62%, respectively, with the addition of 10 mg/L RD to the CSTR at a SRT of 10 days. The removals were 69%, 69%, 67%, 65%, 66% and 65% for BbF, BkF, BaP, IcdP, DahA and BghiP, respectively, at a RD concentration of 10 mg/L and a SRT of 10 days. As the RD concentrations were increased from 10 to 15 mg/L at a SRT of 10 days (See Table 6.6). The yields of ACT, FLN, PHE, ANT and CRB PAHs increased from 69%, 68%, 70%, 60%, 60% to 87%, 86%, 85%, 83%, 85%, respectively. The removal efficiencies of FL, PY, BaA and CHR PAHs also increased from 44%, 52%, 60%, 45% to 80%, 79%, 76%, 82%, respectively. The removals of BbF, BkF, BaP, IcdP, DahA and BghiP were 82%, 80%, 77%, 85%, 84% and 84%, respectively. As the RD concentrations were increased from 15 to 25 mg/L at a SRT of 5 days, the yields of ACT, FLN, PHE, ANT and CRB decreased from 87%, 86%, 85%, 83%, 85% to 74%, 70%, 65%, 73%, 79%, respectively, at a RD dose of 25 mg/L and a SRT of 10 days. Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs also decreased from 80%, 79%, 76%, 82% to 73%, 72%, 70%, 72%, respectively. In addition, the BbF, BkF, BaP, IcdP, DahA and BghiP PAHs yields were 65%, 65%, 68%, 46%, 34% and 35%, respectively, at a RD concentration of 25 mg/L and at a SRT of 10 days in the CSTR system (Table 6.6).

The maximum PAH yields with three, four, five and six rings varied between 94%-97%, 93%-95%, 95%-96% and 95%-96%, respectively, at a RD concentration of 15 mg/L and a SRT of 25 days (Table 6.6). The yield of 3-rings PAHs removals were almost 96% and 96% for ACT and PHE PAHs, respectively. The administration of 15 mg /L RD increased the PAH removals from 59%, 60% and 47% up to 97%, 94% and 94%, respectively, for FLN, ANT and CRB PAHs (Table 6.6). Removals of 4-ring PAHs (FL, PY, BaA and CHR) were significantly higher in the presence of 15 mg/L RD (93%, 95%, 94% and 94%, respectively) in comparison to without RD conditions (71%, 60%, 37% and 65%). The PAHs removal

efficiencies increased from 74%, 45%, 67%, 73% and 57% up to 95%, 96%, 95%, 96% and 96% for BbF, BkF, BaP, IcdP and DahA PAHs.

When the SRT were increased from 25 to 40 days, the yields of ACT, FLN, PHE, ANT and CRB PAHs yields increased from 62%, 62%, 60%, 64% and 68% to 83%, 70%, 60%, 62% and 62%, respectively, at a RD 10 mg/L (Table 6.6). Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs yields also increased from 57%, 53%, 40%, 58% to 58%, 57%, 51%, 60%, respectively, when 10 mg/L RD was added to the CSTR at a SRT of 40 days. The yields were 60%, 32%, 60%, 74%, 78% and 74% for BbF, BkF, BaP, IcdP, DahA and BghiP PAHs, respectively, at a RD concentration of 10 mg/L and SRT of 40 days. As the RD concentrations were increased from 10 to 15 mg/L at a SRT of 25 days ACT, FLN, PHE, ANT and CRB PAHs removals increased from 83%, 70%, 60%, 62%, 62% to 93%, 78%, 67%, 69%, 68%, respectively (Table 6.6). The removal efficiencies of FL, PY, BaA and CHR PAHs also increased from 57%, 53%, 40%, 58% to 58%, 57%, 51%, 60%, respectively. The removals of BbF, BkF, BaP, IcdP, DahA and BghiP PAHs were obtained as 69%, 43%, 65%, 89%, 83% and 87%, respectively, at a RD concentration of 15 mg/L at a SRT of 40 days. As the RD concentrations were increased from 15 to 25 mg/L at SRT of 40 days, The yields of ACT, FLN, PHE, ANT and CRB PAHs decreased from 93%, 78%, 67%, 69%, 68% to 70%, 74%, 60%, 60%, 59%, respectively (Table 6.6). Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs also decreased from 60%, 59%, 61%, 60% to 55%, 50%, 52%, 53%, respectively, with the addition of 25 mg/L RD to the CSTR at a SRT of 25 days. The BbF, BkF, BaP, IcdP, DahA and BghiP PAHs removals were found as 65%, 59%, 60%, 60%, 62% and 64%, respectively, at a RD concentration of 25 mg/L and at a SRT of 40 days in the CSTR system (Table 6.6).

The results of this study showed that 15 mg/L RD increased the removal efficiencies of both lower (3, 4 ring PAHs) and higher (5 and 6 ring PAHs) molecular weight PAHs compounds. The maximum total PAH biodegradation removals (96%) were achieved at a SRT of 25 days and at a RD concentration of 15 mg/L. The yields of 3 and 4 ring PAHs removal was almost 96%, 97%, 96%, 94%,

95% and 94 % for ACT, FLN, PHE, CRB, PY and BaA, respectively. In addition, BbF, BkF, BaP, IcdP, DahA and BghiP PAHs yields were obtained as 95%, 96%, 95%, 96%, 96% and 95%, respectively, at a RD concentration of 15 mg/L and at a SRT of 25 days in the CSTR system.

6.3.5.2 Effects of Increasing SR Biosurfactant on the Individual PAH Removals in the CSTR System at Increasing SRTs

The yields of ACT, FLN, PHE, ANT and CRB PAHs increased from 56%, 59%, 60%, 53% and 47% to 57%, 56%, 55%, 58% and 58%, respectively, at a SR 10 mg/L and at a SRT of 5 days. Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs also increased from 34%, 44%, 54%, 34% to 51%, 52%, 59%, 51%, respectively. BbF, BkF, BaP, IcdP, DahA and BghiP PAHs removal efficiencies were found as 55%, 53%, 53%, 54%, 54% and 54%, respectively, at a SR concentration of 10 mg/L and a SRT of 5 days. When the SR concentrations were increased from 10 to 15 mg/L at SRT of 5 days, the yields of ACT, FLN, PHE, ANT and CRB PAHs increased from 57%, 56%, 55%, 58%, 58% to 62%, 63%, 63%, 62%, 64%, respectively. The removal efficiencies of FL, PY, BaA and CHR PAHs also increased from 51%, 52%, 59%, 51% to 59%, 60%, 65%, 66%, respectively, at a SR concentration 10 mg/L and a SRT of 5 days. The removals of BbF, BkF, BaP, IcdP, DahA and BghiP PAHs were 64%, 65%, 65%, 63%, 64% and 65%, respectively. When the SR concentrations were increased from 15 to 25 mg/L at SRT of 5 days, The yields of ACT, FLN, PHE, ANT and CRB PAHs decreased from 62%, 63%, 63%, 62%, 64% to 57%, 60%, 60%, 60%, 59%, respectively, at a SR 25 mg/L and at a SRT of 5 days. Similarly, the removal efficiencies of FL, PY, BaA and CHR also decreased from 59%, 60%, 65%, 66% to 50%, 53%, 51%, 57%, respectively. The BbF, BkF, BaP, IcdP, DahA and BghiP removals were 59%, 60%, 58%, 57%, 57% and 57%, respectively, at a SR concentration of 25 mg/L and at a SRT of 5 days in CSTR system (Table 6.7).

ACT, FLN, PHE, ANT and CRB PAHs yields increased from 57%, 60%, 63%, 60% and 55% to 69%, 68%, 70%, 64% and 62%, respectively, as the SRT were

increased from 5 to 10 days at a SR concentration of 10 mg/L and at a SRT of 10 days. Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs also increased from 44%, 52%, 60%, 45% to 58%, 59%, 63%, 59%, respectively. The removals were 69%, 69%, 67%, 65%, 66% and 65% for BbF, BkF, BaP, IcdP, DahA and BghiP PAHs, respectively. As the SR concentrations were increased from 10 to 15 mg/L at a SRT of 10 days the yields in ACT, FLN, PHE, ANT and CRB PAHs increased from 69%, 68%, 70%, 64%, 62% to 84%, 80%, 82%, 79%, 80%, respectively. The removal efficiencies of FL, PY, BaA and CHR PAHs also increased from 44%, 52%, 60%, 45% to 69%, 67%, 71%, 70%, respectively at a SR concentration of 15 mg/L. The removals of BbF, BkF, BaP, IcdP, DahA and BghiP PAHs were 82%, 80%, 77%, 85%, 84% and 84%, respectively, at a SR concentration of 15 mg/L at a SRT of 10 days. As the SR concentrations were increased from 15 to 25 mg/L, at a SRT of 5 days, the yields of ACT, FLN, PHE, ANT and CRB PAHs decreased from 84%, 80%, 82%, 79%, 80% to 74%, 70%, 65%, 60%, 48%, respectively. In addition, the removal efficiencies of FL, PY, BaA and CHR PAHs also decreased from 69%, 67%, 71%, 70% to 53%, 60%, 58%, 61%, respectively. The BbF, BkF, BaP, IcdP, DahA and BghiP PAHs removals were 65%, 65%, 68%, 46%, 34% and 35%, respectively, at a SR concentration of 25 mg/L and at a SRT of 10 days in CSTR system (Table 6.7).

ACT, FLN, PHE, ANT and CRB PAHs yields increased from 80%, 59%, 74%, 78% and 80% to 90%, 89%, 90%, 81% and 82%, respectively, as the SRT were increased from 10 to 25 days at a SR concentration of 10 mg/L. Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs also increased from 71%, 60%, 33%, 65% to 76%, 68%, 58%, 80%, respectively. The removals were 79%, 78%, 73%, 70%, 80% and 78% for BbF, BkF, BaP, IcdP, DahA and BghiP PAHs, respectively.

Table 6.7 Effect of increasing SR concentrations on individual PAH removals at increasing SRTs in CSTR system (n=3, mean values)

	PAH removal efficiencies at SRT 5 days				PAH removal efficiencies at SRT 10 days				PAH removal efficiencies at SRT 25 days				PAH removal efficiencies at SRT 40 days			
PAHs	C[a]	SR[b]10	SR[c]15	SR[d]25	C[a]	SR[b]10	SR[c]15	SR[d]25	C[a]	SR[b]10	SR[c]15	SR[d]25	C[a]	SR[b]10	SR[c]15	SR[d]25
ACT	56±1.50	57±2.09	62±0.64	57±0.99	57±1.67	69±1.91	84±2.32	74±0.94	80±2.11	90±1.66	82±1.89	76±1.62	62±1.37	83±2.03	93±2.68	70±2.11
FLN	59±1.25	56±0.61	63±1.91	60±1.67	60±1.56	68±2.35	80±2.22	70±1.67	59±1.01	89±2.16	83±2.16	63±0.68	62±0.87	70±1.67	78±2.90	74±1.95
PHE	60±0.85	55±1.01	63±0.57	60±0.67	63±1.03	70±0.88	82±1.64	65±2.68	74±0.80	90±0.94	80±1.34	65±0.94	60±0.59	60±0.69	67±1.51	70±0.94
ANT	53±2.31	58±1.34	62±0.61	60±0.82	60±0.88	64±2.34	79±0.38	60±1.06	78±0.54	81±2.16	87±1.49	68±1.08	64±1.92	71±2.09	74±1.38	60±2.45
CRB	47±1.90	58±1.28	64±0.38	59±1.04	55±0.67	62±2.02	80±1.64	48±0.69	80±1.68	82±0.54	86±1.52	58±1.36	68±1.64	70±2.35	74±1.57	58±1.97
FL	34±1.45	51±1.82	59±1.09	50±1.34	44±1.42	58±0.38	69±0.99	53±0.58	71±0.91	76±2.39	82±1.57	60±1.93	57±1.34	62±1.69	80±1.10	55±0.69
PY	44±1.35	52±0.43	60±0.24	53±1.64	52±1.64	59±0.11	67±1.87	60±1.11	60±1.68	68±1.99	79±1.58	59±2.20	53±2.81	61±1.38	79±0.69	50±0.66
BaA	54±1.61	59±0.59	65±1.52	51±0.56	60±0.74	63±0.98	71±1.38	58±1.61	33±1.97	58±2.16	79±0.68	60±1.54	40±0.21	59±1.45	69±1.92	53±0.38
CHR	34±0.84	51±0.76	66±1.43	57±0.37	45±0.64	59±1.64	70±0.33	61±1.37	65±0.53	80±0.91	81±2.16	64±0.66	58±0.37	68±1.60	73±1.85	56±0.49
BbF	44±0.95	55±1.34	64±1.38	59±1.69	45±0.55	69±0.64	82±0.84	65±1.06	74±1.35	79±0.64	83±2.04	53±1.85	50±0.94	60±1.62	69±0.48	65±1.67
BkF	50±1.02	53±1.67	65±1.83	60±1.97	52±0.81	69±0.37	80±1.34	65±0.37	45±1.29	78±2.04	84±1.38	48±1.96	50±1.26	32±1.37	43±1.95	59±1.86
BaP	51±1.47	53±1.83	65±1.00	58±0.67	53±1.07	67±1.84	77±0.64	68±1.88	67±1.67	73±1.34	83±1.06	54±2.54	47±1.04	60±0.79	65±0.49	60±1.80
IcdP	43±0.84	54±0.79	63±0.55	57±2.46	53±1.54	65±1.66	85±1.67	46±1.91	73±1.60	70±0.64	82±0.67	67±0.68	43±1.29	74±0.86	89±0.60	60±1.67
DahA	34±0.55	54±0.61	64±0.96	57±0.67	47±0.61	66±048	84±1.54	34±0.61	57±0.37	80±0.86	84±0.82	61±0.93	42±0.76	78±1.68	83±1.68	62±1.53
BghiP	38±0.98	54±1.04	65±0.67	57±1.12	43±0.39	65±1.55	84±0.59	35±0.83	76±0.79	78±1.01	81±0.83	43±1.54	40±0.96	74±2.02	87±1.94	64±0.99

a: Control without biosurfactant; b, c and d: SR 10, SR 15 and SR 25: Surfactin concentrations (mg/L)

The individual PAHs yields were at between 80%-87%, 79%-82%, 83%-84% and 81%-84% with three, four, five and six rings at SR concentration of 15 mg/L and a SRT of 25 days (Table 6.7). The yield of 3-rings PAHs removals were almost 82% and 87% for ACT and ANT PAHs, respectively. The administration of 15 mg/L SR increased the PAH removals from 59%, 74% and 78% up to 83%, 80% and 86%, respectively, for FLN, PHE and CRB PAHs (Table 6.7). Removals of 4-ring PAHs (FL, PY, BaA and CHR) were significantly higher in the presence of 15 mg/L SR (82%, 79%, 79% and 81%, respectively) in comparison to without SR conditions (71%, 60%, 33% and 65%). The PAHs removal efficiencies increased from 45%, 67% and 57% up to 83%, 83% and 84% for BbF, BaP and DahA PAHs, respectively.

As the SRT were increased from 25 to 40 days, the yields of ACT, FLN, PHE, ANT and CRB PAHs increased from 62%, 62%, 60%, 64% and 68% to 83%, 70%, 60%, 71% and 70%, respectively, at a 10 mg/L SR and at a SRT of 40 days, respectively. Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs also increased from 57%, 53%, 40%, 58% to 62%, 61%, 59%, 68%, respectively, at a 10 mg/L SR and at a SRT of 40 days. The yields were 60%, 32%, 60%, 74%, 78% and 74% for BbF, BkF, BaP, IcdP, DahA and BghiP, respectively. ACT, FLN, PHE, ANT and CRB PAHs removals increased from 83%, 70%, 60%, 71%, 70% to 93%, 78%, 67%, 74%, 74%, respectively, when the SR concentrations were increased from 10 to 15 mg/L. The removal efficiencies of FL, PY, BaA and CHR PAHs increased from 62%, 61%, 59%, 68% to 80%, 79%, 69%, 73%, respectively. The removals of BbF, BkF, BaP, IcdP, DahA and BghiP PAHs were 69%, 43%, 65%, 89%, 83% and 87%, respectively. As the SR concentrations were increased from 15 to 25 mg/L at a SRT of 40 days, the yields of ACT, FLN, PHE, ANT and CRB PAHs decreased from 93%, 78%, 67%, 74%, 74% to 70%, 74%, 70%, 60%, 58%, respectively, at a SR concentration of 25 mg/L and at a SRT of 40 days. Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs also decreased from 80%, 79%, 69%, 73% to 55%, 50%, 53%, 56%, respectively. BbF, BkF, BaP, IcdP, DahA and BghiP removals were 65%, 59%, 60%, 60%, 62% and 64%, respectively, at a SR concentration of 25 mg/L and at a SRT of 40 days in CSTR system (Table 6.7). A

significant correlation was not observed between PAH yields and physicochemical properties for SR biosurfactants (R^2= 0.56, F=13.98, P = 0.05).

The maximum total PAH biodegradation removals (85%) were achieved at a SRT of 25 days and at a SR concentration of 15 mg/L. The yields were 80-87%, 79-82%, 83-84%, 81-84% for PAHs with three, four, five and six rings, respectively. The maximum individual 3-4 ring PAHs yields were obtained as 82%, 87%, 86% and 81% for ACT, ANT, CRB and CHR respectively. Among all PAHs, the yields in 5 and 6 ring PAHs were determined as 84%, 83%, 82% and 84% for BkF, BaP, IcdP and DahA, respectively, at a SR concentration of 15 mg/L and at a SRT of 25 days in the CSTR system.

6.3.5.3 Effects of Increasing EM Biosurfactant on the Individual PAH Removals in the CSTR System at Increasing SRTs

The yields of ACT, FLN, PHE, ANT and CRB PAHs increased from 56%, 59%, 60%, 53% and 47% to 57%, 62%, 63%, 59% and 56%, respectively, at an EM 10 mg/L and at a SRT of 5 days. The removal efficiencies of FL, PY, BaA and CHR PAHs also increased from 34%, 44%, 54%, 34% to 50%, 52%, 62% and 55%, respectively, at a 10 mg/L EM and at a SRT of 5 days. In addition, BbF, BkF, BaP, IcdP, DahA and BghiP removals were 55%, 53%, 53%, 54%, 54% and 54%, respectively. As the EM concentrations were increased from 10 to 15 mg/L at a SRT of 5 days ACT, FLN, PHE, ANT and CRB PAHs increased from 57%, 62%, 63%, 59%, 56% to 73%, 74%, 76%, 66%, 67%, respectively. The removal efficiencies of FL, PY, BaA and CHR PAHs increased from 50%, 52%, 62%, 55% to 69%, 60%, 66%, 63%, respectively. The yields of BbF, BkF, BaP, IcdP, DahA and BghiP PAHs were obtained as 64%, 65%, 65%, 63%, 64% and 65%, respectively, at an EM concentration of 15 mg/L at a SRT of 5 days. As the EM concentrations were increased from 15 to 25 mg/L at a SRT of 5 days, The yields of ACT, FLN, PHE, ANT and CRB PAHs decreased from 73%, 74%, 76%, 66%, 67% to 57%, 60%, 60%, 60%, 59%, respectively, at an EM concentration of 25 mg/L and at a SRT of 5 days. Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs decreased

from 69%, 60%, 66%, 63% to 58%, 50%, 59%, 58%, respectively. The BbF, BkF, BaP, IcdP, DahA and BghiP removals were 59%, 60%, 58%, 57%, 57% and 57%, respectively, at an EM concentration of 25 mg/L and a SRT of 5 days (Table 6.8).

As the SRT were increased from 5 to 10 days, the yields of ACT, FLN, PHE, ANT and CRB PAHs increased from 57%, 60%, 63%, 60% and 55% to 69%, 68%, 70%, 64% and 62%, respectively, at a concentration of 10 mg/L EM, as the SRT were increased from 5 to 10 days. Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs also increased from 54%, 59%, 67%, 60% to 75%, 74%, 76%, 79%, respectively, at a 10 mg/L EM and at a SRT of 10 days. The removals were 69%, 69%, 67%, 65%, 66% and 65% for BbF, BkF, BaP, IcdP, DahA and BghiP PAHs, respectively, at an EM concentration of 10 mg/L and at a SRT of 10 days. As the EM concentrations were increased from 10 to 15 mg/L at a SRT of 10 days ACT, FLN, PHE, ANT and CRB PAHs increased from 69%, 68%, 70%, 60%, 60% to 84%, 80%, 82%, 70%, 77%, respectively. The removal efficiencies of FL, PY, BaA and CHR PAHs increased from 54%, 59%, 67%, 60% to 75%, 74%, 76%, 79%, respectively at EM concentration of 15 mg/L. The removals of BbF, BkF, BaP, IcdP, DahA and BghiP PAHs were 82%, 80%, 77%, 85%, 84% and 84%, respectively, at an EM concentration of 15 mg/L at a SRT of 10 days (Table 6.8).

As the EM concentrations were increased from 15 to 25 mg/L at SRT of 5 days, the yields of ACT, FLN, PHE, ANT and CRB PAHs decreased from 84%, 80%, 82%, 70%, 77% to 74%, 70%, 65%, 61%, 64%, respectively, at a EM 25 mg/L and at a SRT of 10 days. Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs decreased from 74%, 76%, 79%, 82% to 70%, 68%, 70%, 65%, respectively. The BbF, BkF, BaP, IcdP, DahA and BghiP PAHs removals were 65%, 65%, 68%, 46%, 34% and 35%, respectively, at an EM concentration of 25 mg/L and at a SRT of 10 days (Table 6.8).

Table 6.8 Effect of increasing EM concentrations on individual PAH removals at increasing SRTs in CSTR system (n=3, mean values)

	PAH removal efficiencies at SRT 5 days				PAH removal efficiencies at SRT 10 days				PAH removal efficiencies at SRT 25 days				PAH removal efficiencies at SRT 40 days			
PAHs	C[a]	EM[b] 10	EM[c] 15	EM[d] 25	C[a]	EM[b] 10	EM[c] 15	EM[d] 25	C[a]	EM[b] 10	EM[c] 15	EM[d] 25	C[a]	EM[b] 10	EM[c] 15	EM[d] 25
ACT	56±1.50	57±1.68	73±1.94	57±1.06	57±1.67	69±2.69	84±2.94	74±0.90	80±2.11	90±2.15	87±2.35	76±1.50	62±1.37	71±1.60	78±2.03	70±2.65
FLN	59±1.25	62±2.19	74±0.91	60±1.68	60±1.56	68±2.64	80±1.09	70±1.84	59±1.01	89±2.00	86±1.65	63±1.62	62±0.87	70±1.89	78±1.56	74±1.84
PHE	60±0.85	63±0.59	76±0.68	60±2.16	63±1.03	70±0.97	82±1.64	65±1.69	74±0.80	90±1.68	80±1.59	65±1.38	60±0.59	60±1.25	67±0.68	70±0.30
ANT	53±2.31	59±0.68	66±1.68	60±2.46	60±0.88	60±1.68	70±0.81	61±2.16	78±0.54	70±1.10	84±1.06	80±1.34	64±1.92	68±0.50	76±1.65	69±0.90
CRB	47±1.90	56±1.20	67±1.34	59±0.63	55±0.67	60±1.05	77±0.90	64±1.56	80±1.68	83±0.60	88±0.90	80±1.20	68±1.64	68±0.90	70±0.35	60±1.15
FL	34±1.45	50±1.64	69±1.52	58±0.95	44±1.42	54±1.55	75±1.18	60±1.46	71±0.91	76±1.19	81±0.80	73±0.90	57±1.34	60±2.10	68±1.84	58±1.67
PY	44±1.35	52±1.80	60±1.09	50±1.16	52±1.64	59±1.7	74±2.56	70±2.10	60±1.68	69±0.54	80±0.45	69±1.90	53±2.81	59±1.67	69±1.67	58±0.90
BaA	54±1.61	62±0.40	66±1.10	59±184	60±0.74	67±2.37	76±0.61	68±0.84	33±1.97	57±0.87	79±1.54	66±2.08	40±0.21	51±2.45	65±2.11	60±1.39
CHR	34±0.84	55±0.67	63±1.28	58±1.92	45±0.64	60±2.29	79±0.34	70±0.58	65±0.53	81±1.38	83±1.56	71±1.37	58±0.37	62±1.68	67±0.38	58±1.55
BbF	44±0.95	55±0.39	64±1.69	59±2.38	45±0.55	69±0.50	82±0.80	65±0.41	74±1.35	82±2.64	85±1.88	53±2.15	50±0.94	60±0.64	69±1.67	65±0.97
BkF	50±1.02	53±1.69	65±2.06	60±2.69	52±0.81	69±0.38	80±1.59	65±1.55	45±1.29	78±1.90	84±1.64	48±1.65	50±1.26	32±0.86	43±1.28	59±1.67
BaP	51±1.47	53±2.16	65±1.67	58±0.57	53±1.07	67±0.92	77±2.31	68±1.28	67±1.67	80±2.11	82±1.52	54±0.92	47±1.04	60±0.76	65±1.61	60±2.31
IcdP	43±0.84	54±0.67	63±0.39	57±0.49	53±1.54	65±1.48	79±1.11	46±1.91	73±1.60	89±0.64	91±1.09	67±0.63	43±1.29	74±1.19	89±0.49	60±0.50
DahA	34±0.55	54±1.70	64±0.49	57±0.58	47±0.61	66±1.97	84±0.37	34±0.67	57±0.37	80±0.90	92±0.60	61±0.59	42±0.76	78±1.38	83±1.45	62±1.60
BghiP	38±0.98	54±1.64	65±1.39	57±1.95	43±0.39	65±2.02	84±2.01	35±1.69	76±0.79	89±1.00	91±0.30	43±0.90	40±0.96	74±0.60	87±1.50	64±1.84

a: Control without biosurfactant; b, c and d: EM 10, EM 15 and EM 25: Emulsan concentrations (mg/L)

ACT, FLN, PHE, ANT and CRB PAHs yields increased from 80%, 59%, 74%, 78% and 80% to 87%, 86%, 80%, 84% and 88%, respectively, as the SRT were increased from 10 to 25 days at an EM concentration of 10 mg/L. Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs also increased from 71%, 60%, 33%, 65% to 81%, 80%, 79%, 83%, respectively. The removals were 85%, 84%, 82%, 91%, 92% and 91% for BbF, BkF, BaP, IcdP, DahA and BghiP PAHs, respectively.

The removals were at between 80%-88%, 79%-83%, 82%-85% and 91%-92% for individual PAHs with three, four, five and six rings at EM concentration of 15 mg/L and at a SRT of 25 days (Table 6.8). The removal efficiencies of 3-rings PAHs were almost 87% and 88% for ACT and CRB PAHs, respectively. The administration of 15 mg/L EM increased the PAH removals from 59%, 74% and 78% up to 86%, 80% and 84%, respectively, for FLN, PHE and ANT PAHs (Table 6.8). Removals of 4-ring PAHs (FL, PY, BaA and CHR) were significantly higher in the presence of 15 mg/L EM (81%, 80%, 79% and 83%, respectively) in comparison to control reactor (71%, 60%, 33% and 65%). The PAHs removal efficiencies increased from 74%, 45% and 67% up to 85%, 84% and 82% for BbF, BkF and BaP PAHs. The removal efficiencies of 6-rings PAHs were almost 91% and 92% for IcdP and BghiP PAHs, respectively (Table 6.8).

The yields of ACT, FLN, PHE, ANT and CRB PAHs increased from 62%, 62%, 60%, 64% and 71% to 70%, 60%, 68%, 68% and 60%, respectively, when the SRT were increased from 25 to 40 days at an EM 10 mg/L. Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs also increased from 57%, 53%, 40%, 58% to 60%, 59%, 51%, 62%, respectively, at a 10 mg/L EM and at a SRT of 40 days. The yields were 60%, 32%, 60%, 74%, 78% and 74% for BbF, BkF, BaP, IcdP, DahA and BghiP PAHs, respectively, at an EM concentration of 10 mg/L and at a SRT of 40 days. As the EM concentrations were increased from 10 to 15 mg/L at SRT of 25 days ACT, FLN, PHE, ANT and CRB PAHs increased from 71% to 70%, 60%, 68%, 68%, 60% to 78%, 78%, 67%, 76%, 70%, respectively. The removal efficiencies of FL, PY, BaA and CHR PAHs increased from 60%, 59%,

51%, 62% to 68%, 69%, 65%, 67%, respectively. The removals of BbF, BkF, BaP, IcdP, DahA and BghiP PAHs were obtained as 69%, 43%, 65%, 89%, 83% and 87%, respectively, at an EM concentration of 15 mg/L at a SRT of 40 days (Table 6.8).

The addition of EM concentrations up to 15 mg/L decreased ACT, FLN, PHE, ANT and CRB PAHs removal efficiencies at SRT of 40 days. These PAHs yields decreased from 78%, 78%, 67%, 76%, 70% to 70%, 74%, 70%, 69%, 60%, respectively, at an EM 25 mg/L and at a SRT of 40 days. Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs also decreased from 68%, 69%, 65%, 67% to 58%, 58%, 60%, 58%, respectively, at a 25 mg/L EM and SRT of 25 days. The BbF, BkF, BaP, IcdP, DahA and BghiP PAHs removals were 65%, 59%, 60%, 60%, 62% and 64%, respectively, at an EM concentration of 25 mg/L and at a SRT of 40 days (Table 6.8). A significant correlation was not observed between PAH yields and physicochemical properties for EM biosurfactants (R^2= 0.53, F=12.89, P = 0.05).

The maximum total PAH biodegradation removals (85%) were achieved at a SRT of 25 days and at an EM concentration of 15 mg/L. The yields of 3 and 4 ring PAHs removal was almost 87%, 86%, 88% and 83% for ACT, FLN, CRB, PY and CHR, respectively. In addition, the BbF, BkF, IcdP, and DahA PAHs (5-6 ring PAHs) yields were obtained 85%, 84%, 91% and 92%, respectively, at an EM concentration of 15 mg/L and at a SRT of 25 days in the CSTR system.

As seen in Table 6.8 the mean maximum removals varied between 95 and 96% for BbF, BkF, BaP, IcdP, DahA and BghiP PAHs with five and six benzene rings at 15 mg/L RD at a SRT of 25 days. The yields were between 96 and 97% (for ACT, PHE and FLN PAHs) and 93- 95% (for FL, PY and BaA PAHs) for the PAHs with three and four benzene rings.

The yields were at between 80-83%, 79-82%, 83-84% and 81-84% for PAHs with three, four, five and six rings, respectively at a SR concentration of 15 mg/L and a SRT of 25 days (Table 6.8). The removals were at between 80-88%, 79-83%, 82-

85% and 91-92% for PAHs with three, four, five and s ix rings, respectively, at EM concentration of 15 mg/L and a SRT o f 25 days (Table 6.8).

In this step of the study, similar to the previous findings (Tables 6.7 and 6.8), no significant difference between PAH yields with three, five and six rings observed in the CSTR although PAHs with more benzene rings became increasingly less soluble in water with increasing number of benzene rings and molecular weight and with decreasing Henry's law constants (R^2 = 0.89, F = 2.36 p = 0.05). In other words, although DahA and BghiP PAHs were the most hydrophobic types, a significant correlation was not observed between the removal percentages of these PAHs and their physicochemical properties (R^2 = 0.59, F = 13.04, p = 0.01) in contrast to the studies performed by Jeong et al. (2004) and Wei et al. (2005). They found that the PAHs with high benzene rings are removed at low yields compared to the PAHs with low benzene rings although they were studied in biosurfactant added aerobic reactors containing biosurfactants.

The high yields in PAHs with high benzene rings could be attributed to the low vapor pressure (for IcdP 1.25E-10 mmHg, for DahA 2.49E-10 mmHg, for IcdP 1.00E-10 mmHg) and low Henry's law constants (for IcdP 3.48E-07 atm m^3/mol, for DahA 1.23E-07 atm m^3/mol, for IcdP 3.31E-07 atm m^3/mol**)** (See Table 2.2 in Chapter two).

Furthermore, the resistance of aerobic sludge biomass to PAHs after acclimation periods, the type of PAH degrading bacteria, the type of biosurfactants, the optimum biosurfactant dose used for maximum PAH removals, the sludge age and the main removal mechanisms of PAHs can significantly affect the yields of PAHs with high benzene rings.

The existence of surfactants could increase the solubility of PAHs with three, five and six benzene rings and they were probably used with PAHs as the carbon and energy source (Barker et al., 2006).

Yin et al. (2009) showed that the addition of 200 mg/L, RD increased the PHE, ANT PAH removals in an oil industry wastewater (Yin et al., 2009). Zhu and Zhang (2008) reported that a RD concentration of 50 mg/L increased the PHE and CHR PAH yields (Zhu and Zhang, 2008). This optimum RD concentration is significantly higher than that the RD dose used in this study. Yin et al. (2009) found that the biodegradation of PAHs could be promoted by reducing the interfacial tension of wastewater (Yin et al., 2009). The studies performed by Lei et al. showed that little or no PAH degradation was observed at high biosurfactant concentrations (Lei et al., 2005). The negative observations regarding the biosurfactants can be explained by one or more of the following effects: (a) toxicity of surfactants due to surfactant-induced permeabilization or lysis of the bacterial cell membrane, (b) toxicity of biosurfactant enhanced aqueous PAH concentrations, (c) prevention of bacterial adhesion to the hydrophobic PAHs. Decreases in bacteria concentrations a high biosurfactant concentration could be attributed to the reduced bioavailability of the biosurfactant as a barrier to hinder direct contact of bacterial cells to micellar-phase PAHs (Zhu and Zhang, 2008).

6.3.5.4 Effect of RD Biosurfactant on the Aerobic Biodegradation of Individual PAHs under Constant SRT and HRT

Although the aerobic CSTR system was efficient for all ring PAH removal, the addition of 15 mg/L RD increased the yields of the all PAHs removals in the petrochemical industry wastewater as mentioned in previous section among the surfactants used, it was found that 15 mg/L RD is the optimum dose for the maximum total PAH and COD removals from the raw petrochemical industry wastewater in an aerobic CSTR reactor at a SRT of 25 days. Therefore, the individual PAH yields were investigated at a 15 mg/L RD at a SRT of 25 days and at a HRT of 5 days.

The yield of 3-ring PAH removal was almost 78% and 80% for ANT and ACT PAHs, respectively. The administration of 15 mg/L RD increased the PAH removals from 72%, 78% and 80% up to 80%, 92% and 99%, respectively, for CRB, ANT and

ACT PAHs (Table 6.9). Removals of 4-ring PAHs (FL, PY and CHR) were significantly higher in the presence of 15 mg/L RD (71%, 60% and 79%, respectively) in comparison to RD-free conditions (69%, 60% and 65%). Treatment with RD (15 mg/L) caused a significant increase of 5 and 6-ring PAHs degradation. The PAHs removal efficiencies increased from 45%, 67% and 57% up to 54%, 73% and 86% for BkF, BaP and DahA PAHs. However, no significant effect was detected in the case of some 3-ring PAHs (FLN, PHE) and one 5-ring PAH (BbP) removals (Table 6.9). In other words, PAHs treatment with RD slightly increases the FLN, PHE and BbP PAHs removals. In this study it was found that PAH treatment with RD was beneficial for the degradation of all ring PAHs with a total PAHs removal yield of 87% in comparison to 74% in the RD-free case. The efficiency for the removal of PAHs is not in relation to the number of aromatic rings. Aerobic degradation in the CSTR process was very efficient for all ring PAHs removal. The results of this study show that 15 mg/L RD increased the removal efficiency of both lower (3 and 4 ring PAHs) and higher (5 and 6 ring PAHs) molecular weight PAHs compounds. These results could be attributed to the combining effects of activated sludge which is resistant to PAHs, to the type of biosurfactant and the dose. The results found in this study are in contrast to the findings of Manoli and Samara (2008).

They found that 3-4 benzene rings PAHs were biodegraded more rapidly than 5-6 benzene rings PAHs. PAH removals did not decrease from high ring PAHs to low-ring PAHs. We found the aerobic biodegradation of PAHs to be independent of the carbon chain length in contrast to the findings of Pathak et al., (2009) who reported that lower carbon number PAHs are degraded more rapidly. Among all PAHs, only for 2, 3 and 4 ring PAHs were high removals (56% - 67%) obtained. Whang et al., (2008) found that PAHs of > 3 benzene rings remained almost unchanged in the aerobic tank.The PAH removals found in this study are higher than those reported in the literature. Sartoros et al., (2005) found that the addition of biosurfactant at 25 ^{o}C increased the overall mineralization of anthracene and pyrene to 50% and 44% from 33.0% and 27.6%, respectively.

Table 6.9 PAH removal efficiencies in the effluent of an aerobic CSTR reactor at a SRT of 25 and HRT of 5 days with and without RD (n=3, mean±SD)

PAHs	Number of rings	Influent PAH conc. (ng/mL)	Effluent PAH conc. (ng/mL) (without RD)	PAH removal efficiency (%)	Effluent PAH conc. (ng/mL) (with 15 mg/L RD)	PAH removal efficiency (%)
ACT	3	29.43±0.09	5.91±0.02	80±0.20	0.38±0.09	99±0.20
FLN	3	9.38±0.06	3.87±0.06	59±2.40	1.76±0.03	81±0.30
PHE	3	15.01±0.02	3.85±0.01	74±3.06	3.85±0.09	74±0.56
ANT	3	3.61±0.04	0.77±0.03	78±2.06	0.29±0.04	92±0.78
CRB	3	0.90±0.06	0.25±0.04	72±1.90	0.17±0.09	80±0.30
FL	4	2.98±0.07	0.91±0.06	69±2.78	0.87±0.03	71±0.03
PY	4	2.19±0.05	0.89±0.03	60±4.01	0.87±0.06	60±0.10
BaA	4	0.36±0.03	0.24±0.01	33±1.23	0.22±0.09	37±0.34
CHR	4	0.72±0.02	0.25±0.01	65±2.67	0.15±0.06	79±0.56
BbF	5	0.08±0.08	0.02±0.03	71±1.98	0.02±0.08	74±0.04
BkF	5	0.09±0.04	0.05±0.01	45±1.78	0.04±0.07	54±0.02
BaP	5	0.07±0.06	0.02±0.01	67±2.89	0.01±0.05	73±0.03
IcdP	6	0.12±0.02	0.03±0.06	73±4.78	0.01±0.05	87±0.56
DahA	6	0.27±0.07	0.11±0.05	57±6.05	0.03±0.03	86±1.01
BghiP	6	0.09±0.03	0.02±0.02	76±4.06	0.01±0.09	84±0.67
Total PAHs		65.32±0.05	17.25±0.11	74±2.56	8.76±0.07	87±2.34

The addition of Tween 60 caused an acceleration of the FLN degradation process from 34% to 54% Whang et al., (2008). Das et al. (2008) showed that a marine biosurfactant produced from the *Bacillus circulans* could increase the bioavailability and consequent degradation of anthracene from 45% to 69%. In a study performed by Kolomytseva et al. (2009) the biodegradation of FLN was increased to 67% by a biosurfactant produced from *Rhodococcus rhodochrous.* The older studies showed that the biosurfactants enhance the solubility of PAHs and motilization of PAHs in a CSTR reactor (Rosenberg et al., 1999). They showed that the addition of tween-80 increased the FLN solubility and the biodegradation rate 5-20 folds, respectively. Biosurfactants are preferred over synthetic surfactants because they are more cost-effective, less toxic and easily biodegradable. Applications of biosurfactants increase the solubility of PAHs, and thus, facilitate their biodegradation. Surfactants are shown to increase the surface area of the PAHs. In the study performed by Whang et al., (2008) it was found that the diesel solubility increased with surfactin addition up to 40 mg/L while the diesel biodegradation percentage increased up to 94%.

In this study, the RD increased significantly the PAHs removal efficiencies. Since the effect of surfactants on PAHs biodegradation depends on a number of factors, including the type of surfactant and the applied level, the specificity of PAHs, and the identity of the microorganisms present in this study, we conclude that the 15 mg/L RD was effective in the petrochemical industry bacteria treating PAHs.

RD, dosed as above, improved the rates of bioconversion and, probably, in the initial buildup of PAH intermediates decreased the toxicity of PAHs thus facilitating their consumption by the microorganism, as reported by Das et al. (2008).

Although petroleum hydrocarbon biodegradation is usually a slow process due to the hydrophobic nature of the contaminating PAHs, in this study high removal of PAHs was obtained. The yields of PAHs degradation were between 37% and 99%; between 74% and 99%, 37% and 79%, 54% and %74, 84% and 87% for three (ACT, FLN, PHE, ANT, CRB), four (FL, PY, BaA, CHR), five (BkF, BaP) and six ring (DahA, BghiP) PAHs, respectively at SRT of 25 days with 15 mg/L RD.

6.3.5.5 Variation of PAH Degrading Bacteria versus Increasing Biosurfactant Concentrations

The critical micelle concentration (CMC) is the solution concentration at which biosurfactants molecules begin to self-associate to form stable aggregates known as micelles (Anand et al., 2010). At the CMC, solutions containing biosurfactants exhibit drastic changes in physical and chemical properties such as surface tension, electrical conductivity, and detergent activity (Akhter et al, 1997; Tan et al., 2010).

The studies performed by Whang et al. (2008), Mazaheri-Assadi and Tabatabaee (2010) and Swaranjit and Randhir (2010) showed that the critical micelle concentrations (CMC) of SR, RD and EM biosurfactants were 45 mg/L, 50 mg/L and 10-30 mg/L, respectively, to treat the PAHs and diesel contaminated waters. In this study, in order to improve the PAH biodegradation yields, the influence of biosurfactants on the total PAH biodegradation yields were studied using three different biosurfactants, namely RD, EM and SR at a SRT of 25 days in the CSTR system. Based on the CMCs of biosurfactants given above the effects of each surfactant were evaluated at doses CMC, slightly above the CMC, and below the CMC on the viability of PAH degrading bacteria and PAH yields. The results showed that the PAH degrading bacteria numbers and the PAH yields decreased significantly in the CMC values and in the values above and below CMCs given in the literature (See Table 6.10). The variations of total PAH yields and PAH degrading bacteria numbers versus biosurfactant concentrations (CMC dose, below CMC dose and higher CMC dose) was given in Table 6.11 for SRT of 25 days.

Table 6.10 Critical micelle concentration (CMC) values in literature

CMC value for RD (mg/L)	**References**
50	Whang et al. (2008)
1-200	Mulligan (2009)
120	Pornsunthorntawee et al.(2008)
1-200	Van Hamme et al.(2006)
50	Yin at al. (2009)
50-300	Sotirova at al. (2009)
110-150	Lang (2002)
28,80	Zhang et al.(2009)
CMC value for SR (mg/L)	**References**
45	Whang et al. (2008)
40	Mulligan (2009)
25	Pornsunthorntawee et al. (2008)
<25	Maier (2003)
CMC value for EM (mg/L)	**References**
100-500	Kokare et al. (2007)
critical micelle dilution (CMD) 1/10-1/100 (w/v)	Chamanrokh et al.(2010)
163	Zhao et al. (2011)

As shown in Table 6.11 the growth of PAH degrading bacteria decreased as the RD, EM and SR bisurfactant concentrations were increased from 25 mg/L to 45 mg/L and 55 mg/L, from 15 mg/L to 50 mg/L and 65 mg/L and from 10 mg/L to 25 mg/L and 40 mg/L, respectively (Table 6.11). The PAH degrading bacterial growth decreased from $28x10^4$ colony/100 mL to $22x10^2$ colony/100 mL as a the SR concentration was increased from 25 to 55 mg/L (Table 6.11). The PAH degrading bacterial growth decreased from $30x10^4$ colony/100 mL to $21x10^2$ colony/100 mL and as a the RD concentration was increased from 35 to 65 mg/L. The PAH degrading bacterial growth decreased from $19x10^3$ colony/ 100 mL to $6x10^2$ colony/100 mL as a the EM concentration was increased from 25 to 55 mg/L (Table 6.11).

Table 6.11 Variations of total PAH yields and PAH degrading bacteria numbers versus biosurfactant concentrations (SRT= 25 days, CMC, below CMC and higher CMC)

	WB-C	SR			RD			EM		
		25	45	55	15	50	65	10	25	40
BN	$39x10^{6}\pm 8x10^{5}$	$28x10^{4}\pm 6x10^{3}$	$26x10^{3}\pm 4x10^{2}$	$22x10^{2}\pm 3x10^{1}$	$30x10^{4}\pm 3x10^{3}$	$27x10^{3}\pm 3x10^{2}$	$21x10^{2}\pm 4x10^{1}$	$19x10^{3}\pm 2x10^{2}$	$18x10^{3}\pm 3x10^{2}$	$6x10^{2}\pm 6x10^{1}$
TPY	65.38±0.60	60±0.84	57±1.01	50±0.85	92±1.11	55±0.97	44±0.58	60±0.94	58±1.10	44±0.87

WB-C: Without biosurfactant, control; BN: PAH degrading bacteria number (colony/100 mL); TPY: Total PAH yield (%); SR: Surfactin concentrations (mg/L); RD: RD concentrations (mg/L); EM: Emulsan concentrations (mg/L); CMC: Cell micelle concentration of biosurfactants (mg/L)

The growth of PAH degrading bacteria was significantly delayed or inhibited by the biosurfactants at high levels, such as SR (>25 mg/L), RD (15>mg/L), EM (10>mg/L). As the SR, RD and EM biosurfactant concentrations were increased from 25 to 55 mg/L, from 15 to 65 mg/L and from 10 to 40 mg/L, respectively, the total PAH degradation yields decreased at all biosurfactants (Table 6.11). The highest PAH biodegradation yields (92%) and bacteria numbers ($30x10^4$) were achieved at a RD concentration of 15 mg/L (Table 6.11). The PAH yields remained lower for the 55 mg/L SR and 40 mg/L EM administered samples (50% and 44%, respectively) at a SRT of 25 days.

A process that occurs below the CMC, when biosurfactant monomers increase the contact angle between the water and hydrophobic contaminant, promotes the separation of contaminant from water and finally displaces the PAH from the water (Mazaheri-Assadi and Tabatabaee, 2010).

High RD, EM and SR concentration caused inhibition to the aerobic microorganism. A plausible explanation of these inhibitions is that free energy of adhesion was expected to increase by surfactants, which diminished bacterial adhesion to PAHs surfaces (Whang et al., 2008). Adsorption of biosurfactants onto nonpolar, hydrophobic surfaces is primarily by dispersion force interaction and results in aggregation of surfactant molecules in aqueous solution (Whang et al., 2008). In this study it was found that the concentrations of surfactants even were below the CMCs, the surfactant molecules reached saturation, and micelle-accommodated PAH began to form at this point. Hence, surfactants could entirely prevent the cells from contacting the surface of PAH and the PAH micelle; thus, degradation did not occur any more (Mazaheri-Assadi and Tabatabaee, 2010). The solubilization process occurs above the CMC, when the PAHs are partitioned from the water into the hydrophobic core of surfactant micelles (Swaranjit and Randhir, 2010).

Solubilization depends on the type and dose of the surfactant, the hydrophobicity, the surfactant-water interactions, and the time that the contaminant has been in

contact with the water. A variety of factors and mechanisms have been proposed to explain the inhibition process, including cellular toxicity from interaction of surfactant molecules with cell membranes or cell membrane bound proteins, inhibition of enzymes of the catabolic pathway either by association with the enzyme or with the substrate, decreased bioavailability due to sequestration of the substrate compound into surfactant micelles, or the accumulation of toxic intermediates due to incomplete metabolism incurred from substrate-surfactant interactions. Stelmack et al. (1999) also observed similar inhibitions on ANT degradation by two bacterial strains, *Mycobacterium s*p. and *Pseudomonas sp*., by Triton X-100 and Dowfax 8390. In the present study, the growth of both bacteria was slightly increased with low biosurfactant concentration (10 mg/L). It was observed that *Microbacterium sp*. did not grow when the RD level exceeded 40 mg/L (Al-Halbouni et al., 2008).

6.3.5.6 Variation of Floc (Zoogloea ramigera) and Pseudomonas aeruginosa Bacteria versus Increasing Biosurfactant Concentrations

In this step of the study the influence of three biosurfactants (RD, EM and SR) on the growth and viability of the cells of floc bacteria (*Zoogloea ramigera*) and *Pseudomonas aeruginosa* were investigated at a constant SRT of 25 days, since he optimum sludge age for maximum total PAH and COD_{dis} removals was found to be 25 days (See section 6.3.1). These two organisms were selected since the predominant bacterial population in CSTR was comprised of 93% Gram-negative bacteria principally *Pseudomonas aeruginosa* (53%), *Zoogloea ramigera* (floc bacteria, 30%), *Flavobacterium* (7%) and *Comamonas* (3%) in the samples without biosurfactant (Table 6.12). According to the biochemical tests results it was found that the Voges Proskaver, the Indol, the Oxidase and Methyl Red tests were negative while the catalase test was positive. Gram staining showed that the isolated bacteria were Gram negative and rod shaped *Pseudomonas* bacteria. Among the biochemical tests applied to the colonies in M-ZR agar the flagella, pigment formations and hydrolysis of gelatin tests were positive while the acid production from glucose and hydrolysis of starch tests were negative.

Table 6.12 Bacterial population of sludge in the CSTR without biosurfactant

	Bacteria number (coloni/100 mL)	Percent ratio in total bacteria population (%)
Pseudomonas aeruginosa	$6x10^4 \pm 1x10^3$	53
Zoogloea ramigera (floc bacteria)	$3x10^4 \pm 1x10^2$	30
Flavobacterium	$8x10^3 \pm 2x10^1$	7
Comamonas	$3x10^3 \pm 1x10^1$	3

Gram staining result showed that the Gram-negative, rod-shaped bacterium that formed characteristic cell aggregates surrounded by gelatinous matrices was *Zoogloea ramigera*. Table 6.13 shows the effects of different concentrations of RD, EM and SR on the growth of *Zoogloea ramigera* and *Pseudomonas aeruginosa* bacteria in the CSTR system.

The presence of RD supported and stimulated the cell growth with respect to the control whereas EM and SR biosurfactants did not encourage the growth of floc bacteria and *Pseudomonas aeruginosa* significantly. The viability of bacteria cells decreased in the control CSTR system, which contained only raw petrochemical wastewater without biosurfactant. The addition of 10 mg/L RD to the CSTR system resulted in an enhancement of cell growth compared to the control (Table 6.13). However, in the presence of 15 mg/L RD the cell growth reached the highest values. Comparison of the cell growth and viability with different concentrations of RD also revealed that such surfactant is utilized by the floc and *Pseudomonas aeuruginosa* bacteria probably as an additional carbon source in the CSTR system (Al-Halbouni et al., 2008).

Table 6.13 Effects of biosurfactants on the bacterial cell number in CSTR system treating PAHs (n=3, mean values)

Bacteria number (colony/100 mL)	Control	Rhamnolipid concentration (mg/L)			Emulsan concentration (mg/L)			Surfactin concentration (mg/L)		
		10	15	25	10	25	75	10	40	50
Zoogloea ramigera	$2x10^{2}\pm 1x10^{1}$	$4x10^{3}\pm 2x10^{2}$	$8x10^{7}\pm 1x10^{6}$	$9x10^{5}\pm 2x10^{4}$	$3x10^{2}\pm 2x10^{1}$	$2x10^{5}\pm 3x10^{4}$	$2x10^{4}\pm 1x10^{3}$	$4x10^{2}\pm 2x10^{1}$	$5x10^{4}\pm 1x10^{3}$	$6x10^{2}\pm 1x10^{1}$
Pseudomonas aeruginosa	$6x10^{2}\pm 1x10^{1}$	$4x10^{3}\pm 1x10^{2}$	$6x10^{8}\pm 2x10^{6}$	$9x10^{4}\pm 3x10^{2}$	$3x10^{2}\pm 1x10^{1}$	$2x10^{5}\pm 2x10^{4}$	$2x10^{4}\pm 1x10^{3}$	$2x10^{2}\pm 2x10^{1}$	$5x10^{4}\pm 2x10^{3}$	$6x10^{3}\pm 1x10^{1}$

As shown in Table 6.13, the growth of floc (*Zoogloea ramigera*) and *Pseudomonas aeruginosa* bacteria increased, as the RD, EM and SR bisurfactant concentrations were increased from 10 to 10, 25 and 40 mg/L, respectively. These levels of biosurfactants were beneficial to both the floc and *Pseudomonas aeruginosa* bacteria growth. The maximum bacterial growth was between 10^7 and 10^8 colony in 100 mL wastewater sample at 15 mg/L RD concentration. The numbers of bacteria were 10^5 and 10^4 colony in 100 mL wastewater samples for 25 and 40 mg/L EM and SR biosurfactants, respectively. However, the growth of *Zoogloea ramigera* and *Pseudomonas aeruginosa* bacteria were significantly delayed or inhibited by the added surfactants at high levels, such as RD (>15 mg/L), EM (>25 mg/L) and SR (>40 mg/L).When the concentrations of RD,EM and SR surfactants were <15 mg/L, <25 mg/L and <40 mg/L, respectively, no obvious effects on the growth of bacteria were noticed, indicating that these biosurfactants did not affect the uptake of the hydrophobic PAHs by the floc and *Pseudomonas bacteria*.

6.3.5.7 Effect of SRT on the Biomass Production through Hydrophobic PAH Degradation in the Presence of Biosurfactant RD

When the CSTR system SRTs (5, 10, 25 and 40 days) has been selected, the biomass (MLVSS) concentration was determined. The total biomass in the system is a function of the reactor volume and the biomass concentration in the reactor. In order to detect the effects of increasing RD concentrations on biomass in CSTR systems the MLVSS concentrations were measured at different sludge ages and RD concentrations. As the RD concentrations were increased from 10 to 15 mg/L, the MLVSS concentrations increased from 1850, 1950, 2000 and 2150 mg/L to 2120, 2400, 2890 and 3450 mg/L at SRTs 5, 10, 25 and 40 days, respectively (Figure 6.6).

The highest MLVSS concentration was obtained at RD and SRT concentrations of 15 mg/L and 25 days. For RD concentrations >15 mg/L the MLVSS concentrations decreased. The lowest MLVSS concentrations were obtained as 1500, 1900, 2000 and 2100 mg/L at 150 mg/L RD concentration for SRTs 5, 10, 25 and 40 days, respectively. The MLVSS concentration decreased at high RD concentrations since

high RD concentrations probably negatively affect the biomass treating the PAHs in petrochemical industry wastewaters.

Although some studies showed that the longer the SRT, the higher the biomass concentration in the aerobic reactors treating hydrocarbons at low toxicity level in this study the highest MLVSS was found at a SRT of 25 days. In this study, the MLVSS in the CSTR system with an SRT of 5 days was around 2050 mg/L, whereas it was around 3400 mg/L in the CSTR system with an SRT of 25 days at a RD concentration of 15 mg/L. Because of the difference in MLVSS, microorganisms in the CSTR system containing 15 mg/L RD at SRT of 25 days had a chance to take up about 2.2 times more hydrophobic PAH substrates than those in the CSTR containing 150 mg/L RD at a SRT 5 days. This might have led to the higher PAH and COD capability of activated sludge at a SRT 25 days. The MLVSS concentrations in SRT of 40 days decreased to 2850 mg/L at a RD concentration of 15 mg/L while this parameter was measured as 2400 mg/L at a RD concentration of 50 mg/L. The activated sludge process with longer SRT normally contains a higher amount of inert biomass and this might contribute to the lower MLVSS content as reported by Liaoa et al. (2006). In addition, high RD concentrations such as 50 mg/L and 150 mg/L exhibited toxicity to the biomass, through long contact times of bacteria with hydrophobic PAHs at long SRTs.

The acute toxicity test results performed with 15 mg/L RD showed that this RD dose was not toxic to the *Vibrio fisheri* and *Daphnia magna* test organisms (See section 6.6). Furthermore to number of P*seudomonas aeruginosa* and PAH degrading bacteria (*Pseudomonas putida*) and *Zoogloea ramigera* numbers increased at a RD concentration of 15 mg/L (See section 6.3.5.6)

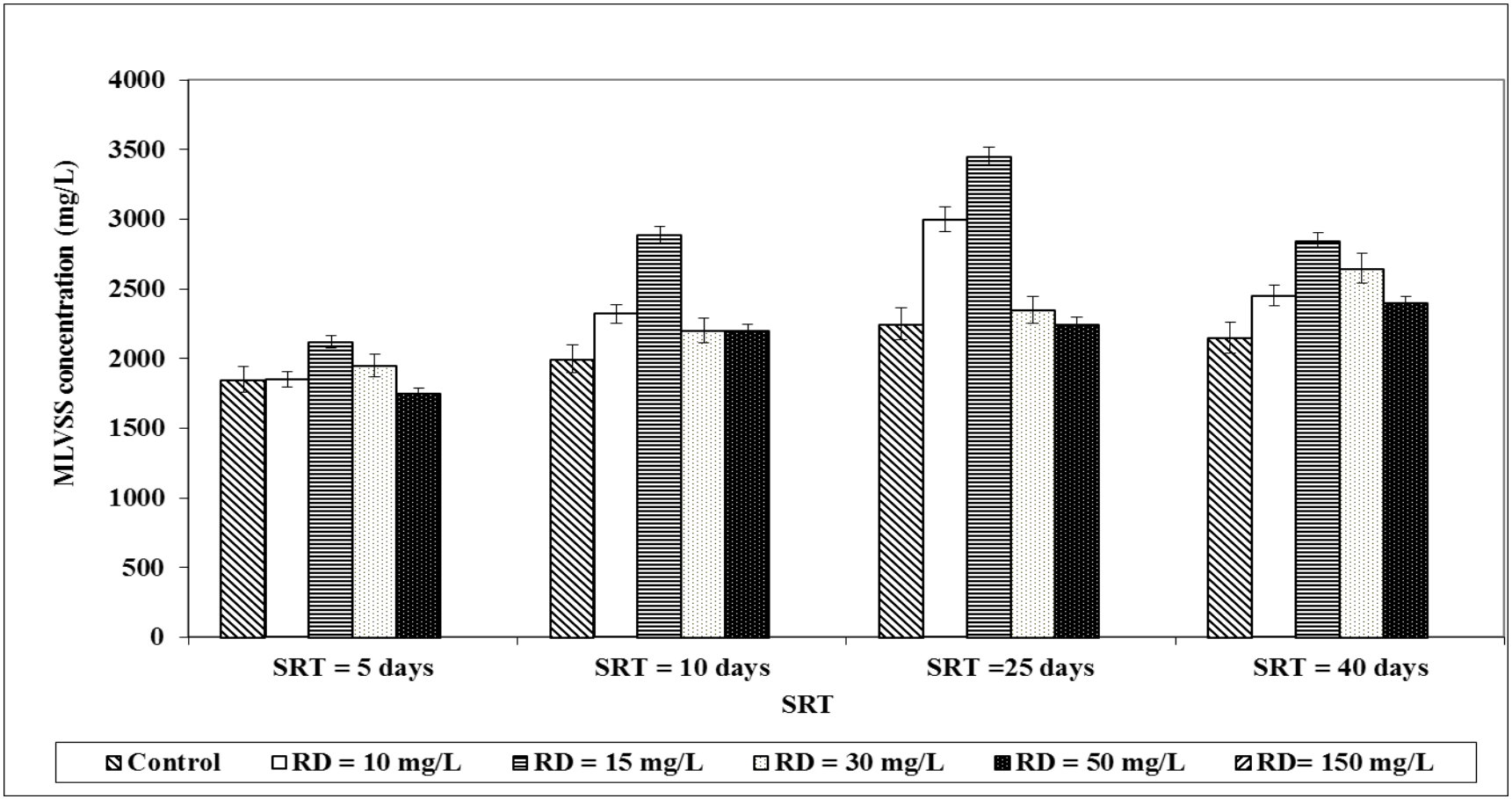

Figure 6.6 Effects of SRTs and RD concentrations on the MLVSS variations in CSTR system treating petrochemical wastewater

Similarly, Yuan et al., (2009) observed that faster growing microorganisms were able to uptake less RD at optimum sludge ages treating refractory organics at low SRTs. An optimum RD dose and SRT are required for maximum PAH biodegradations. In addition Ramdani et al. (2010) showed that as the RD concentration was increased, the PAH uptake and storage capability of mixed cultures decreased in aerobic batch reactors. Furthermore, it was indicated that a maximum RD storage ultimate biodegradation of PAHs might occur at optimum SRTs and RD concentrations in activated sludge reactors. In this study the present case conformed to the literature evidence, since it is obvious that SRT, as well as RD, could greatly influence the PAH biodegradation capability. The MLVSS concentrations increased from 1850 mg/L to 2250 mg/L as the SRT increased from 5 to 25 days in the CSTR system in the absence of RD (Table 6.14). For SRTs >25 days the MLVSS concentrations in the activated sludge system decreased to 2050 mg/L (Figure 6.6). Table 6.14 shows the MLVSS and VSS concentrations in the CSTR at different SRTs.

Table 6.14 The MLVSS and VSS concentrations in the CSTR at different SRTs

	SRT 5 days	SRT 10 days	SRT 25 days	SRT 40 days
MLVSS	1850±24	2000±26	2250±23	2050±15
VSS	1090±18	1260±22	1575±14	1230±12
VSS/ MLVSS	0.59±0.09	0.63±0.10	0.70±0.14	0.60±0.16

At a SRT of 5 days, the active bacteria (MLVSS) constitute about 59% of the VSS; the remaining biomass is not active while the MLVSS constitute about 70% of the VSS at a SRT of 25 days (Table 6.14). Apart from the reason that short SRT sludge possessed higher bacterial activity high SRTs are required for the destruction of refractory hydrophobic PAHs. Heterotrophic bacteria in activated sludge are characterized by relatively large maximum specific growth rates, resulting in low values of SRT (Yuan et al. 2009). The range of SRTs necessary for efficient removal of soluble organic matter from municipal wastewater is generally between 0.5-1.5 days (Al-Halbouni et al. 2008). In practice, a safety factor is employed to guard

against upsets in treatment performance and account for uncertainty in the kinetic parameters and influent characteristics, as well as natural variability in the microbial community.

In general, a higher SRT results in a greater amount of biomass in the system and therefore a higher MLVSS concentration. However at long biomass retention times the MLVSS concentration decreased since the aged biomass could not survive thus resulting in cell lysis. Several studies have demonstrated that the MLVSS concentration decreased at long SRTs when the CSTR system is treating refractory organics such as petrochemical wastewaters. From our results, it was found that shorter SRTs may cause low biomass capacity than that selected under longer SRTs. The SRT might have affected the biomass growth in the aerobic activated sludge system via the difference in refractory organic compounds.

The results of this study showed that the MLVSS concentrations in the CSTR system treating hydrophobic PAHs with 5 and 6 benzene rings in samples containing 15 mg/L RD is significantly higher than in those containing high concentrations of RD and no RD (Figure 6.6). RD biosurfactant can solubilize certain amounts of hydrophobic PAHs in their interior and allow a fast PAH diffusion from the aqueous media to the bacteria in CSTR system as reported by Zheng et al. (2007).

6.3.5.8 Influences of SRT on SVI and Floc Size in the Presence and Absence of RD

The long-term effect of SRT on sludge settling and floc size distribution are reflected by the median floc sizes and SVI at different STRs and RD concentrations in Figures 6.7 and 6.8. The results of the study showed that better sludge settling was found at SRTs of 10 and 25 days in CSTR systems containing 15 mg/L RD. The SVIs were 39.2 and 40.3 mL/g MLVSS for the aforementioned sludge ages, respectively (Figure 6.7). The average floc size varied between 44 and 52 μm in the SRTs in question, respectively (Figure 6.8).

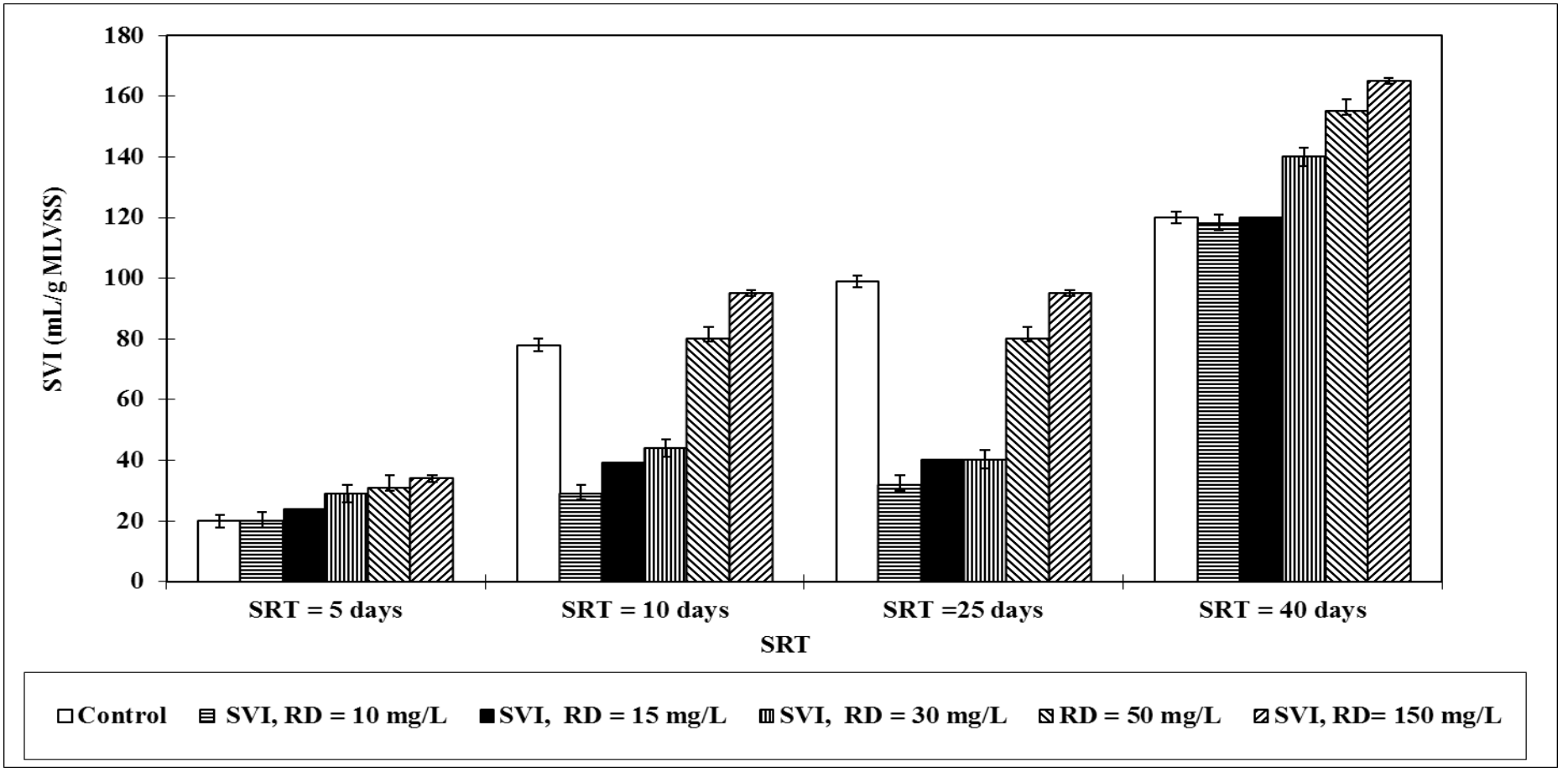

Figure 6.7 Effect of SRT and RD concentrations on SVI in the CSTR system treating PAH

The SVIs were 78 and 99 mL/g MLVSS while the floc diameters increased to 67 μm and 87 μm in the control CSTR system in the absence of RD at SRTs 10 and 25 days, respectively. The results showed that the SVI is high and the floc size is bigger in the CSTR system in the absence of RD at SRTs of 10 and 25 days (Figures 6.7 and 6.8). High SVIs contributed to the growth of flamentous bacteria while big flocs hindered the contact between oxygen, bacteria and PAH substrat resulting in death regions inside flocs. Very thin flocs, so called “point flocs” were obtained at low SRTs. Short SRTs like 5 days had a negative effect on SVI (20 mL/g MLVSS) in control CSTR systems compared to the CSTR containing 15 mg/L RD.

The CSTR system containing 15 mg/L RD had bigger floc (92 μm) and higher SVI (120 mL/g MLVSS) values than that of the SRT of 40 days. SVI had values 24 mL/g MLVSS at 5 day SRTs (average floc size was 16 μm) in the CSTR system containing 15 mg/L RD (Figures 6.7 and 6.8). However, SVI for sludge obtained at 25 days SRT without RD was 99 mL/g MLVSS, which is considerably higher, compared to all the other CSTR systems. In general, it can be concluded that as the SRTs were raised the SVIs values and floc sizes increased. On the other hand as the RD concentrations were increased the SVI values increased while the sizes of the flocs decreased after an optimum floc diameter at optimum SRT and RD dose. The RD concentrations up to 15 mg/L increased the sludge setttling at an optimum SRT of 25 days. This could be attributed to the unifying and binding properties of surfactant to the bacterial excretes and extracellular microbial products resulting in more compact floc formation at this SRT. At 15 mg/L RD a significant strong linear correlation between sludge settleability, floc size and 25 days SRT in the CSTR system treating petrochemical industry wastewater was obtained (R^2=0.83, F=2.92, p=0.01)

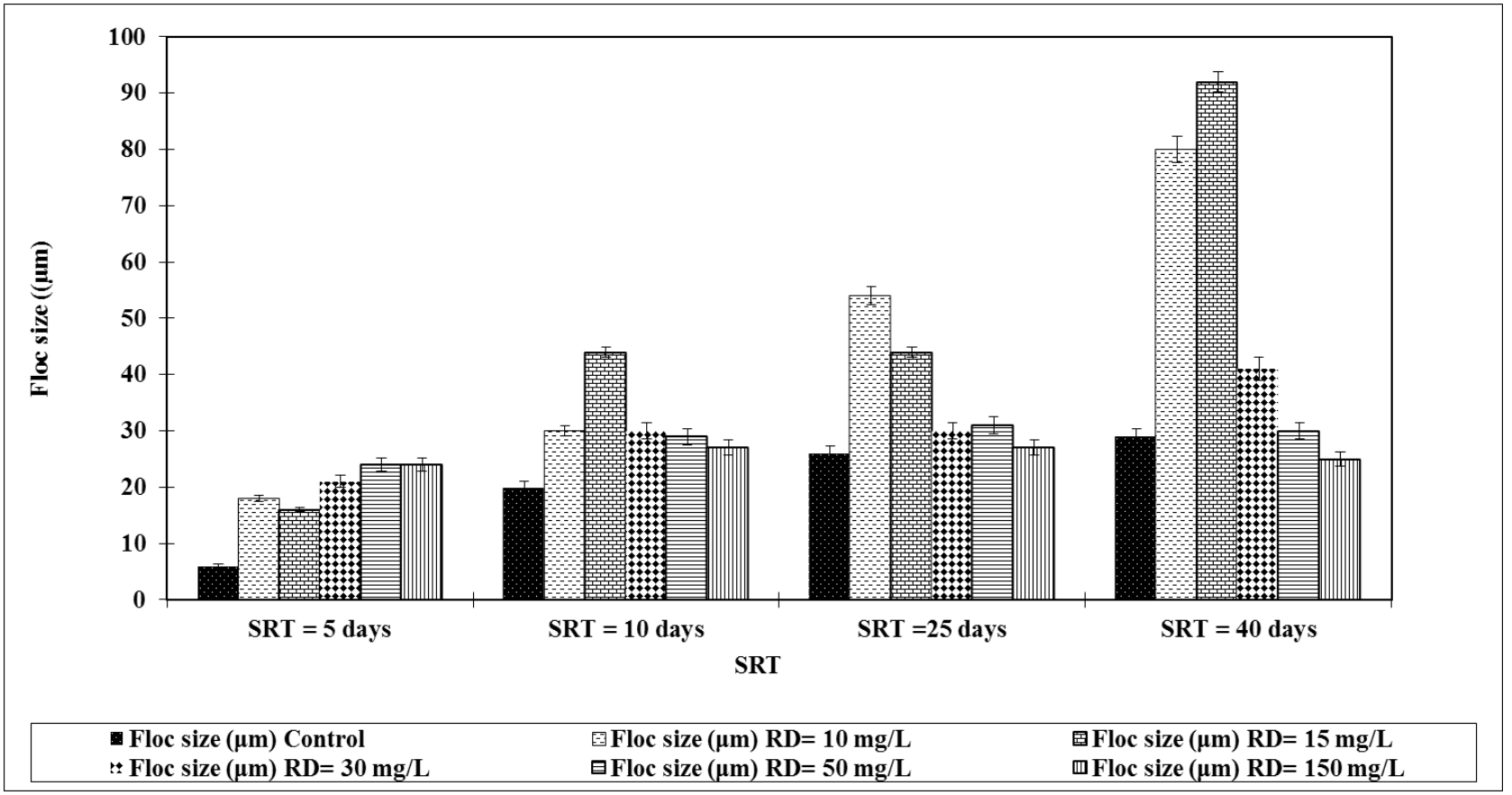

Figure 6.8 Effect of SRT and RD concentrations on floc sizes in the CSTR system treating PAH

Similarly Jin et al. (2003) found a positive correlation between the SVI of activated sludge and sludge flocs with low content of filaments. It can be suggested that negative surface charge was increased with increasing SRT and this negatively affected the sludge settling properties. According to Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, increased negative surface charge will result in increased repulsive electrostatic interactions between approaching surfaces, which means less bonding ability with other surfaces including bacteria with low excretes (Jin et al. 2003). Wilen et al. (2003) concluded that proteins in extracellular polymeric substances have a strong positive correlation with negative surface charge and flocculation ability of activated sludge. Polymeric constituents correlated well with average floc size and SRT at 25 days and resulted in producing sludge with lower SVI. Poor correlation between sludge settleability at longer SRT and floc size indicates that other factors like sludge morphology, density and surface properties are important in determining sludge settling characteristics. The SVI was higher for the higher SRTs and lower SRTs of 5 and 40 days, thus indicating a better filterability of the activated sludge at an optimum SRT of 25 days (Figure 6.7). The determined differences of SVI parameters with regard to different SRTs were statistically significant by high probabilities (p) for different means in the paired t-test: p was 99.9% for SVI.

F/M was one of the most significant factors influencing floc size distribution and a larger mean or median floc size was associated with a higher F/M (or a lower SRT). In contrast, Andreadakis (1993) found that the median floc size at an SRT of 1.1 d was around 20 mm and smaller than that (35–45 mm) of SRTs from 4.2 to 17.4 d. Generally, an overload of organic substrates would lead to the breakup of flocs, but sludge would exhibit poor flocculation under very low F/M condition (or very high SRT) (Eckenfelder and Musterman, 1995). These results indicate that the significance of F/M ratio or SRT on floc size distribution depends on the range of F/M or SRT tested. Therefore, a suitable F/M ratio or SRT is always desirable for effective separation of sludge flocs from treated effluent. The stability of floc size distribution at different SRTs might be explained by the growth rate of cells. At lower SRTs, more substrates are available for sludge microorganism growth.

6.3.5.9 PAH Metabolites at Increasing RD Concentrations at a SRT of 25 Days

The study the inter-metabolites produced from the degradation of ACT, FLN, BaP, IcdP and DahA PAHs are summarized in Table 6.15 for 15 and 25 mg/L RD concentrations. NAP-5,8, dihyrox-1,4-indenoquinone (for ACT); 9 hydroxyflourene (for FLN); 9-phenantrol (for PHE); pyrene-8-hydroxy-7-carboxylic acid (for BaP); 5,8, dihyrox-1,4-indenoquinone (for IcdP) and BaA-BaA-7,12dione (for DahA) are the metabolites of ACT, FLN, PHE, BaP, IcdP and DahA PAHs, respectively, in the CSTR reactor with *Zoogloea ramigera* under aerobic conditions. The concentrations of these metabolites mentioned above are high and removed with high yields at the end of the aerobic treatment in the CSTR reactor at a RD concentration of 15 mg/L. The levels of metabolites decreased and they removed with low yields at 25 mg/L RD concentration in the CSTR.

PAH metabolizing bacteria could degrade 22.16 ng/mL ACT (initial ACT concentration= 29.33 ng/mL) 7.27 ng/mL FLN (initial FLN concentration= 9.39 ng/mL), 12.98 ng/mL PHE (initial PHE concentration= 15.01 ng/mL), 0.06 ng/mL BaP (initial BaP concentration= 0.07 ng/mL), 0.11 ng/mL IcdP (initial IcdP concentration= 0.13 ng/mL) and 0.007 ng/mL DahA (initial DahA concentration= 0.27 ng/mL) with yields of 96%, 97%, %95, 96% and %96, respectively, at SRT of 25 days at a RD concentration of 15 mg/L, respectively (See Table 6.15). As the RD concentrations were increased from 15 to 25 mg/L at a SRT 25 of days, ACT, FLN and PHE PAHs removals decreased from 96%, 97% and %96 to 76%, 63%, and 65%, respectively. BaP, IcdP and DahA PAHs removals decreased from 95%, 96% and 96% to 61%, 67% and 74%, respectively (Table 6.15).

Table 6.15 Variations of metabolite products in different PAHs versus increasing RD concentrations at a SRT of 25 days

		RD 15 mg/L					RD 25 mg/L				
Initial PAH conc. (ng/mL)	Metabol. conc. (ng/mL)	Metabol. conc. (ng/mL)	Effleunt PAH conc. (ng/mL)	Yield PAH (%)	Effleunt metabol. conc. (ng/mL)	Yield Metabol. (%)	Metabol. conc. (ng/mL)	Effleunt PAH conc. (ng/mL)	Yield PAH (%)	Effleunt metabol. conc. (ng/mL)	Yield Metabol. (%)
ACT :29.43	NAP and 5,8-dihyrox-1,4-indenoquinone	17.27 3.89	1.17	96	0.034 0.078	98	0.038- 0.04	7.06	76	0.01 0.002	2.90
FLN: 9.39	9- hydroxyflourene	7.27	0.28	97	0.21	97	0.97	3.37	63	0.009	1
PHE:15.01	9-phenantrol	12.98	0.60	96	0.25	98	1.10	5.25	65	0.02	2.11
BaP: 0.07	pyrene-8-hydroxy-7-carboxylic acid	0.06	0.035	95	0.004	92	0.001	0.027	61	0.00003	3
IcdP: 0.13	5,8-dihyrox-1,4-indenoquinone	0.11	0.005	96	0.004	92	0.002	0.043	67	0.00004	2
DahA:0.27	BaA, BaA-7,12dione and 1,2-benzenedicarboxaldehyde	0.004 0.002 0.001	0.011	96	0.011	0	0.004 0.002 0.001	0.07	74	0.07	0

Under these conditions, the yields of metabolite products (NAP-5,8 and dihyrox-1,4-indenoquinone originating from ACT PAH; 9 hydroxyflourene originating from FLN PAH and 9-phenantrol originating from PHE PAH) also drastically decreased from 98%, 97%, 98% to 2.9%, 1% and 2.11% (Table 6.15). Similarly, the removal efficiencies of the metabolites namely pyrene-8-hydroxy-7-carboxylic acid and 5,8, dihyrox-1,4-indenoquinone originating from BaP and IcdP PAHs decreased from 92%, 92% to 3%, 2% as the RD was increased from 15 to 25 mg/L at 25 days SRT in the aerobic CSTR system. BaA and BaA-7,12dione metabolites originating from DahA PAH were accumulated as the RD concentration increased from 15 to 25 mg/L. In other words the aforementioned PAH metabolites were not removed at 25 mg/L RD concentration in the CSTR system (Table 6.15).

In the presence of 15 and 25 mg/L RD the metabolites produced from ACT, FLN, PHE, BaP, IcdP and DahA PAHs were analysed in the samples taken from the effluent of the CSTR at a SRT of 25 days. NAP, 5,8-dihyrox-1,4-naphthoquinone, 9-hydroxyflourene, 9-hydroxyflourene and 9-phenantrol are the metabolites detected from the aerobic biodegradation of ACT, FLN and PHE in the CSTR at a 15 mg/L RD concentration (Table 6.15). From 29.43 ng/mL ACT, 9.39 ng/mL FLN and 15.01 ng/mL PHE, 17.27 ng/mL NAP, 3.89 ng/mL 5,8-dihyrox-1,4-naphthoquinone, 7.27 ng/mL 9-hydroxyflourene, 12.98 ng/mL 9-phenantrol were produced. Although the ACT, FLN and PHE PAH yield were 96%, %97 and 96% in the CSTR, the effluent pollutant concentration (1.17 ng/mL for ACT, 0.28 ng/mL for FLN and 0.60 ng/mL for PHE) consisted only of its aerobic metabolites (0.034 ng/mL NAP and 0.078 ng/mL 5,8-dihyrox-1,4-naphthoquinone for ACT, 0.21 ng/mL 9-hydroxyflourene, 0.25 ng/mL 9-phenantrol) at a 15 mg/L RD concentration.

From 0.07 ng/ mL BaP, 0.13 ng/mL IcdP and 0.27 ng/mL DahA, 0.06 ng/mL pyrene-8-hydroxy-7-carboxylic acid, 0.11 ng/mL 5,8-dihyrox-1,4-naphthoquinone, 0.004 ng/mL BaA, 0.002 ng/mL BaA-7,12dione, 0.001 ng/mL 1,2-benzenedicarboxaldehyde were produced. Although the BaP, IcdP and DahA PAH yields were 95%, %96 and 96% in the CSTR, the effluent pollutant concentration (0.0035 ng/mL for BaP, 0.005 ng/mL for IcdP and 0.011 ng/mL for DahA) consisted

only of its aerobic metabolites (0.004 ng/mL pyrene-8-hydroxy-7-carboxylic acid, 0.004 ng/mL 5,8-dihyrox-1,4-naphthoquinone, 0.004 ng/mL BaA, 0.002 ng/mL BaA-7,12 dione, 0.001 ng/mL 1,2-benzenedicarboxaldehyde) at a 15 mg/L RD concentration.

As the RD concentrations were increased from 15 to 25 mg/L ACT, FLN, PHE, BaP, IcdP and DahA PAH removals were between 61% and 76%. 29.43 ng/mL ACT, 9.39 ng/mL FLN and 15.01 ng/mL PHE converted to 0.038 ng/mL NAP, 0.04 ng/mL 5,8-dihyrox-1,4-naphthoquinone, 0.97 ng/mL 9-hydroxyflourene, 1.10 ng/mL 9-phenantrol respectively, throughout aerobic treatment at a 25 mg/L RD concentration in the CSTR. Although these metabolites were removed with yield of 2.9%, 1% and 2.11% the effluent PAHs concentration consisted of ACT (7.06 - 0.012=7.048 ng/mL), FLN (3.37–0.009= 3.36 ng/mL) and PHE (5.25-0.02 = 5.23 ng/mL) not from metabolites **(**Table 6.15). 0.07 ng/mL BaP, 0.13 ng/mL IcdP and 0.27 ng/mL DahA, converted to 0.001 ng/mL pyrene-8-hydroxy-7-carboxylic acid, 0.002 ng/mL 5,8-dihyrox-1,4-naphthoquinone, 0.004 ng/mL BaA, 0.002 ng/mL BaA-7,12 dione, 0.001 ng/mL 1,2-benzenedicarboxaldehyde, respectively, throughout aerobic treatment at a 25 mg/L RD concentration in the CSTR. Although these metabolites were removed with yield of 3%, 2% and 0% the effluent PAH concentration consisted of BaP (0.027 ng/mL), IcdP (0.043 ng/mL) and DahA (0.07 ng/mL) not from metabolites (Table 6.15). The concentrations of these metabolites mentioned above are high and removed with high yields at the end of the aerobic treatment in the CSTR reactor at a RD concentration of 15 mg/L. The levels of metabolites decreased and they removed with low yields at 25 mg/L RD concentration in the CSTR (Table 6.15).

Gordon and Dobson (2001) studied FLN degradation by *P. alcaligenes* PA-10 and identified four intermediates formed during FLN degradation i.e. 9-fluorenone-1-carboxylic acid, 9-hydroxy-1-fluorene carboxylic acid, 9-fluorenone and 9-fluorenol (Rehman et al., 2009). However, Kelley et al (1993) identified 10 intermediates as 8-hydroxy-7-methoxyfluoranthene, 9-hydroxyfluorene, 9-fluorenone, 1-acenaphthenone, 9-hydroxy-1-fluorenecarboxylic acid, phthalic acid, 2-

carboxybenzaldehyde, benzoic acid, phenylacetic acid and adipic acid in FLN degradation by *Mycobacterium* sp (Gordon and Dobson, 2001). Su et al.(2009) found that BaP with five benzene rings can be degraded to one ring oxidation metabolite, benzo[*a*]pyrene *cis*-7,8-dihydrodiol, with 1,2 dioxygenase enzyme (Su et al., 2009). It was reported that the enzyme activities and the produced metabolite levels decreased at high RD and SR biosurfactant concentrations (Su et al., 2009; Rehmann et al., 2009). The differences in metabolic products may be linked to relative differences in bacteria, metabolic pathways of organism, wastewater and PAH degrading enzymes.

Sometimes the PAH substrate could not be bound to the active centre of the extracellular enzymes produced by the PAH degrading biomass. Therefore, the PAH could not be biocatalyzed by these enzymes since high biosurfactant concentrations probably block the active sites of the enzymes, thus preventing the biodegradation and the removal of PAHs. Therefore the PAHs and their metabolites were accumulated and they are competed together for the active sites of substrate (PAHs).

Karsa and Porta (2001) have reported the involvement of monoxygenases and dioxygenases synthesized by bacteria in FLN degradation. It was found that degradations of NAP, PHE and FLN were initiated by the naphthalene dioxygenase, phenanthrene 3,4-dioxygenase and fluoranthene 1,2 oxygenase enzymes by dioxygention process (Su et al., 2009). Most of the PAH degradation was prevented at high SR concentrations since the concentration of the extracellular ezymes (oxygenase) was produced by *Pseudomonas* sp. and *Mycobacterium* sp. (Gordon and Dobson, 2001; Rehmann et al., 2009). Dioxygenase promotes both the uptake and release of compounds from the cells through modifications and play an important role in the oxidization of PAHs by initial ring oxidation and ring cleavage processes (Pinyakong et al., 2000).

6.3.6 Main Removal Mechanisms of Total and Individual PAHs from Real Petrochemical Wastewater under Aerobic Batch Conditions

Some batch studies were performed to determine the main degradation mechanisms (biodegradation, volatilization and adsorption) of the total and individual 15 PAHs under aerobic conditions in lab scale stirred aerobic reactors. Furthermore, the effects of PAHs biosorption on biodegradation of PAHs was investigated with *Pseudomonas putida* and *Escherichia coli* cultures at 15 mg/L RD concentration in batch reactors.

6.3.6.1 Total PAH Removal and Adsorption of PAHs in Aerobic Batch Reactors Containing 15 mg/L RD

The studies performed with batch reactors showed that the total PAH removal efficiencies were between 39.13 and 92.16% and between 16.26 and 30.83% for three and four ring PAHs, respectively (Table 6.16). The total PAH removals varied at between 51.89%-54.43% and between 69.42%-82.17% for five and six ring PAHs, respectively. It was observed that the PAHs such as BbF, BkF, BaP, IcdP, DahA, and BghiP with high molecular weight were removed with total treatment efficiencies of 54.43%, 66.39%, 51.89%, 82.17%, 69.42% and 79.99%, respectively. The total removal efficiencies in ANT, CRB, FL, PY, and CHR PAHs were found to be lower, for example, 42.49%, 39.13%, 30.83%, 23.35% and 16.26%. This could be attributed to the low sludge age of 2 days, which is not sufficiently long for uptake of the aforementioned PAHs by the biomass through biological degradation in the batch reactor. The high PAH removal efficiencies in continuous operation of the CSTR could be attributed to high SRT of 25 days (Sponza & Gök, 2010).

Table 6.16 Total PAH removals and PAHs percentages sorbed to sludge (biomass) in aerobic batch reactors treating petrochemical wastewater containing 15 mg/L RD (n=3, mean±SD)

	Total PAH removals (incubation time: 2 days)				Adsorption (incubation time: 2 days)			Sorption (incubation time: 2 days)			Accumulation (incubation time: 2 days)
	column 1	column 2	column 3	column 4	column 5	column 6	column 7	column 8	column 9	Column 10	Column 11
PAHs	PAH (ng/mL)	PAH (ng/ mL)	Removal Efficiency (%)	Removal Efficiency (%)	PAH (ng/ mL)	PAH (ng/mL)	Removal Efficiency (%)	PAH (ng/g d.w.)	PAH (ng/g d.w.)	PAH (ng/g d.w.)	PAH accumulated in sludge (%)
ACT	17.01±1.2	1.33±0.01	92.16±0.20	79.00±0.87	17.01±1.56	16.28±0.10	4.30±0.09	4.89±0.06	5.06±0.06	0.16±0.003	3.40±0.90
FLN	16.95±1.3	6.13±1.01	63.81±0.10	49.20±0.42	17.01±1.27	16.28±0.10	4.30±0.08	4.89±0.07	5.06±0.04	0.16±0.003	3.39±0.60
PHE	13.35±0.9	3.28±0.99	75.42±0.12	70.70±0.28	16.95±1.09	16.28±1.03	3.90±0.11	3.48±0.08	3.60±0.07	0.11±0.004	3.38±0.34
ANT	3.13±0.1	1.80±0.01	42.49±0.13	31.70±0.11	13.35±0.09	12.89±1.02	3.40±0.03	7.70±0.09	7.96±0.08	0.26±0.002	2.20±0.01
CRB	0.46±0.01	0.22±0.01	39.13±0.14	21.34±0.37	3.13±1.03	3.03±0.01	2.90±0.02	5.27±0.06	5.38±0.06	0.11±0.001	4.84±0.02
FL	1.14±0.01	0.78±0.01	30.83±0.11	12.08±0.04	0.46±0.01	0.44±0.01	5.00±0.02	0.27±0.001	0.29±0.001	0.01±0.0001	3.22±0.01
PY	2.00±0.01	1.66±0.01	23.35±0.01	11.74±0.07	1.14±0.01	1.09±0.01	3.90±0.01	11.20±0.09	11.56±0.01	0.36±0.001	1.88±0.001
BaA	0.59±0.01	0.61±0.02	25.86±0.02	10.54±0.04	2.00±0.01	1.96±0.01	2.05±0.01	24.82±0.34	25.28±1.02	0.46±0.001	2.93±0.001
CHR	1.22±0.02	1.02±0.01	16.26±0.01	9.38±0.03	0.59±0.01	0.57±0.01	3.01±0.01	6.57±0.08	6.77±0.09	0.19±0.0002	5.36±0.01
BbF	0.16±0.01	0.07±0.01	54.43±0.01	15.42±0.07	1.22±0.01	1.15±0.01	6.03±0.01	14.84±0.04	15.64±0.67	0.79±0.002	4.62±0.001
BkF	0.21±0.01	0.07±0.01	66.39±0.02	23.87±0.02	0.16±0.01	0.15±0.01	5.03±0.03	1.47±0.01	1.54±0.05	0.06±0.0001	5.57±0.003
BaP	0.26±0.01	0.12±0.01	51.89±0.01	13.48±0.01	0.21±0.01	0.20±0.01	6.03±0.03	1.08±0.001	1.15±0.01	0.06±0.0002	4.39±0.056
IcdP	0.26±0.01	0.04±0.001	82.17±0.01	22.97±0.04	0.26±0.01	0.25±0.01	5.04±0.08	2.70±0.01	2.82±0.01	0.11±0.0002	5.80±0.023
DahA	0.50±0.02	0.15±0.001	69.42±0.001	21.90±0.02	0.26±0.01	0.25±0.01	6.09±0.08	0.61±0.001	0.64±0.001	0.03±0.0001	6.80±0.06
BghiP	0.24±0.01	0.04±0.001	79.99±0.001	19.31±0.01	0.50±0.01	0.46±0.01	7.04±0.02	1.03±0.001	1.10±0.002	0.07±0.0001	6.08±0.09

Column 1: PAH concentrations in the influent wastewater of aerobic batch reactor (ng/mL); Column 2: PAH concentrations in the effluent wastewater of aerobic batch reactor (ng/mL); Column 3: PAH removal efficiencies of aerobic batch reactor (%); Column 4: PAH removal efficiencies of aerobic batch reactor without RD (%); Column 5: PAH concentrations in the influent wastewater (ng/mL); Column 6: PAH concentrations in the effluent wastewater (ng/mL); Column 7: PAH removal efficiencies of aerobic batch reactor (%); Column 8: Initial PAH concentrations in autoclaved sludge (ng/g.dw); Column 9: Final PAH concentrations in autoclaved sludge (ng/g.dw); Column 10: PAH concentrations of accumulate in autoclaved sludge (ng/g.dw); Column 11: Percentages of accumulated PAH in autoclaved sludge (%)

The total PAH removal efficiencies were between 39.13%-92.16% and between 16.16% -30.83% for three and four ring PAHs, respectively, (Table 6.16) while the total yields in PAHs with five and six benzene rings were between 54.43%-66.39% and 69.42%-82.17% at a RD concentration of 15 mg/L (Column 3 in Table 4.17).

The administration of 15 mg/LRD increased the total PAH removals from 79.00%, 70.00% and 21.34% up to 92.16%, 75.42% and 42.49%, respectively, for ACT, PHE and CRB PAHs (Table 4.17). Removals of 4-Ring PAHs (FL, PY, BaA) were significantly higher in the presence of 15 mg/L RD (30.83%, 23.35% and 25.86%, respectively) in comparison to rhamnolipid-free conditions (12.08%, 11.74% and 10.54%). Treatment with rhamnolipid (15 mg/L) caused a significant increase of 5 and 6-ring PAHs degradation. The PAHs removal efficiencies increased from 23.87%, 13.48% and 22.97% up to 66.39%, 51.89% and 82.17% for BkF, BaP and IcdP PAHs. However, no significant effect was detected in the case of some 2-ring PAHs (FLN, PHE) and one 5-ring PAH (BbP) removals (Column 4 in Table 6.16).

The results of the study showed that a low part of the PAHs were accumulated in the biomass /sludge in the CSTR. The PAH accumulated in sludge varied at between 2.20%-4.84% and at between 1.88%-5.36% for three and four ring PAHs, respectively (Column 11 in Table 6.16). It was observed that the PAHs such as ACT, PHE, CRB and CHR PAHs with low molecular weight were removed with treatment efficiencies of 3.40%, 3.38%, 4.84% and 5.36%, respectively, via adsorption (Column 11 in Table 6.16). From individual PAHs 4.62% of BbF, 5.57% of BkF, 4.39% of BaP, 6.80% of IcdP, 6.80% of DahA, and 6.08% of BghiP were not metabolized in biomass and remained in the sludge, probably adsorbed onto the biomass (Column 11 in Table 6.16).

Trably and Patureau (2007) found significant abiotic losses via adsorption (9%-38%) for the lightest PAHs (FLN, PHE and ANT), while biodegradation occurred for all PAHs. More than 80% of the lightest PAHs were removed in aerobic reactors at a HRT of 20 days and at an OLR of 1.2 kg COD/m^3.d (Trably and Patureau, 2007).

During the past thirty years, several different treatment technologies have been tested in efforts to remove the PAHs. Among them, biodegradation has shown particular promise as a safe and cost-effective option (Chauhan et al, 2008). In spite of their xenobiotic properties, a variety of genera of gram positive and negative bacteria have been isolated and characterized for their ability to utilize PAHs in aerobic reactor systems.

6.3.6.2 Volatilization of PAHs in Aerobic Batch Reactors Containing 15 mg/L RD

It was observed that the removal of PAHs from the petrochemical industry wastewater via volatilization varied between 0.69% and 5.92% (See Table 6.17). Low PAHs removals were obtained in high molecular weight PAHs (BaA, BbF, BkF, BaP, IcdP, DahA and BghiP) with volatilization, while high PAHs removals were obtained in PAHs containing only few rings (ACT, FLN, PHE and CRB) in the aerobic batch reactor.

The maximum volatilization yields of 3 and 4 ring PAH removal was 5.92% and 3.07% for FLN and FL, respectively. The maximum volatilization 5 and 6 ring PAHs removal efficiencies were obtained as 1.18% and 1.97% for BaP and DahA PAHs, respectively. The minimum volatilization yield of 3 and 4 ring PAH removal was almost 4.23% and 0.69% for ANT and BaA, respectively. The minimum volatilization yield of 5 and 6 rings PAH was 0.86% and 1.08% for BkF and BghP, respectively.

PAH removals varied at between 4.23%-5.92% and between 0.69%-3.07% for three and four ring PAHs, respectively (Table 6.17). Five and six ring PAHs yields varied between 0.86% and 1.18% and between 1.08% and 2.26%, respectively. It was observed that the PAHs such as ACT, FLN, PHE and CRB PAHs with low molecular weight were removed 5.34%, 5.92%, 5.72% and 5.42%, respectively, via volatilization in aerobic batch reactor. The removal efficiencies of BaA, BbF, BkF, DahA and BghiP PAHs were obtained as 0.69%, 0.98%, 0.86%, 1.97% and 1.08%, respectively (Table 6.17).

Table 6.17 PAHs concentrations (ng/mL) and removal percentages through volatilization in aerobic batch reactor (n=3, mean±SD)

PAH	PAH [a]	PAH [b]	PAH removal (%)	PAH [c]	PAH removal eff. by volatilization (%)
ACT	17.01±0.01	15.76±0.09	7.34±0.99	19.57±0.98	5.34±0.98
FLN	16.95±0.01	15.41±0.11	9.06±0.77	21.35±0.56	5.92±0.45
PHE	13.35±0.02	12.03±0.45	9.86±0.05	21.06±0.78	5.72±0.23
ANT	3.13±0.01	2.99±0.05	4.26±0.05	6.15±0.07	4.23±0.21
CRB	0.46±0.01	0.43±0.001	5.51±0.06	2.55±0.01	5.42±0.41
FL	1.14±0.01	1.10±0.005	3.10±0.08	3.54±0.01	3.07±0.06
PY	2.00±0.01	1.98±0.005	1.40±0.01	2.81±0.01	1.38±0.06
BaA	0.59±0.002	0.59±0.004	0.70±0.001	0.42±0.002	0.69±0.006
CHR	1.22±0.001	1.21±0.005	1.00±0.001	1.23±0.005	0.99±0.007
BbF	0.16±0.001	0.16±0.002	1.04±0.003	0.16±0.002	0.98±0.003
BkF	0.21±0.002	0.21±0.003	0.88±0.002	0.19±0.005	0.86±0.005
BaP	0.26±0.004	0.26±0.004	1.20±0.004	0.32±0.002	1.18±0.009
IcdP	0.26±0.005	0.26±0.002	2.31±0.002	0.62±0.006	2.26±0.007
DahA	0.50±0.003	0.49±0.003	2.02±0.003	1.00±0.001	1.97±0.003
BghiP	0.24±0.001	0.24±0.003	1.14±0.004	0.27±0.004	1.08±0.006

a: PAH concentrations in wastewater at the start experiment (ng/mL), b : PAH concentrations in wastewater at the end of operation time (ng/mL); c :PAH concentrations in reactor headspace after operation time (ng/m^3).

Manoli and Samara (2008) found significant abiotic losses via volatilization (1%-2%) for the total PAHs removals in municipal activated sludge system. Low removals due to volatilization were also predicted by Byrns (2001) (20.2% ACT, 1.1% for Acenapthene, 0.49% for ANT, 0.006% for PY, 0% BaP) in activated sludge. Blanchard et al., 2004 reported that volatilization is insignificant removal mechanism for PAHs with a greater number of fused rings such as CHR and BkF PAHs in municipal treatment plant which is activated sludge processes (Blanchard et al., 2004). High abiotic losses were observed for all PAHs at 55 °C, which was attributed to volatilization. In another study, the continuous decreases in PAH concentration were attributed to volatilization and to sorption (Santos et al., 2008)

6.3.6.3 Bio-Sorption of PAHs in Aerobic Batch Reactors Containing 15 mg/L RD

Bio-sorption of a PAH (BaP) was examined using activated sludge samples taken from the refinery, from the municipal treatment plants (non-PAH degrader bacteria) and from the *Pseudomonas putida* culture known as PAH-degrader. In all cases, the PAHs sorption was described adequately by a linear sorption model as mentioned by Haritash and Kaushik, (2009). As mentioned in Table 6.18 the linear model used to determine the partition coefficient (K_p) allows to determine the sorption capacity of three different sources of biomass with 95% confidence intervals

Table 6.18 Partition coefficients of aqueous BaP for *Pseudomonas putida,* municipal wastewater activated sludge and petrochemical industry activated sludge

Adsorbent	K_p (L/g biomass)	Lower 95%	Lower 95%	R^2
Pseudomonas putida	1.99	1.87	2.11	0.99
Municipal wastewater activated sludge	29.80	28.40	31.21	0.99
Petrochemical industry activated sludge	2.98	2.86	3.09	0.99

Low K_p values for refinery activated sludge (approximately 2.98 L/g) and *Pseudomonas putida* (1.99 L/g) confirm that biosorption is not an important mechanism involved in the fate of PAHs in the activated sludge system taken from the petrochemical industry while high K_p values (29.8 L/g) in the municipal activated sludge showed that biosorption is an important PAH removal process (See Table. 6.18).

6.3.6.4 Effects of PAH Bio-Sorption on Biodegradation of BaP in Aerobic CSTR Reactor

In order to investigate the influence of non PAH-degrading biomass on PAHs degradation, experiments were conducted in which BaP removals were compared with aerobic reactor systems containing the PAH degrading bacteria (*Pseudomonas putida*) and those containing the PAHs degrading-bacteria taken from the petrochemical industry wastewaters and non PAHs-degrading bacteria taken from the municipal activated sludge in Izmir. 40 ng/mL BaP was completely degraded on incubation days of 2 and 4 by *Pseudomonas putida* and petrochemical biomass as PAHs degrading biomass (See Figure 6.9).

In this study the samples of activated sludge taken from the petrochemical industry treatment plant and a municipal treatment plant exhibited different BaP equilibrium sorption characteristics (Figure 6.10). The bacteria with the highest sorption capacity belong to a group known as *fecal coliform* found in the municipal activated sludge plant while no significant sorption was observed *in Pseudomonas putida* and petrochemical industry floc bacteria (Figure 6.10).

Stringfellow and Alvarez-Cohen (1999) showed that only as little as 0.4% of the activated sludge biomass was metabolically active for PAHs degradation. This indicates that the sorption of PAHs to non-degrading biomass could potentially influence the degradation of PAHs in refinery wastewater treatment systems. In contrast to these findings, in our study it was found that activated sludge from the petrochemical industry has a low K_p and low sorption capacity (See Figure 6.10).

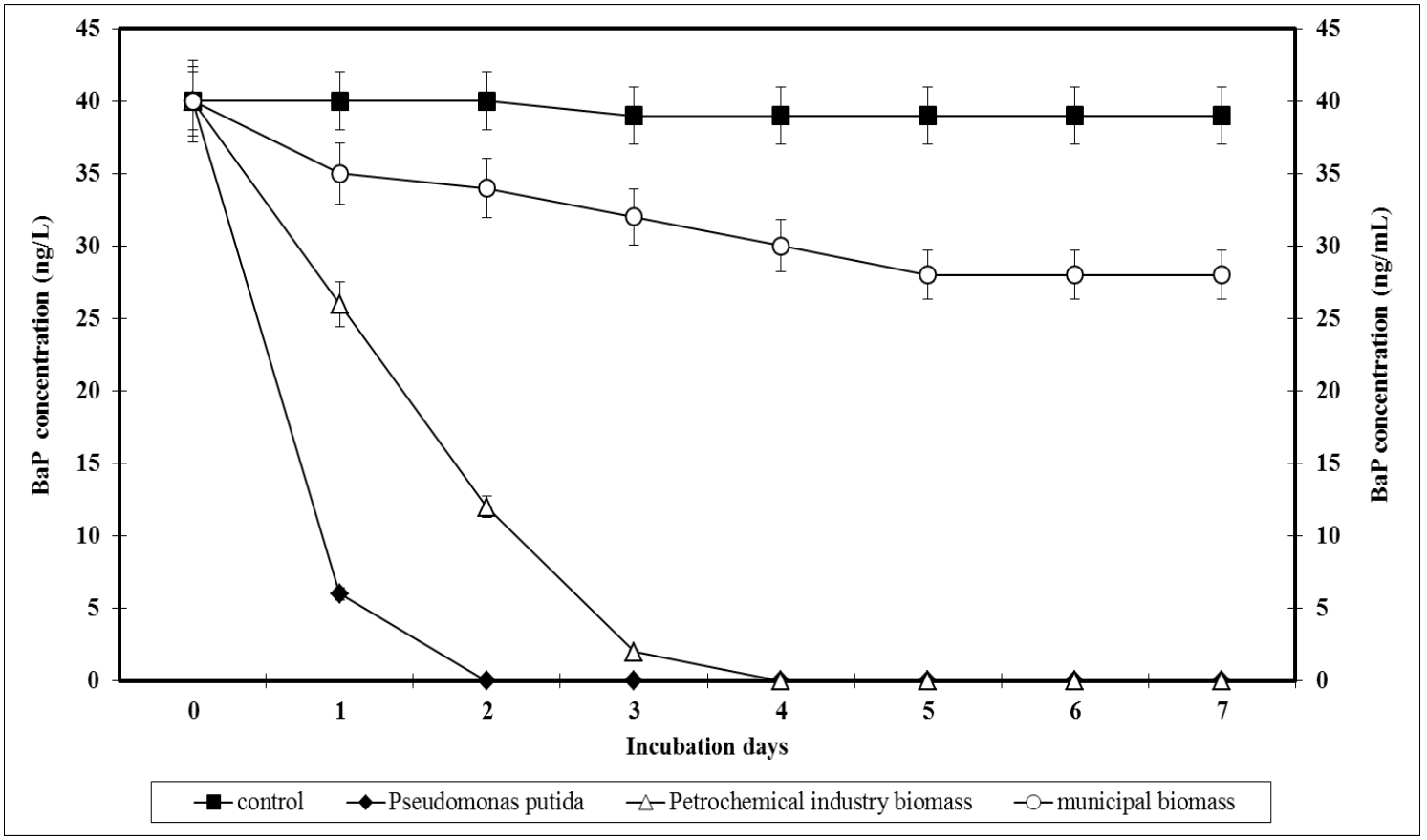

Figure 6.9 Utilization of BaP by PAH-degrading and non degrading biomass

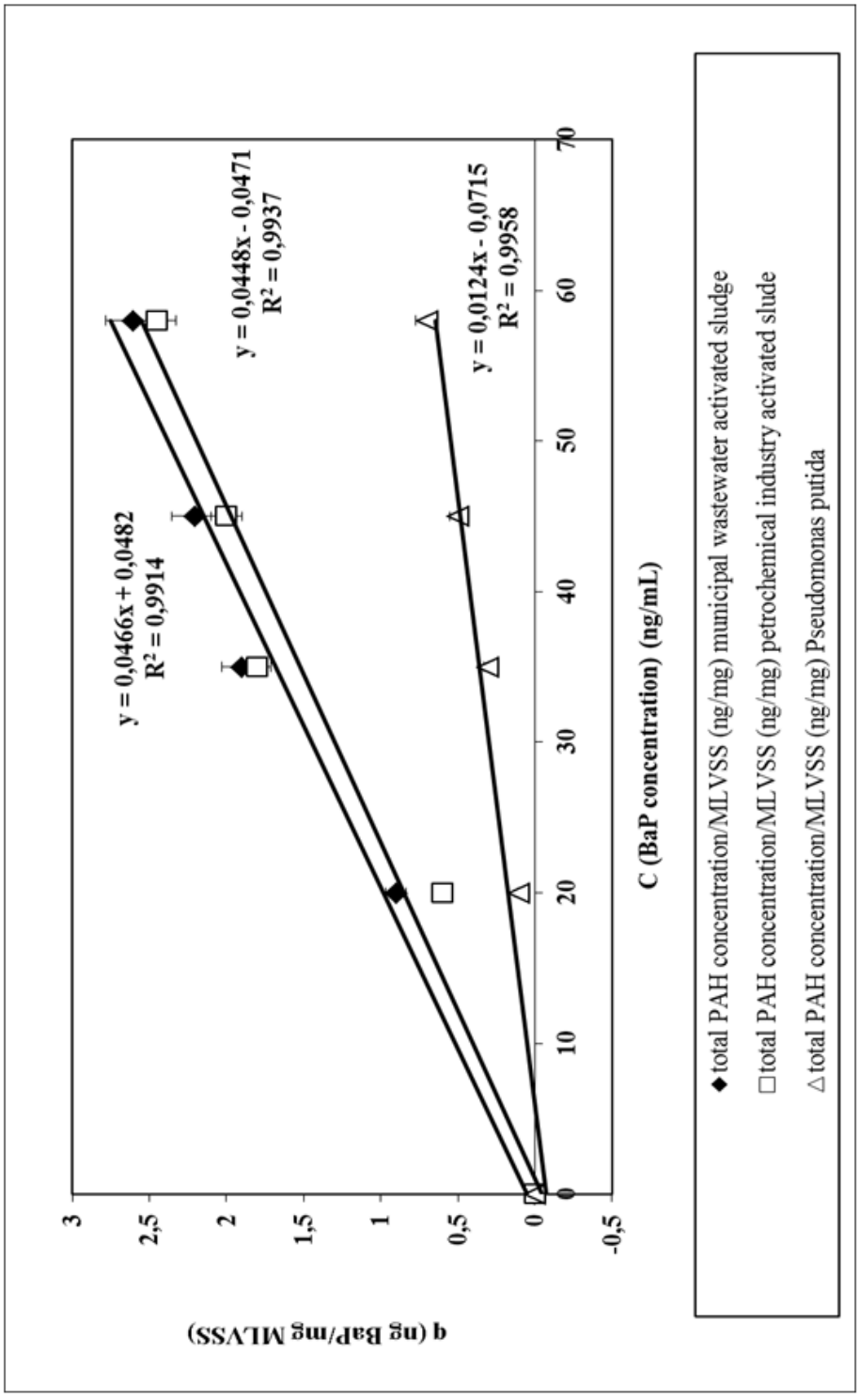

Figure 6.10 Variation of BaP concentration versus PAH adsorbed per unit mass of adsorbent (biomass) (n=3, mean).

A low amount of BaP was used by the municipal sludge while the control did not metabolize the BaP at all. A significant reduction in the rate of BaP degradation was observed in the presence of non-degrading PAHs biomass suggesting that PAHs sequestration by non-degraders does not reduce the biodegradation of PAHs to the degrading bacteria. It can be concluded that the removal of PAHs with sorption is negligible and the sorbed PAH, would, probably, ultimately be biodegraded although a small part of the sorbed PAHs was immediately used for biodegradation.

6.3.6.5 Main Removal Mechanisms of PAHs

Table 6.19 shows the maximum and minimum removal percentage for short chain (ACT, FLN, PHE, ANT, CRB, FL, PY, BaA, and CHR) and long chain (BbF, BkF, BaP, IcdP, DahA and BghiP) PAHs with and without RD among the PAH removal mechanisms in aerobic batch reactor. Although sorption, volatilization (by air stripping) and biodegradation are declared as the major removal mechanisms considered in aerobic batch reactors (Haritash & Kaushik, 2009), in this study we found that biodegradation is the main removal mechanism of PAH removal. This is in contrast to Haritash and Kaushik, (2009), who maintained that sorption and volatilization (by air stripping) are the major removal mechanisms taking place in aeration basins. For all PAHs biological removal appeared to be the predominant removal mechanism (94-97%) in the aerobic batch reactor (Table 6.19). Biodegradation was found to make only a small contribution (4%) to a few PAHs (only for ACT, FLN and PHE PAHs) while volatilization seems to be unimportant for all PAHs (Table 6.19).

The maximum and minimum removal percentage for short chain and long chain PAHs with and without RD among the PAH removal mechanisms are illustrated Table 6.19.

Table 6.19 Maximum and minimum removal percentage for short chain and long chain PAHs with and without RD among the PAH removal mechanisms in aerobic batch reactor

Main removal mechanism	Without Rhamnolipid		15 mg/L Rhamnolipid	
Molecule formula	$C_{12}H_8$-$C_{16}H_{10}$ Minimum and maximum in short chain PAHs	$C_{18}H_{12}$-$C_{22}H_{12}$ Minimum and maximum in long chain PAHs	$C_{12}H_8$-C_{16}-H_{10} Minimum and maximum in short chain PAHs	$C_{18}H_{12}$-$C_{22}H_{12}$ Minimum and maximum in long chain PAHs
Total PAH removal (%)	33-80	45-76	37-99	54-87
Biological PAH removal (%)	9.38-79.00	13.48-23.87	16.26-92.16	51.89-79.99
Adsorption PAH removal (%)	0.50-3.98	0.62-3.98	2.05-5.00	5.03-7.04
Volatilization PAH removal (%)	0.30-1.34	0.69-1.26	0.69-5.92	0.86-2.26
Sorption in sludge (%)	0.04-3.19	0.88-2.58	1.88-4.84	4.62-6.80

The yield of total PAHs was 33-80% and 45-76% for minimum and maximum in short and long chain PAHs, respectively, without RD aerobic batch reactor. The administration of 15 mg/L RD, the total PAH removals were obtained as 27-99% and 54-87% for minimum-maximum in short and long chain PAHs, respectively. Removals of minimum and maximum in long chain PAHs were significantly higher in presence of 15 mg/L RD. Percent removal efficiencies of PAHs were 9.38-79.00% and 13.48-23.87% for minimum and maximum in short and long chain PAHs, respectively, via biological in without RD reactor. The presence of 15 mg/L RD, the PAHs removal was obtained as 16.26-92.16% and 51.89-79.99% for minimum and maximum in short and long chain PAHs, respectively (Table 6.19).

In this study it was found that total PAH treatment with RD was low beneficial for the adsorption with minimum and maximum in short and long chain PAHs removal yield of 2.05-5.00% and 5.03-7.04% in comparison to 0.50-3.98% and 0.62-3.98% in minimum and maximum in long and short chain PAHs, respectively, in the RD free

case aerobic batch reactor (Table 6.19). The studies performed with aerobic batch reactors showed that the minimum and maximum in short and long chain PAHs removal efficiencies were obtained as 0.69-5.92% and 0.86-2.26%, respectively, with 15 mg/L RD, via volatilization. It was observed that the minimum and maximum in short and long chain PAHs were removed with treatment efficiencies of 0.30-1.34% and 0.69-1.26%, respectively, without RD (Table 6.19).

The yield of PAHs was 0.04-3.19% and 0.88-2.58% for minimum and maximum in short and long chain PAHs, respectively, in sorption mechanism without RD aerobic batch reactor (Table 6.19). The administration of 15 mg/L RD, PAHs removals were obtained as 1.88-4.84% and 4.62-6.80% for minimum and maximum short and long chain PAHs, respectively.

In a model considering the major abiotic and biotic processes in an aerobic reactor it was found that the PAHs with log K_{ow} < 5 are expected to be removed mainly through degradation (up to 95%), whereas PAH compounds with log K_{ow} > 6.5 will not be degraded (Lohmann and Lammel, 2004). In this model, the contribution of individual PAHs to the total removal of PAHs was classified according to the octanol-water coefficient (K_{ow}) and the Henry's law constant values of individual PAH compounds (Makkar and Rockne, 2003).

6.3.6.5.1 PAH Removal Modelling. Although in this study it was found that biodegradation is the main removal mechanism of all PAHs in aerobic activated sludge reactor containing RD a fate model of PAHs should be estimated to quantify the actual adsorption, volatilization, the biodegradation potential when the removal efficiencies are low in PAHs with high molecular weights. The removal mechanism of PAHs should be considered in the case of failure of activated sludge processes. Three models were generated to simulate the experimental data. Volatilization (vol), adsorption (ad) and biodegradation (bio) terms, in a general PAHs FATE model under steady-state biomass balance on PAHs, become (Sponza, 2005):

$$V\,(dS/dt) = Q\,(S_o-S)+R_{bio}+R_{ad}+R_{vol} \qquad (6.1)$$

Where, Q is influent flow rate (L/d), S_o is influent PAHs concentration (mg/L), S is effluent PAHs concentration (mg/L), R_{bio} is PAHs accumulation caused by biologically degradation rate (mg/d), R_{ad} is PAHs accumulation caused by adsorption degradation rate (mg/d) and R_{vol} is PAHs accumulation caused by volatilization gas phase rate (mg/d). At steady-state conditions the accumulation term V (dS/dt) in Eq. (6.1) is equal to zero. Hence Eq. (4.1) at time t based on the change during the time interval Δt simplifies to

$$0 = Q\,(So\text{-}S)+R_{bio}+R_{ad}+R_{vol} \qquad (6.2)$$

The rates of PAHs removed by adsorption onto biological solid (biomass), volatilization and biodegradation are expressed as the following equations, respectively (Chiu 1993; Sponza, 2005):

$$R_{bio}=-k_1\,X_a\,S\,V \qquad (6.3)$$

$$R_{ad}=Q_w\,X_v\,K_p\,S \qquad (6.4)$$

$$R_{ad}=4.48x10^{-5}\,(K_{ow})^{0.67}Q_w\,X_v\,S \qquad (6.5)$$

$$R_{vol}=G.H_c \qquad (6.6)$$

Where k_1 (k/K_s) is first order biological reaction constant (L/mg.day), k is maximum specific substrate utilization rate (1/day), K_s is half velocity constant (mg/L), X_a is biomass concentration in aerobic reactor (mg/L), V is aeration basin volume (L), Q_w is waste flow rate (L/day), X_v is concentration of solids in sludge (mg/L), K_p is sorption partition coefficient, K_{ow} octanol/water partition coefficient (m^3 H_2O/m^3 octanol), G is gas flow rate (L/day) and H_c is Henry's law constant (atm m^3/mole). Applying a k_1 biodegradation constant [(k-maximum specific substrate utilization rate 0.00045 1/day)/(K_s - half velocity constant 0.01431 mg/L)] of $3.15x10^{-5}$ 1/day in Eq. (6.1) determined the study biodegradation is seen to be major mechanism of PAHs removal and contributed around 94% (See Table 6.20). The differences in PAHs removal percentages between this section and early sections could be due to the variation in petrochemical industry wastewater depended to

processes in the petrochemical industry. Since the raw wastewater exhibited different characteristics the composition on wastewater varied sometimes.

The contributions to removal of volatilization and adsorption were calculated by substituting a partition coefficient K_p [$_{(4.48x10^{-5})}$ $(K_{ow})^{0.67}$ (L/mg)] [octanol/water partition coefficient, K_{ow}, $10^{3.36}$-$10^{6.63}$ (m^3 H_2O/m^3 octanol)] (Manoli and Samara, 2008), flow rate Q (1 L/d), volumetric gas flow rate G (360 L/d), universal gas constant R ($8.206x10^{-5}$ m^3 atm/K.mole) and Henry's law constant Hc- $4.4x10^{-4}$-$3.31x10^{-7}$ (dimensionless) (Lohmann and Lammel, 2004) in Eqs. (6.4)–(6.6). The predicted volatilization accounted for 1.0% (Table 6.20). In terms of mass removed, biodegradation accounted for 5.08 mg/day while adsorption and volatilization accounted for 0.16 mg/day and 0.058 mg/day, respectively (See Table 6.20)

Table 6.20 Comparison of PAH removal mechanisms according to tentative data and suggested model in the aerobic batch reactor

Mechanism of PAH transformation	PAH[a] From the models (mg/day)	PAH[a] From the experiments (mg/day)	PAH[b] From the models (%)	PAH[b] From the experiments (%)
Biological *(R_{bio})*	5.95	5.08	94.1	95.8
Adsorption *(Rad)*	0.17	0.16	2.9	3.1
Volatilization *(Rvol.)*	0.050	0.058	1.0	1.1

a:Transformation rate of PAH; b:Transformation efficiency (%) PAH

Values of biokinetic coefficients can be directly used in modeling for degradation of wastewaters containing PAHs. These models can also provide indications of relative degradation, adsorption and volatilization rates of PAHs in the activated sludge systems.

The concentrations of PAHs measured in the influent and effluent samples of the aerobic CSTR reactors were compared to the predicted values for biodegradation, volatilization and adsorption. A good agreement between the experimentally

calculated percent removals of PAHs and those predicted by the FATE model was observed (Table 6.20).

6.3.6.5.2 The Fates of PAH and RD Biosurfactant at a RD Concentration of 15 mg/L at an Optimum SRT of 25 days. The degradations and accumulations of PAHs and RD were examined in the CSTR system with the presence of varying RDs additions and SRTs. Usually PAH and biosurfactant accumulation are not desirable in the CSTR system due to environmental concerns and these are regarded as important factors in various applications. When the SRT was increased from 10 to 25 days, the overall removal efficiency of PAHs by the CSTR system increased from 85% to 96% at a 15 mg/L RD (R^2= 0.87, F = 2.02, p = 0.01) (See section 6.3.4).

The possible fate of PAHs in CSTR system most likely includes being discharged with the final effluent and waste sludge, accumulating in the system, and being degraded by microorganisms in activated sludge. The fate of PAHs was calculated based on the following mass balance equations:

$$\text{PAH-M}_{\text{inlet}} - \text{PAH-M}_{\text{outlet}} = \text{PAH-M}_{\text{sludge}} + \text{PAH-M}_{\text{biodegradation}} + \text{PAH-M}_{\text{accumulation}} \quad (6.7)$$

$$\text{RD- M}_{\text{inlet}} - \text{RD- M outlet} = \text{RD- M}_{\text{sludge}} + \text{RD- M}_{\text{biodegradation}} + \text{RD- M}_{\text{accumulation}} \quad (6.8)$$

The unit in Eq. (6.7) was ng/day while the unit in Eq. (6.8) was ng/day. Here M_{inlet}, M_{outlet}, and M_{sludge} are PAH masses in the inlet, effluent, and waste sludge, respectively. They were calculated based on the relevant PAH concentrations. $M_{accumulation}$ is PAH mass accumulated in the CSTR system. $M_{biodegradation}$ is PAH mass degraded by the activated sludge in CSTR which was obtained from Eq. (6.7). With a similar equation, the mass balance of RD was investigated in the CSTR system. The PAH concentrations measured in different parts of the CSTR were shown in Table 6.21. It was found that the PAHs were mainly biodegraded in the CSTR. 94–97% of PAHs was biodegraded, 1.1–1.5% and 0.7–1.2% of PAHs were accumulated in the sludge and in the aerobic reactor, respectively, while 0.9–1.3% of the PAHs were in the effluent of the CSTR (Table 6.21).

Table 6.21 Fates of PAHs and 15 mg/L RD in the CSTR system at a SRT of 25 days (n =3, mean values)

PAHs	A[r n]	PAH [b]	PAH [c]	PAH [d]	PAH [e]	PAH [f]	RD [g]	RD [h]	RD [i]	RD [j]	RD [k]
ACT	3	29.4386	0.9606	1.1775	0.1092	96					
FLN	3	9.3800	0.3538	0.2814	0.0984	97					
PHE	3	15.0104	1.6367	0.6004	0.4502	96					
ANT	3	3.6110	0.4124	0.2166	0.0034	94					
CRB	3	0.9010	0.0037	0.0540	0.0017	94					
FL	4	2.9831	0.0347	0.2088	0.0061	93					
PY	4	2.1927	0.0448	0.1095	0.0054	95	15.01-5.09	0.20-.38	0.25-0.40	0.23-0.41	14.31-6.61
BaA	4	0.3612	0.0012	0.0216	0.0021	94					
CHR	4	0.7237	0.0051	0.0434	0.0019	94					
BbF	5	0.0803	0.0041	0.0040	0.0021	95					
BkF	5	0.4912	0.0920	0.0019	0.0025	96					
BaP	5	0.0723	0.0028	0.0036	0.0018	95					
IcdP	6	0.1292	0.0788	0.0064	0.0035	95					
DahA	6	0.2797	0.0023	0.0167	0.0074	94					
BghiP	6	0.0966	0.0092	0.0048	0.0027	95					
Total		65.3214	3.6422	2.7506	0.6984	96					

a [r n]: Ring number in PAHs; PAH[b]: (PAH inlet), influent PAH concentration (ng/mL); PAH[c]: (PAH $_{acc.}$),concentration of PAH accumulated in sludge (ng/gdw.); PAH[d]: (PAH outlet.), PAH concentration in effluent (ng/mL); PAH[e]: (PAH $_{CSTR}$), PAH concentration in aeration tank (ng/mL); PAH[f]: PAH removal efficiency (%); RD[g]: (RD_{inlet}), RD concentration in inlet (ng/mL); RD[h]: (RD $_{outlet}$), RD concentration in outlet (ng/mL); RD[i]: (RD $_{sludge}$), RD concentration in sludge (mg/L); RD[k]: (RD_{CSTR}), RD concentration in aeration tank (ng/mL);RD[l]: (RD $_{biodeg}$), RD concentration biodegraded in aeration tank (mg/mL)

The concentrations of RD in the aerobic activated sludge and in the effluent were in the range of 0.25–0.40 mg/L and 0.20–0.38 mg/L, respectively, at initial RD concentrations of 15.01–15.09 mg/L (Table 6.21), suggesting that 14.31–14.61 mg/L RD was biodegraded throughout 94–97% PAH treatment in the CSTR at a SRT of 25 days. It was shown that the removal of RDs from the aqueous phase of activated sludge caused the increase in surface tension in the activated sludge system. This decrease in RD concentration in the aqueous phase could be due to the biological utilization as carbon sources by the microorganism as reported by Lei et al. (2005). The quick adsorption of RDs on biomass could largely cause the initially rapid decrease in surface tension in the beginning upon aeration while the microbial biodegradation of RDs dominated the decrease in surface tensions at a late stage when the PAHs were consumed. Based on the present data, we observed that PAHs can be efficiently degraded in the CSTR along with a high RD removal efficiency. The optimal SRT for both RD and PAH removal must be 25 days. The decrease in RD concentration in the aqueous phase could be due to uptake by the bacteria following the adsorption of activated sludge as well as waste PAHs for only a few minutes or the biological utilization as carbon sources by the microorganism (Yuan et al., 2009). If optimum surfactant concentrations are applied, the hydrophobic contaminants such as PAHs can be solubilized by incorporation into surfactant, i.e., aggregates of biosurfactant molecules where the hydrophobic moieties form a core which is insulated from the aqueous environment by the outward-oriented hydrophilic moieties.

6.3.6.5.3 DO Utilization, BOD_5/COD ratio, Oxygen Utilization (OU) and Oxygen Utilization Rate (OUR) variations with and without 15 mg/L RD at a SRT of 25 days in the CSTR System. The variations in BOD_5, BOD_5/COD ratios, DO utilization, Oxygen Utilization (OU) and Oxygen Utilization Rate (OUR) were investigated in the CSTR reactors with and without 15 mg/L RD biosurfactant at the optimum SRT of 25 days throughout 35 days of continuous operation in the CSTRs. In the RD amended CSTR the BOD_5/COD ratio increased from 0.23 to 0.37 indicating the biodegradability of the effluent wastewater compared to CSTR without RD (Table 6.22).

Table 6.22 Variation of BOD_5, BOD_5/COD ratios, DO utilization and OUR with/without RD at a SRT of 25 days (n = 3, mean values)

CSTR	COD [a]		BOD_5 [b]		BOD_5 /COD [c]		PAH [d]		DO [e]	OU [f]	OUR [g]
	I [h]	E [i]	I [h]	E [i]	I [k]	E [i]	I [h]	E [i]			
15 RD[m]	2650±4.24	328±2.27	650±5.20	127±2.00	0.23±0.02	0.37±0.04	54.57±0.02	3.11±0.01	4±0.1- 6±0.1	489±3.20	189±2.50
0 RD[n]	2650±6.75	1278±3.14	650±5.20	340±1.50	0.23±0.02	0.26±0.04	54.57±0.02	20.19±0.01	3±0.1 - 4±0.1	287±1.40	74±3.60

COD[a]: Total COD concentration (mg/L); BOD_5[b]: BOD_5 concentration (mg/L); BOD_5 /COD[c]: BOD_5 to COD ratio; PAH[d]: PAH concentration (ng/mL); DO [e]: Dissolved oxygen utilization (mg/L); OU[f]: Final cumulative oxygen utilization (mg/L); OUR[g]: Oxygen utilization rate (mg/L.h); I[h]: Influent concentration; E[i]: Effluent concentration; I[k]: Ratio in influent; E[l]: Ratio in effluent; m: CSTR reactor containing 15 mg/L RD; n: CSTR reactor containing no RD.

The biodegradability of an industrial wastewater is dependent upon BOD_5/COD ratio. It has generally been accepted that when a biodegradability ratio is greater than 0.3, this represents a readily biodegradable effluent (Tchobanoglous et al., 2003). An increase in the BOD_5/COD ratio indicates an improvement in the biodegradability of the petrochemical wastewater containing PAHs due to formation of inter-metabolite-products more biologically degradable. The aerobic transformation of PAHs into more biodegradable inter-metabolites in the CSTR increased the biodegradability ratio of the petrochemical wastewater (See section 6.3.5.9 in Table 6.15). Meanwhile, the OUR levels increased from 70 to 198 mg/L.h, compared to the CSTR containing no RD.

The COD_{total} and PAH yields were 88% and 96%, respectively, in the CSTR containing 15 mg/L RD while the BOD_5 concentration decreased from 650 to 127 mg/L yielding a BOD_5 removal efficiency of 80% (See Table 6.22). BOD_5 removal efficiency remained as 47% in the CSTR containing no RD. In the CSTR with 15 mg/L RD, the OUR increased since the PAHs in wastewater could be effectively uptaken by the biomass together with RD. As a result, the MLVSS concentrations in the CSTR containing RD were higher than in the reactor without RD (See section 6.3.5.7 in Figure 6.6). Therefore, the DO utilization levels and OUR of microorganisms increased in the CSTR with RD. It was reported that the OUR provides a suitable measure of cell metabolic activity and information about the effects of different conditions on cell viability (Lee et al., 2003). Haritash and Kaushik (2009) and Mohan et al. (2006) mentioned similar data with the DO levels in biological reactor containing different biosurfactants at optimum dose throughout aerobic PAH degradation (Haritash and Kaushik, 2009; Mohan et al., 2006).

6.3.6.5.4 Effects of Environmental Conditions on PAH Yields.

6.3.6.5.4.1 Effects of Temperature on PAH Yields. Increasing the temperature from 21 to 25 to 45 ^{o}C showed that total PAH yields increased from 95% up to 99% in the presence of 15 mg/L RD in CSTR as reported by Nie et al. (2010) (Table 6.23). Higher temperatures increase the solubility and mass transfer rates of PAHs. It

was reported that the diffusion coefficient and the solubility of PHE in water increase by factors of 1.5 and 2.5 when the temperature is raised from 20 to 45 ^{o}C.

Table 6.23 Effects of temperature on mean total PAH yields (n = 3, mean values)

Parameter	Temperature (^{o}C)		
	21-25	45	60
Mean total PAH yields (%)	95±0.04	99±0.05	65±0.90

Similar enhancement of degradation rates at high temperatures has been previously observed in tests with PY and ANT (Feitkenhauer and Marki, 2003). Increasing of temperature to 60 ^{o}C reduced the total PAH yields significantly since the microorganisms present in activated sludge were mesophilic. Possibly these microorganisms could not acclimate to the high temperatures. A second factor that could limit the PAH yields at high temperatures is the low aqueous solubility of oxygen at high temperatures. Another factor that could lower PAH yields could be the lowering of octanol–water partition coefficients at higher temperatures (Feitkenhauer and Marki, 2003).

6.3.6.5.4.2 Effects of DO and ORP on PAH Yields. The impact of oxic, anaerobic and anoxic conditions on the fate of PAHs was investigated. Five incubation conditions were chosen: two bioreactors were subjected to alternations in aeration (oscillating conditions). One of these reactors was aerated with 2–3 mg/L DO while the second reactor was aerated with 4–5 mg/L DO. These reactors were operated continuously for 35 days. Another reactor was operated without DO under anaerobic conditions while the last reactor was also operated under anoxic conditions (DO = 0 mg/L) by adding 25 mg/L NO_3-N to permanent anoxia. Permanent oxic (4–5 mg/L DO) and sequential oxic/anoxic oscillation conditions showed total PAHs removal of about 95% and 78%, respectively, after 35 days of operation, at a SRT of 25 days at 15 mg/L RD concentration (Table 6.24). The reactor containing 2–3 mg/L DO

exhibited low PAH yield compared to oxygenated samples with 4–5 mg/L DO. The PAH yields decreased to 61% under anaerobic conditions (Table 6.24).

Table 6.24 Effects of DO and ORP on mean total PAH yields (n = 3, mean values)

Parameter	DO [a], ORP [b]				
	2-3 [a]; + 45[b]	4-5 [a] ; + 145[b]	0 [a]; -340 [b]	AN [c]; + 8[b]	SA/AN [d]; +145[c] +8[c]
Mean total PAH yields (%)	84	95	61	83	78

DO [a]: dissolved oxygen concentration (mg/L); ORP [b]: oxidation reduction potential in low, high-oxygenated, anaerobic and anoxic conditions (mV); AN [c]: Anoxic conditions (DO = 0 mg/L); SA/AN [d]: sequential aerobic/anoxic conditions

The addition of anaerobic sludge to the CSTR increased the PAH yields to 89%. The inoculum effect was more significant under the non-oxygen condition (Chang et al., 2003). The efficiency of the enriched consortia indicated that bioaugmentation with the enriched consortia from sediments was useful in bioremediation of PAHs in anaerobic contaminated sediment (Chang et al., 2003). In the case of oscillating conditions, two days of aeration following the anoxic period was sufficient to reach the same percentage as under oxic conditions. The availability of oxygen exerts a strong influence on PAH yields. Very low PAH yields without oxygen have been reported (Cravo-Laureau et al., 2011). In very low oxygen environments, there may be microbial degradation of PAHs with low molecular weights via non-oxygen dependent mechanisms (Cravo-Laureau et al., 2011; Janbandhu and Fule, 2011). Under the low-oxygen condition with the inoculation of the enriched consortium the anaerobic biodegradation of some three-rings (FL, PHE) and four -rings (FLN, PY) PAHs were detected (Janbandhu and Fule, 2011). The oxidation reduction potentials (ORP) were measured as +145, +45, −340, and +8 mV in high, low oxygenated, anaerobic and anoxic reactors, respectively (Table 4.25). ORP value is considered an important factor affecting the growth of anaerobic bacteria, especially for those which required a lower ORP range and were sensitive to higher ORP values, such as methanogens (Chang et al., 2003).

6.3.6.5.4.3 Effects of Electron Acceptors on PAH Yields. The effects of three electron acceptors (Fe^{+2}; NO_3^{-1} and SO_4^{-2}) on the PAH yields were investigated in CSTR through high-oxygenated (4-6 mg/L DO) and un-oxygenated anaerobic conditions (0 mg/L DO). 25–45 mg/L Fe^{+2}, 56–98 mg/L NO_3^{-1} and 30–65 mg/L SO_4^{-2} were added to the reactors. The effect of Fe^{+2} on the PAH yields were found to be insignificant under oxidative aerobic conditions (4–6 mg/L DO). Good PAH consumption was obtained in presence of NO_3^{-1} and SO_4^{-2} under anaerobic conditions while the PAH yields were not changed under oxic conditions (Table 6.25).

Table 6.25 Effects of electron acceptors (Fe^{+2}, NO_3^{-1}, SO_4^{-2}) on mean total PAH yields (n = 3, mean values)

Parameter	Electron acceptors (Fe^{+2}, NO_3^{-1}, SO_4^{-2}) (mg/L)					
	Fe^{+2} (25-45 mg/L)		NO_3^{-1} (56-98 mg/L)		SO_4^{-2} (30-65 mg/L)	
	DO: 4-5	DO: 0	DO:4-5	DO: 0	DO :4-5	DO: 0
Mean total PAH yields (%)	95	98	95	99	95	99

DO: dissolved oxygen concentration (mg/L)

The reason for this is the destruction of PAHs by the utilization of nitrate and sulphate as electron acceptors under reductive anaerobic conditions resulting in high PAH yields, in the presence of anaerobic specific bacteria. These data agree with the results obtained by Chang et al. (2003) under anaerobic PAH degradation (Chang et al., 2003).

6.3.6.5.4.4 Effect of pH on PAH Yields. The pH was monitored through the experiment. No significant pH reductions were observed in aerated CSTRs while a significant reduction in pH was observed due to acidification and volatile fatty acid (VFA) productions in anaerobic CSTRs (Table 6.26).

Table 6.26 Effects of pH on mean total PAH yields (n = 3, mean values; pH: −log[H])

Parameter	pH		
	4	7	10
Mean total PAH yields (%)	56±1.22	95±1.10	53±1.45

The maximum PAH yields were obtained at pH 7 and 8 for aerobic and anaerobic conditions, respectively. The accumulation of H^+ or other acidic metabolites could reduce the pH (Table 6.26). If the activity of methanogenic bacteria is slowed down by unfavorable environmental conditions they will not utilize the VFAs at approximately the same rate as the VFAs produced by the acid formers. The pH change may signify metabolic activity leading to production of acidic or alkaline metabolites during breakdown of PAHs. Kim et al. (1999) observed that acidic pH conditions promote uptake of PAHs for degradation (Kim et al., 1999). Therefore, monitoring the pH of the media may be used to check the progress of PAH degradation.PAH degradation depends on the environmental conditions, number and type of the microorganisms and the nature and chemical structure of the chemical compound being degraded. They are biodegraded/biotransformed into less complex metabolites, and through mineralization into inorganic minerals, H_2O, CO_2 (aerobic) or CH_4 (anaerobic) and the rate of biodegradation depends on pH, temperature, oxygen, microbial population, degree of acclimation, accessibility of nutrients, chemical structure of the compound, cellular transport properties, and chemical partitioning in growth medium.

6.3.7 Effects of Increasing HRTs on Removal of COD_{dis} and Total PAHs with and without 15 mg/L RD Biosurfactant at a SRT of 25 days

6.3.7.1 Effects of Increasing HRTs on Removal of COD_{dis} and Total PAHs without RD Biosurfactant at a SRT of 25 days

The aerobic CSTR systems were operated through 90 days in order to determine the effect of increasing HRTs on the COD_{dis} and total PAHs removals. Different HRTs (2.5-3.3-5-10 days) were used in aerobic CSTR systems. The CSTR reactors were fed with real petrochemical wastewater without RD biosurfactant. Influent COD_{dis} and total PAHs concentration concentrations were approximately 2850 mg/L and 119.76 ng/mL, respectively. Influent, effluent concentrations and removal efficiencies of COD_{dis} and total PAHs are given in Table 6.26.

The effluent COD_{dis} concentrations were measured as 1026, 827, 656 and 1083 mg/L at HRTs of 2.5, 3.3, 5 and 10 days, respectively. As the HRTs were increased from 2.5 to 3.3 and to 5 days, the COD_{dis} removal efficiencies increased from 64% to 71% and to 77%. The PAHs removal yields also increased from 57% to 63% and 71%, in the CSTR system for the aforementioned HRT increase. The maximum COD_{dis} and total PAHs removal efficiencies were 77% and 71%, respectively, at a HRT of 5 days in the CSTR without RD. As the HRT was increased from 5 days to 10 days, the COD_{dis} and total PAHs removal efficiencies decreased from 77% to 62% and from 71% to 55%, respectively (Table 6.26). The COD_{dis} yield results obtained in this study is lower than those obtained by Shokrollahzadeh et al. (2008) (E=89%, at a HRT of 36 h). In our study the high COD_{dis} concentrations in the effluent could be attributed to the inhibitory effect of the non-degraded PAH and PAH metabolite accumulations under aerobic conditions in the CSTR, which was measured as COD_{dis}. Zhao et al., (2006) investigated the effect of HRT on pre-treat oil field wastewater at eight different HRTs varying between 4 and 16 h by immobilized microorganisms in aerobic reactor. 74% COD removal efficiency was obtained at a HRT of 4 h at an influent COD concentration of 300 mg/L. This result exhibited similar data with our study yields.

Table 6.26 Concentrations of COD_{dis} and total PAHs and removal efficiencies of influent, effluent wastewater in the CSTR system at increasing HRTs and at a SRT of 25 days without RD

	HRT 2.5 days		HRT 3.3 days		HRT 5 days		HRT 10 days	
	COD_{dis} (mg/L)	PAHs (ng/mL)	COD_{dis} (mg/L)	PAHs (ng/mL)	COD_{dis} (mg/L)	PAHs (ng/mL)	COD_{dis} (mg/L)	PAHs (ng/mL)
Influent	2850±11.42	119.76±1.36	2850±14.15	119.76±0.94	2850±16.84	119.76±0.81	2850±6.67	119.76±0.62
Effluent	1026±8.41	51.49±0.04	827±9.32	44.31±0.07	656±6.51	34.73±0.03	1083±4.68	53.89±0.04
Total removal eff. (%)	64±1.41	57±2.10	71±1.92	63±2.04	77±1.84	71±1.35	62±.1.17	55±1.92

6.3.7.2 Effects of Increasing HRTs on Individual PAHs Removal without RD at a SRT of 25 days

Among the HRTs used it was found that the aerobic CSTR system was efficient for all ring PAH removals at a HRT of 5 days (Table 6.27). The PAH yields varied between 54% and 80% at a HRT of 5 days. The yield of 3- and 4-ring PAH removal was almost 80%, 80% and 79% for ACT, CRB and CHR PAHs, respectively at a HRT of 5 days. The yield of 5- and 6-ring PAHs were 54% and 87% for BbF and BaP PAHs, respectively, for the same HRT. The PAH yields were lower at 2.5, 3.3 and 10 days HRTs compared to the 5 days HRT. The total PAH yields were 57%, 63% and 55% for HRTs 5, 10 and 40 days, respectively.

The maximum yield of 3 and 4 ring PAHs removals were 80% for ACT and CRB PAHs. The maximum PAH removals for 5 and 6 ring were %86 and 87% for IcdP and BaP, respectively, at a HRT of 5 days in the CSTR system. The optimum HRT for maximum individual PAHs removals (80%, 80%, 79%, 87%, 86% for ACT, CRB, CHR, BaP, IcdP) in the CSTR was found to be 5 days without RD biosurfactant.

The low PAH yields in low (2,5 , 3.3 days) HRTs could be attributed to the short HRTs which are not sufficiently long for uptake of the PAHs by the biomass through aerobic degradation in the CSTR reactor. The minimum total PAH yields obtained at a HRT of 10 days. This could be attributed to the death of PAH degrading bacteria, and to the aged bacteria without activity to metabolizing the PAHs at long HRTs in the CSTR system

The results obtained in our study agree with the studies performed by Shokrollahzadeh et al. (2008) and Zhao et al. (2006) which they found 80% and 84% PAHs removal efficiencies at a HRT of 4 days in the activated sludge process.

Table 6.27 Individual PAH removal efficiencies in the CSTR at increasing HRTs without RD at a SRT of 25 days

PAHs	Number of rings	Influent PAH concentration (ng/mL)	PAH removal efficiency (%) HRT 2.5 days	PAH removal efficiency (%) HRT 3.3 days	PAH removal efficiency (%) HRT 5 days	PAH removal efficiency (%) HRT 10 days
ACT	3	45.74±0.07	60±1.20	65±1.80	80±1.70	55±1.34
FLN	3	48.18±0.06	59±1.30	64±2.10	64±1.10	57±1.42
PHE	3	22.13±0.08	48±1.80	56±2.20	74±1.40	54±1.21
ANT	3	1.07±0.02	58±2.01	64±1.84	78±1.52	61±1.14
CRB	3	0.69±0.04	46±1.60	49±1.60	80±1.90	63±1.00
FL	4	0.49±0.03	50±1.55	54±1.50	71±2.10	57±1.71
PY	4	0.51±0.04	64±1.30	65±1.43	60±1.61	55±1.60
BaA	4	0.08±0.06	43±1.80	46±1.40	55±1.30	45±1.40
CHR	4	0.17±0.04	45±1.20	46±1.32	79±1.20	51±1.50
BbF	5	0.04±0.02	58±1.10	60±1.50	54±1.40	56±1.32
BkF	5	0.03±0.03	45±0.90	46±1.84	73±1.35	46±1.30
BaP	5	0.03±0.02	56±1.20	59±1.91	87±2.24	56±1.04
IcdP	6	0.18±0.01	53±1.80	54±2.10	86±2.11	62±1.40
DahA	6	0.39±0.03	53±1.80	55±1.70	84±0.99	57±1.20
BghiP	6	0.03±0.02	52±1.60	56±1.35	72±1.42	64±1.14
Total PAHs rem. eff. (%)		119.76±0.04	57±1.30	63±1.50	71±1.61	55±1.25

6.3.7.3 Effect of HRTs on COD_{dis} and Individual PAHs Removal Efficiencies with 15 mg/L RD at a SRT of 25 days in the Aerobic CSTR System

The effect of HRTs on the COD_{dis} and total PAHs removal efficiencies were shown in Table 4.28 in the presence of 15 mg/L RD. The COD_{dis} and total PAHs concentrations were 2850 mg/L and 119.76 ng/mL, respectively, in the influent of the CSTR system. The effluent COD concentrations were measured as 627, 314, 115 and 685 mg/L at a HRT of 2.5, 3.3, 5 and 10 days, respectively (Table 4.28). As the HRTs were increased from 2.50 to 5 days, the COD_{dis} and total PAHs removal efficiencies increased from 78% to 96% and from 74% to 95%, respectively, in the CSTR systems. The maximum COD_{dis} and total PAHs removal efficiencies were 96% and 95%, respectively, at a HRT of 5 days at a 15 mg/L RD in the CSTR. As the HRT increased from 5 days to 10 days, the COD_{dis} and total PAHs removal efficiencies decreased from 96% to 76% and from 95% to 71%, respectively (Table 6.28). As the HRTs were increased from 2.5 to 3.3 days at a 15 mg/L RD and at a SRT of 25 days, The ACT, FLN, and PHE PAHs (PAHs with 3 benzene rings) yields increased from 77%, 73%, 72%, to 85%, 84%, 76%, respectively (Table 6.29). The removal efficiencies of FL and CHR PAHs (PAHs with 4 benzene rings) increased from 74% and 67% to 84% and76%, respectively. The removal efficiencies of BbF and BaP PAHs (PAHs with 5 benzene rings) increased from 63% and 59% to 70% and 67%, respectively. The yields of DahA and BghiP PAHs with 6 benzene rings were 64%, 62% and 71%, 77%, at a RD concentration of 15 mg/L at HRTs of 2.50 and 5 days, respectively (Table 6.29). When the HRTs were increased from 3.3 to 5 days, the yields of ANT and CRB PAHs increased from 79% and 79% to 96% and 93%, respectively in the CSTR system. Similarly, the removal efficiencies of FL and CHR PAHs increased from 84% and 76% to 91% and 89%, respectively as the HRT was increased from 3.3 days to 5 days. The 5 and 6 ring PAHs removals were almost 88%, 89% and 87% for BkF, DahA and BghiP PAHs, respectively, at a HRT of 5 days (Table 6.29). When the HRTs were increased from 5 to 10 days, the yields of 3 and 4 ring PAHs removal decreased to 72% and 71%, respectively, for ACT and CRB at a 15 mg/L RD (Table 6.29).

Table 6.28 Concentrations of COD_{dis} and total PAHs and removal efficiencies of influent, effluent wastewater in CSTR system with 15 mg/L RD at increasing HRTs and at a SRT of 25 days

	HRT 2.5 days		HRT 3.3 days		HRT 5 days		HRT 10 days	
	COD_{dis} (mg/L)	PAHs (ng/mL)	COD_{dis} (mg/L)	PAHs (ng/mL)	COD_{dis} (mg/L)	PAHs (ng/mL)	COD_{dis} (mg/L)	PAHs (ng/mL)
Influent	2850±11.42	119.76±1.36	2850±14.15	119.76±0.94	2850±16.84	119.76±0.81	2850±6.67	119.76±0.62
Effluent	627±8.71	28.74±0.05	314±8.40	20.36±0.04	115±4.80	5.98±0.03	685±6.71	34.73±0.07
Total removal eff. (%)	78±1.85	74±1.10	89±2.10	83±1.31	96±1.21	95±1.75	76±1.36	71±1.82

Table 6.29 Individual PAH removal efficiencies in the CSTR at increasing HRTs with 15 mg/L RD at a SRT of 25 days

PAHs	Number of rings	Influent PAH concentration (ng/mL)	PAH removal efficiency (%) HRT 2.50 days	PAH removal efficiency (%) HRT 3.3 days	PAH removal efficiency (%) HRT 5 days	PAH removal efficiency (%) HRT 10 days
ACT	3	45.74±0.07	77±1.21	85±2.14	96±1.90	72±1.90
FLN	3	48.18±0.06	73±1.56	84±2.10	93±1.82	71±1.30
PHE	3	22.13±0.08	72±1.34	76±1.94	96±1.95	69±1.40
ANT	3	1.07±0.02	64±1.20	74±1.30	93±2.10	69±1.52
CRB	3	0.69±0.04	77±1.42	79±1.46	90±2.20	71±1.34
FL	4	0.49±0.03	74±1.50	84±1.75	91±2.40	75±1.21
PY	4	0.51±0.04	71±1.60	75±1.34	79±1.35	64±1.85
BaA	4	0.08±0.06	69±1.30	70±2.06	88±1.20	67±1.46
CHR	4	0.17±0.04	67±1.54	76±2.11	89±1.10	59±1.40
BbF	5	0.04±0.02	63±1.42	70±1.64	84±1.40	65±1.30
BkF	5	0.03±0.03	61±1.33	65±1.89	88±1.00	58±1.80
BaP	5	0.03±0.02	59±1.97	67±1.90	87±1.35	59±1.71
IcdP	6	0.18±0.01	61±1.67	69±1.60	86±1.67	68±1.65
DahA	6	0.39±0.03	64±2.01	71±1.50	89±1.65	68±1.60
BghiP	6	0.03±0.02	62±1.64	77±1.40	87±1.90	67±2.10
Total PAHs rem. eff. (%)		119.76±0.04	74±1.38	83±1.65	95±1.70	71±1.62

Similarly, the removal efficiencies of FL, PY, BaA and CHR PAHs yields also decreased from 91% and 79%, to 75% and 64% respectively, when the HRTs were increased from 5 to 10 days in presence of 15 mg/L RD. The PAHs removal efficiencies decreased from 89% and 87% to 68% and 67% for BaP and BghiP PAHs, respectively, as the HRTs were increased from 5 to 10 days in presence of 15 mg/L RD (Table 6.29).

In this study it was found that PAH treatment with 15 mg/L RD at a HRT of 5 days was beneficial for the degradation of all PAHs with maximum total PAH removal yield of 96% in the CSTR. It was found that the PAHs removal efficiencies are not related to the number of benzene rings (ANOVA, R^2= 0.56, F=12.34, P=0.01). Aerobic degradation in the CSTR system was very efficient for all ring PAH removals. The results of this study showed that 5 days HRT increased the removal efficiencies of both lower (3, 4 ring PAHs) and higher (5 and 6 ring PAHs) molecular weight PAH compounds in the presence of 15 mg/L RD at a SRT of 25 days. These results could be attributed to the resistance of the activated sludge, to the optimum operational conditions (such as SRT and HRT) and to the optimum RD dose used. In the study performed by Yin et al. (2009) RD was used to promote the biodegradation of the PHE PAH from an oil field wastewater by reducing the surface tension of the wastewater. This contributed to the increase in the PHE PAH yield 23 times at a 50 mg/L RD compared to RD free conditions.

In this study the removal efficiencies of PAHs with three, four, five and six benzene ring PAHs were higher than the PAHs removals obtained by Ma et al. (2008) who found 66 %-40% and 37% yields in PAHs with 2–4 rings, and in PAHs with 5–6 rings with the exception of BbF and IcdP in 4 days HRT. Wang et al. (2007) reported that 93% of the total PAHs in the influent removed through the biological treatment. Only 3 rings PAHs removed efficiently. However Wang et al. (2007) showed that 4, 5, and 6 rings PAHs were not removed during the aerobic treatment process easily. This result could be attributed to the short HRT (1 day) and to the sensitive organisms used in the aerobic reactor in the study performed by Wang et al. (2007).

6.3.8 Removals of COD and COD subcategories

Petrochemical wastewater characterization is now regarded as an indispensable step yielding all the necessary information for a reliable modelling and design of CSTR system, particularly for the treatment of refractory organics. The amount of organic carbon is only meaningful when it is expressed in terms of various fractions with different mechanisms and rates of biodegradation. In this respect, COD fractionation has been introduced as a very useful tool for the evaluation of aerobic activated sludge process treating petrochemical industry wastewater (Sponza & Gök, 2010).

The COD fractionation involves identification of inert COD (COD_i) together with readily biodegradable COD (COD_{rd}), slowly biodegradable COD (COD_{sd}) and metabolic products COD (COD_{mp}) fractions. The COD_{rd} is hypothesized to consist of simple soluble molecules that can be readily absorbed by the organisms, whereas the slowly biodegradable substrate is assumed to be made up of particulate/colloidal/complex organic molecules that require enzymatic breakdown prior to absorption and utilization.

The COD_i fractions of the wastewaters are of importance in meeting the discharge limitations as they by-pass the biological treatment system without being affected by the biochemical reactions and become the major constituent of the effluent. The inert fraction may be further subdivided into COD_i (this fraction in the influent bypasses the system without affecting the biochemical reactions in the reactor) and particulate COD_i (this fraction is initially present in the wastewater or metabolically produced during the aerobic treatment and leaves the process with excess sludge). In the aerobic hydrolysis COD_{sd} is transformed into COD_{rd} and a small fraction of COD_i. The soluble microbial products generated by the hydrolysis of slowly degradable organics to readily degradable organics and by the decay of biomass through endogenous phase are directly converted into stored material in bacterial cells. These stored compounds are subsequently used as a carbon and energy source for growth purposes.

Three reactors namely control (glucose) reactor, petrochemical wastewater reactor (with 15 mg/L RD) and petrochemical wastewater reactor (non-added RD) were batch operated to determine the removal efficiencies of different COD subcategories.

6.3.8.1 Determination of the Total COD (COD_{total}) and Dissolved COD (COD_{dis}) in glucose, RD Added and Non-Added CSTR Systems at a SRT of 25 days

The influent COD_{total} concentrations were determined as 1781 mg/L in the glucose reactor, RD added and non-added reactors, respectively (Table 6.30). The influent COD_{dis} concentrations were determined as 1195 mg/L in the glucose reactor and 1025 mg/L in the RD added and non-added CSTR, respectively (Table 6.30). The effluent COD_{total} concentrations were determined as 245 mg/L, 289 mg/L and 655 mg/L in the glucose, RD added and non-added CSTR, respectively after 17 days of operation time. In addition, the effluent COD_{dis} concentrations were found as 115 mg/L, 172 mg/L and 238 mg/L in the glucose, RD added and non-added CSTR, respectively (See Table 6.30). The COD_{total} removals were around 89%, 88% and 60% in the glucose, RD added and non-added CSTR, respectively (See Table 6.30). The COD_{dis} removal efficiencies were obtained as 92%, 93% and 80% in the glucose, RD added and non-added CSTR, respectively (See Table 6.30). The maximum COD_{dis} removal efficiency was found as 93% in the 15 mg/L RD added CSTR reactor at a SRT of 25 days. The COD_{total} concentrations in the effluent with 15 mg/L and without RD were 289 and 655 mg/L, respectively. These results show that the COD_{total} could not be easily metabolised by the activated sludge bacteria at a SRT of 25 days in the CSTR reactor without RD. The effluent COD_{dis} concentrations was found as 115 mg/L, 172 mg/L and 238 mg/L in the glucose reactor and in the RD added and non-added reactor systems at a SRT of 25 days. The COD_{dis} can be easily used by the bacteria and this type of COD_{dis} can be used easily as carbon source in the glucose and CSTR containing 15 mg/L RD compared to the CSTR reactor without RD.

Table 6.30 Influent, effluent concentrations of COD_{total} and COD_{dis} and removal efficiencies of the reactors at a SRT of 25 days (n=3, mean ± SD)

Reactor type		Parameters	COD_{total} (mg/L)	COD_{dis} (mg/L)
Reactor efficiencies	Control Reactor (glucose)	Influent	1781±23.05	1195±45.90
		Effluent	245±9.04	115±9.89
		Removal Efficiency (%)	89±1.34	92±1.23
	15 mg/L RD (petrochemical wastewater)	Influent	1781±23.56	1025±23.78
		Effluent	289±2.78	172±8.67
		Removal Efficiency (%)	88±1.33	93±0.90
	Without RD (petrochemical wastewater)	Influent	1781±45.78	1025±45.78
		Effluent	655±12.78	238±9.67
		Removal Efficiency (%)	60±1.45	80±0.88

6.3.8.2 Determination of Readily Degradable COD (COD_{rd}) and Slowly Degradable COD (COD_{sd}) in Glucose, RD Added and Non-Added CSTR at a SRT of 25 days

The COD_{rd} and COD_{sd} concentrations in the influent, effluent and removal efficiencies of the reactors are given Table 6.31. The COD_{rd} concentrations were determined as 1589 mg/L, 500 mg/L and 500 mg/L in the influent of the glucose, RD added and non-added reactors, respectively. The COD_{sd} concentration were found as 4 mg/L, 1025 mg/L and 1025 mg/L in the influent the glucose, RD added and non-added reactors, respectively. The effluent COD_{rd} concentrations were determined as 42 mg/L, 109 mg/ L and 158 mg/L in the RD added, non-added and glucose reactors, respectively, at a SRT of 25 days (Table 6.31). The effluent COD_{sd} concentrations were determined as 1 mg/L, 261 mg/L, 1003 mg/L in the glucose, RD added and non-added reactors respectively, at a SRT of 25 days (Table 6.31).

Table 6.31 Influent, effluent concentrations of the COD_{rd} and COD_{sd} and removal efficiencies of the CSTR reactors at a SRT of 25 days (n=3, mean ± SD)

Reactor type		Parameters	COD_{rd} (mg/L)	COD_{sd} (mg/L)
Reactor efficiencies	Control Reactor (glucose)	Influent	1589±4.78	4±0.11
		Effluent	158±2.67	1±0.01
		Removal Efficiency (%)	90±4.89	75±0.01
	15 mg/L RD (petrochemical wastewater)	Influent	500±21.37	1025±14.89
		Effluent	42±3.09	261±4.56
		Removal Efficiency (%)	91±2.67	74±2.98
	Without RD (petrochemical wastewater)	Influent	500±25.89	1025±14.89
		Effluent	109±2.27	1003±2.11
		Removal Efficiency (%)	78±2.27	2±0.01

The COD_{rd} and COD_{sd} were removed with treatment efficiencies of 91%, 74% and 78%, 2% respectively, in RD added and non-added reactors (Table 6.31). The COD_{rd} and COD_{sd} removal efficiencies were found as 90% and 75% in the glucose reactor, respectively. The COD_{dis} removal efficiencies were obtained as 92%, 93% and 80% in the glucose, RD added and non-added CSTR, respectively. The maximum COD_{rd}, COD_{sd} and COD_{dis} removal efficiencies were found as 75%, 74% and 93%, respectively in 15 mg/L RD added reactor**.** As the 15 mg/L RD added in the CSTR reactor at a SRT of 25 days, the COD_{rd} is taken up by the activated sludge bacteria degrading PAHs in a matter of minutes and metabolized, giving rise to a high unit rate of oxygen demand for synthesis. This COD was assumed to consist of simple molecules able to pass through the cell wall and immediately be used for energy and synthesis by the organisms. High removals in COD_{rd} and COD_{sd} indicated the COD_{rd} produced through the hydrolysis of slowly degradable organics was also sufficiently used by the activated sludge bacteria at a 15 mg/L RD and at a SRT of 25 days in the CSTR reactor.

6.3.8.3 Determination of the Inert COD (COD_i) and Metabolic Products COD (COD_{mp}) in Glucose, RD Added and Non-Added CSTR at a SRT of 25 days

The COD_i was measured using the glucose comparison method. This method involves running three batch reactors, two with the wastewater to be studied and the third with glucose. One of the wastewater reactors has the COD_{total}, and the second has the total COD_{dis}, whereas the initial COD_{dis} in the glucose reactor is adjusted to equal COD_{dis} value. The experimental studies are performed until all the biodegradable COD is depleted, where the COD profiles reach a plateau and stay unchanged. The difference between glucose COD_{dis} and wastewater COD gives the COD inert (See Table 6.32).

Table 6.32 Influent, effluent concentrations of COD_i and COD_{mp} and removal efficiencies of the reactors at a SRT of 25 days (n=3, mean ± SD)

Reactor type		**Parameters**	**COD_i (mg/L)**	**COD_{mp} (mg/L)**
Reactor efficiencies	Control Reactor (glucose)	Influent	10±0.98	3±0.70
		Effluent	12±1.01	8±1.09
		Removal Efficiency (%)	-	-
	15 mg/L RD (petrochemical wastewater)	Influent	370±3.08	5±1.78
		Effluent	68±1.98	0±0.05
		Removal Efficiency (%)	82±2.03	99±0.01
	Without RD (petrochemical wastewater)	Influent	370±4.18	5±0.20
		Effluent	432±3.56	25±1.63
		Removal Efficiency (%)	-	-

Figure 6.11 shows the COD_i concentration in the influent of RD added and non-added reactor. Glucose COD_{dis} and COD_{dis} of the wastewater reached steady-state conditions after 6 day of operation in the control reactor for influent wastewater throughout 17 days of continuous operation at a SRT of 25 days.

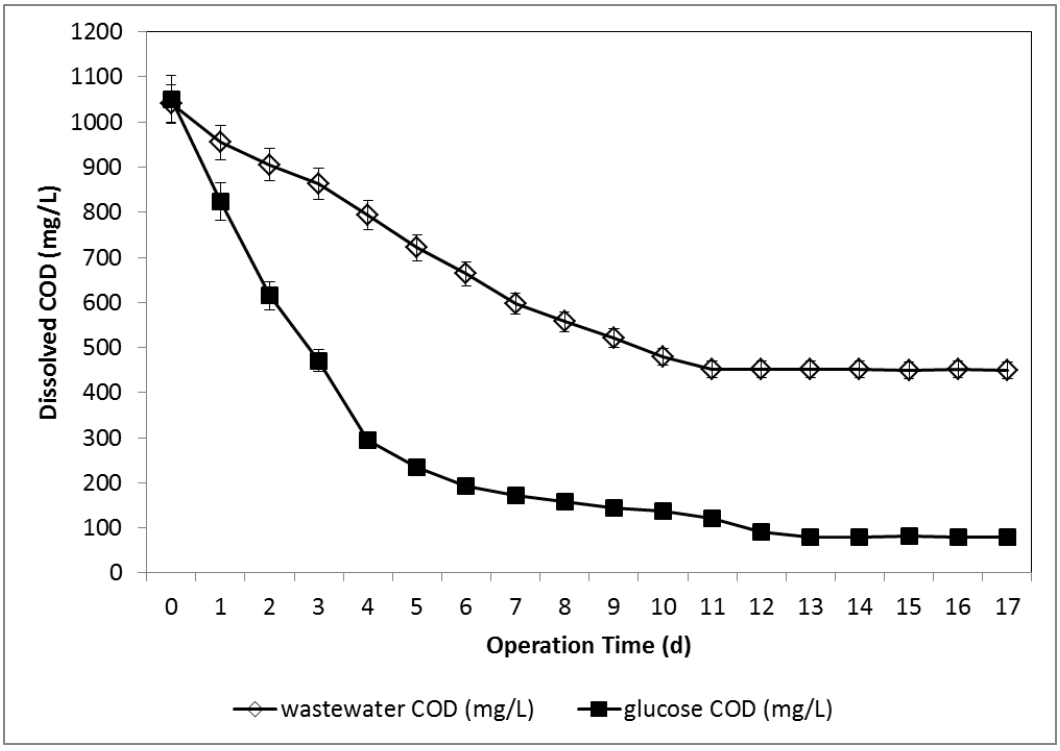

Figure 6.11 Variation of the COD_{dis} concentration with time in the influent of RD added and non-added reactor (COD_i =370 mg/L)

The influent COD_i concentration was determined as 370 mg/L the influent of the RD added and non-added reactors respectively, at a SRT of 25 days (Figure 6.11). The effluent COD_i concentration were determined as 12 mg/L (Figure 6.12), 68 mg/L (Figure 6.13) and 432 mg/L in the glucose reactor, 15 mg/L RD added and non-added CSTR reactors, respectively. The non-degraded COD_i concentrations were measured as 370 mg/L in the influent of the 15 mg/L RD containing reactor, while the COD_i concentrations decreased to 68 mg/L (See Figure 6.13) in the effluent of the reactor resulting in a COD_i removal efficiency of 82% in the CSTR reactor containing 15 mg/L RD (See Table 6.32). This could be explained by the uptake of COD_i to the cell together with hydrolyzed slowly degradable organics through fast PAH and COD diffusions with RD. As shown in Table 6.32, the COD_i concentrations increased from 10 mg/L to 12 mg/L and from 370 to 432 mg/L in the effluent of the CSTR reactor with glucose and without RD, respectively. The COD_i concentrations decreased in the aerobic reactor containing 15 mg/L RD as aforementioned.

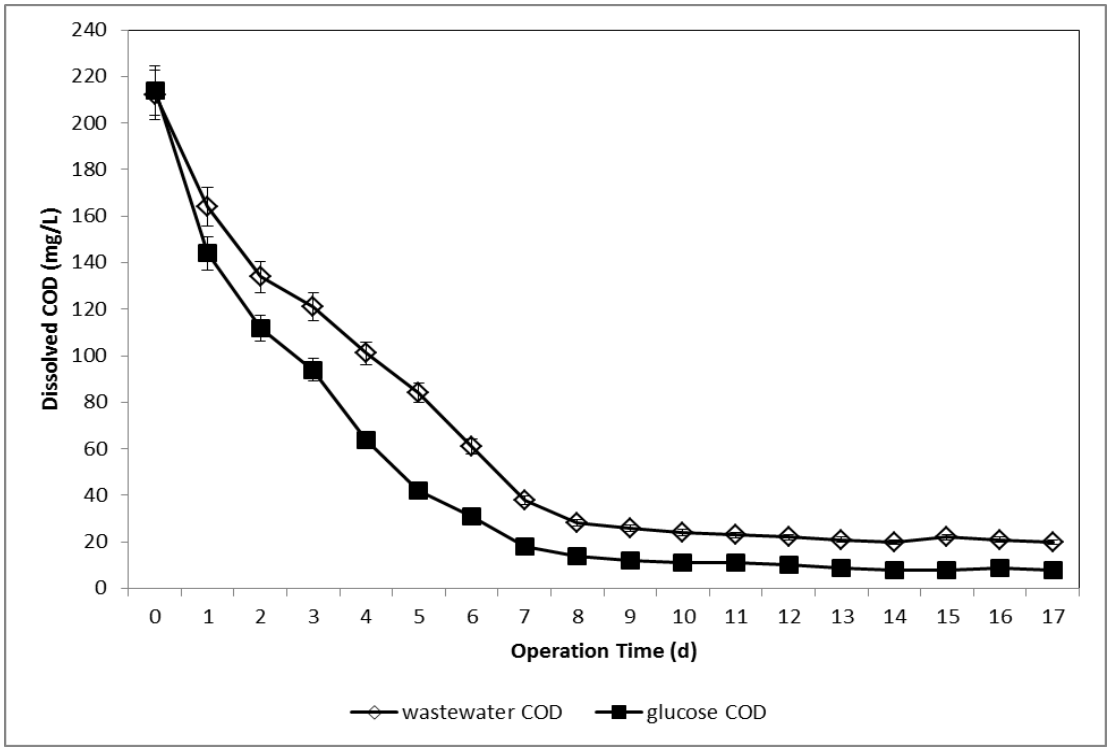

Figure 6.12 Variation of the COD_{dis} concentration with time in the effluent of glucose reactor (COD_i =12 mg/L)

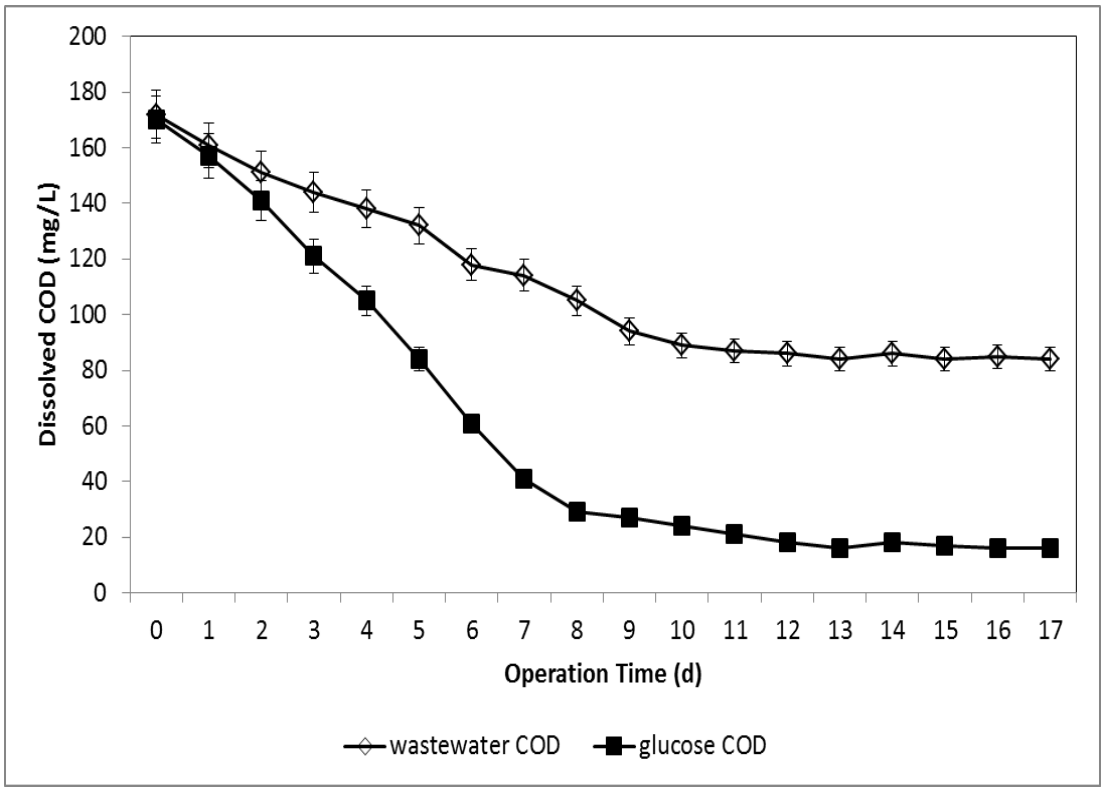

Figure 6.13 Variation of the COD_{dis} concentration with time in the effluent of the 15 mg/L RD added reactor (COD_i =68 mg/L)

The increases in the COD_i concentrations originating from the metabolic products were generated by the microorganisms through aerobic hydrolyzis in RD free reactors. The studies performed by Ekama et al., (1986) showed that the influent COD_i concentration potentially increased due to soluble microbial products generation in activated sludge system treating refractory organics containing no-biosurfactant. The CSTR reactor without RD exhibited low removal efficiencies compared to the 15 mg/L RD added CSTR reactor. This indicates that the biodegradability of the COD_{sd} in petrochemical industry wastewater increased as did COD_i uptaken to the cell with RD under aerobic treatment conditions in the CSTR reactor.

The COD_{mp} concentrations were measured as 3 mg/L, 5 mg/L and 5 mg/L in the influent of glucose reactor, RD added and non-added CSTR reactor, respectively. The COD_{mp} concentrations decreased from 5 mg/L to 0 mg/L, in the effluent of RD added CSTR reactor resulting in a COD_{mp} removal efficiency of 99% (See Table 6.31).COD_{mp} generated by the hydrolysis of slowly degradable organics to readily degradable organics and by the decay of biomass through endogenous phase are directly converted into stored material in bacterial cells with 15 mg/L RD. These stored compounds are subsequently used as a carbon and energy source for growth purposes in the RD added system. Bacteria in the aerobic CSTR process might be able to utilize the COD_{mp} directly and the aforementioned stored components of COD. The study performed by Orhon et al. (1999) reported that the COD_{dis}, and COD_{mp} concentrations were measured 800 and 65 mg/L, respectively, in the synthetic wastewater. The COD_{dis}, COD_i and COD_{mp} were determined 180, 18 and 10 mg/L in domestic wastewater, respectively, while the COD_{dis}, COD_i and COD_{mp} were found 1260, 185 and 125 mg/L in industry wastewater, respectively (Orhon et al., 1999). Selçuk et al. (2006) reported that 33% COD_{dis} removal efficiency was obtained from a simulated textile industry wastewater with an influent COD_{dis} of 950 mg/L. The COD_i and COD_{mp} concentration were determined 280 mg/L, 10 mg/L, respectively, after pre-ozonation process. The COD_i and COD_{mp} efficiencies were found 33% and 7%, respectively. The COD_{dis}, COD_i and COD_{mp} yields found in this study are higher than the yield obtained by Selçuk et al. (2006).

6.4 The Performance of Anaerobic ITBR System Treating the Real Petrochemical Industry Wastewater

6.*4.1 Start-Up Period of the Anaerobic Inverse Turbulent Bed Reactor (ITBR) in a Continuous Mode at a Constant HRT without Biosurfactant*

6.4.1.1 COD_{dis} and Total PAHs Removals in the Anaerobic ITBR System at the Start-up Period

Anaerobic inverse turbulent bed reactors (ITBR) are now commonly used for brewery, food-processing, winery, paper, yeast, pharmaceuticals, chemical industry wastewater (Buffeiere et al., 2000, Cresson et al., 2006; Cresson et al., 2007; Alvarado-Lassman et al., 2008; Sowmeyan and Swaminathan 2007). Several systems were used to adapt the fluidization process for the anaerobic treatment of industry wastewater inverse fluidized beds" or "down-flow fluidized beds" (Garcia-Calderon et al. 1998; Arnaiz et al., 2005) and, more recently, the anaerobic inverse turbulent bed reactor (Arnaiz et al., 2006; Cresson et al., 2007). In the ITBR configuration, the originality arises from the use of a carrier with a specific density lower than the liquid and a fluidization only ensured by an up-flow current of gas (Sanchez et al., 2005). Previous studies on ITBR show several advantages compared to classical up-flow and down-flow fluidization. First, bed height control results automatically from the location of the injection device (Arnaiz et al. 2006). Second, a gas injection is simpler than a liquid recycling, reducing clogging problems. Third, the low energy requirement, because of the low fluidization velocities required (Buffiere et al. 2000; Arnaiz et al., 2003).

Among all phases to run an anaerobic wastewater treatment process, the start-up remains the longest, the most important step and the most difficult period to control. Start-up periods between 2 and 9 months have been reported for continuous operation of fluidized bed reactors under steady-state conditions (Alvarado-Lassman et al., 2008**).** It was reported that the start-up period in the ITBR reactors were shortened (Cresson et al., 2007).

The adaptation period is very important since the bacterial population used as seed is going to be exposed to real petrochemical wastewater containing the PAHs in the anaerobic ITBR system. In order to acclimate the partially granulated biomass in the anaerobic ITBR reactor, this reactor was operated through 300 days fed real petrochemical wastewater under steady-state conditions. The operational conditions were given in detail in Section 5.3 and in Table 5.9.

When the anaerobic ITBR was started to operate with a real petrochemical wastewater containing approximately 2850 mg/L of COD, in the influent, the variations in COD concentrations and COD removal efficiencies were illustrated in Figure 6.14 at a constant HRT of 2.75 days and constants SRT and OLR of 63 days and 1.04 kg COD/m^3day, respectively.

On day 40, 11% COD efficiency was achieved at an OLR of 1.04 kg COD/m^3.day, at a SRT of 63 days and at a HRT of 2.75 days in the anaerobic ITBR system. 19% and 35% COD removal efficiencies were obtained on days 60 and 120, respectively. 55%, 62% and 72% COD removal efficiencies were achieved on 160, 200 and 230 days of continuous operation, respectively. The COD removal efficiencies remained stable as 81% between 240 and 300 days. A steady state condition was defined with a COD removal efficiency of more than 80% and this is considered acceptable for the start-up of an anaerobic reactor and acclimatization to the anaerobic systems (Enright et al., 2007). The studies investigating the anaerobic biodegradation of refractory and high strength with anaerobic ITBR system are limited with a study. Until now, it was found only a research investigating the removal of COD from the petrochemical industry wastewater in the anaerobic ITBR. Ochieng et al. (2003) reported a maximum COD removal efficiency of 36% at a HRT of 1 day in the fluidized bed reactor treating the petroleum wastewaters. The COD yield obtained in our study (81%) study is higher than those obtained by Ochieng et al. (2003). Recent studies showed that the ITBR reactor was the only used to treat the wine distillery wastewater under anaerobic methanogenic conditions (Buffeiere et al., 2000, Alvarado-Lassman et al., 2008; Sowmeyan and Swaminathan; Cresson et al., 2007; Alvarado-Lassman et al., 2008).

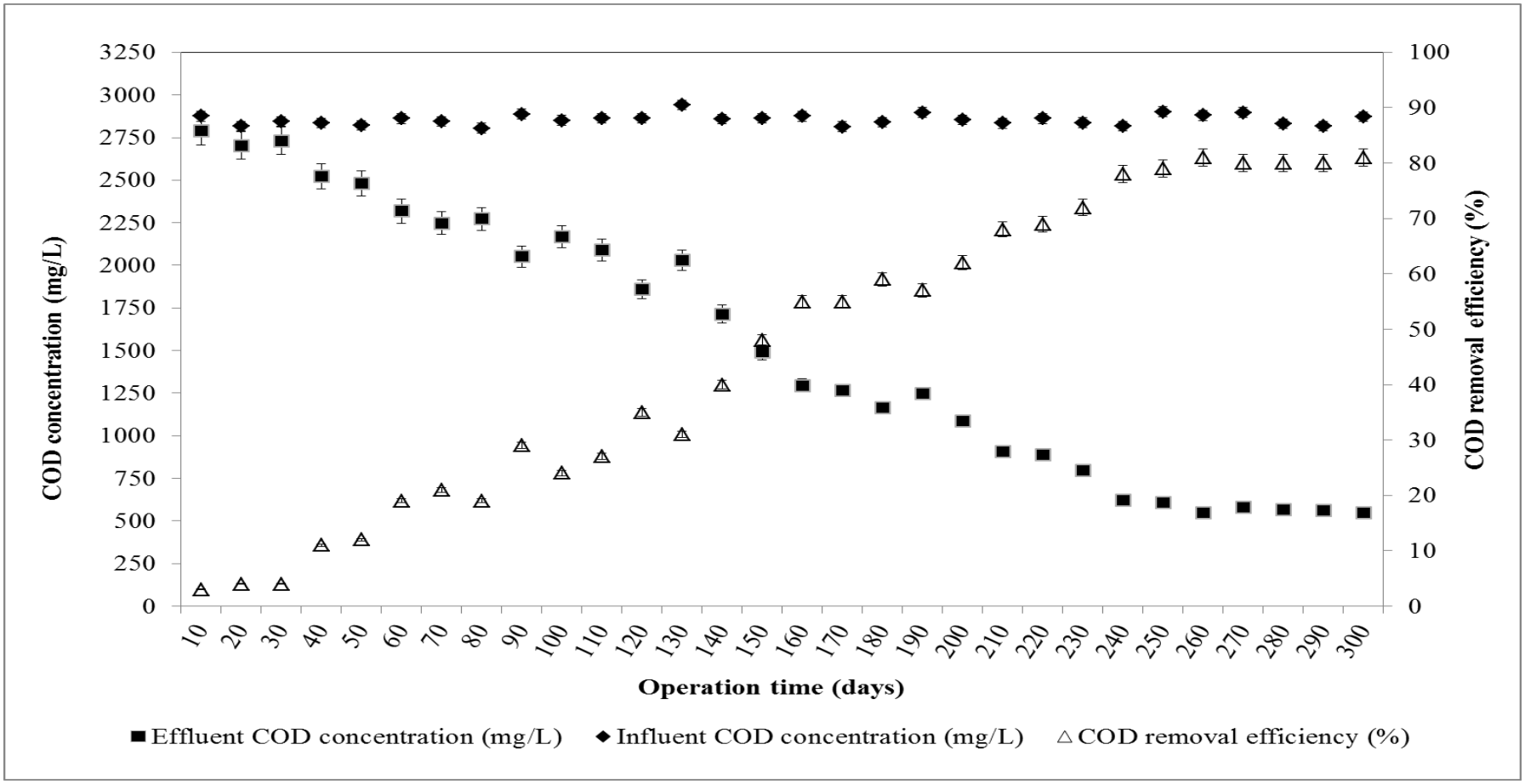

Figure 6.14 Variation of influent, effluent COD concentration (mg/L) and COD removal efficiencies (%)versus 300 day of continuous operation time in the anaerobic ITBR for the start-up period (HRT=2.75 days, SRT= 63 days and OLR=1.04 kg COD/m^3day)

In this study it was reported that the anaerobic ITBR was appeared to be a good option for the anaerobic treatment of high strength wine distillery wastewater. The systems attained high OLR with good COD removal rates and it exhibited a good stability in the reactor system. In this study 78% maximum COD yield was obtained at an OLR 15.8 kg COD/m^3.day.

Cresson et al. (2007) studied the anaerobic removal of wine based wastewater in an ITBR system. The COD removal efficiency attained to 80%, at an OLR of 8 g COD/L.day. Alvarado-Lassman et al. (2008) used an anaerobic inverse fluidized bed reactors (IFBR) to remove the brewery wastewaters. Greater than 90% COD yield was obtained at an OLR of 70 g COD/L.day.

When the anaerobic ITBR was started to operate with a real petrochemical wastewater containing 292 ng/mL of total PAH concentration in the influent, the variations in PAHs concentrations and total PAHs removal efficiencies were illustrated in Figure 2 at a HRT of 2.75 days, at a SRT of 63 days and an OLR of 1.04 kg COD/m^3day.

The steady state conditions were defined with total PAH removal efficiencies higher than 70% on day 210 at a HRT of 2.75 days, at a SRT of 63 days at an OLR of 1.04 kg COD/m^3day. The PAHs concentrations in the effluent varied between 81.21 and 121.39 ng/mL throughout operation period. The effluent total PAHs concentrations were determined as 121.39, 105.12 and 114.67 ng/mL on days 30, 60 and 90 in the anaerobic ITBR, respectively. The effluent concentrations of PAHs were measured as 92.58, 102.20 and 89.21 ng/mL on days 120, 150 and 180. The effluent total PAHs concentrations remained stable as 81.47 ng/mL after 210 days of operation period. The total PAHs removal efficiencies were 58% and 64% on days 30 and 60, respectively. The total PAHs removal efficiencies were obtained as 61%, 68% and 65% on days 90, 120 and 150, respectively. The total PAHs removal efficiencies remained stable as 72% after a continuous operation period of 210 days and remained the same between days 210 and 300. This showed that the anaerobic ITBR system reached steady-state conditions (See Figure 6.15).

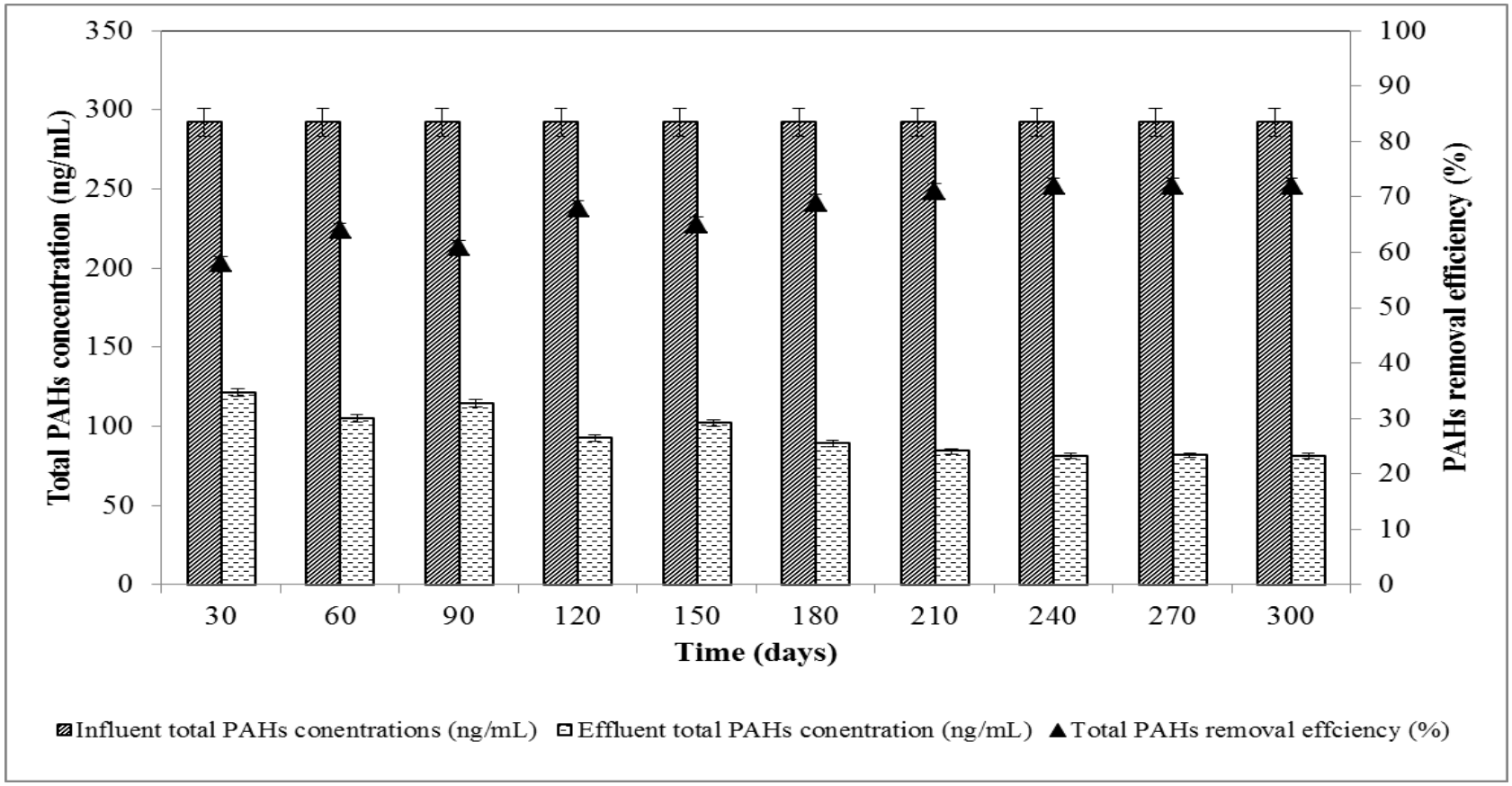

Figure 6.15 Variations of effluent total PAH concentrations (ng/mL) and percentage removal efficiencies versus 300 day of continuous operation time in the anaerobic ITBR for the start-up period (HRT=2.75 days, SRT= 63 days, OLR=1.04 kg COD/m^3day and 106.18 ng PAH/mL.day)

6.4.1.2 Variation of Total Gas, Methane Gas and Methane Percentages in the Start-Up Period in the Anaerobic ITBR System

Figure 6.16 shows the methane gas and methane percentages in the anaerobic ITBR during the start-up period. Total gas, methane gas and the methane percentages were increased with 300 days of continuous operation. The total gas, methane gas and methane percentage reached 3.05 L/day, 1.29 L/day and 42.3%, respectively after 210 days of operation period at a HRT of 2.75 days, at a SRT of 63 days and at an OLR of 1.04 kg COD/m^3day. Total gas, methane gas and methane production rate were measured as 3.2, 1.4 L/day and 43.8%, respectively, at the end of 270 days and remained the same between days 230 and 300. This showed that the anaerobic ITBR reached steady-state conditions. Sowmeyan et al. (2008) studied performance of anaerobic ITBR for treating high strength distillery wastewater during start-up phase. Biogas production was very low during the first 10 days. The biogas and methane production rate at the end of 65 days were 66.1 L/day and 42.9 L CH_4/day, respectively at an OLR of 35 kg COD/m^3.day and at a HRT of 0.16 day. The methane and total gas productions obtained in this study is lower than those by Sowmeyan et al. (2008). The reason of this could be explained by the different wastewater characterization and operation conditions (OLR and HRT) which decrease the activity of methanogens.

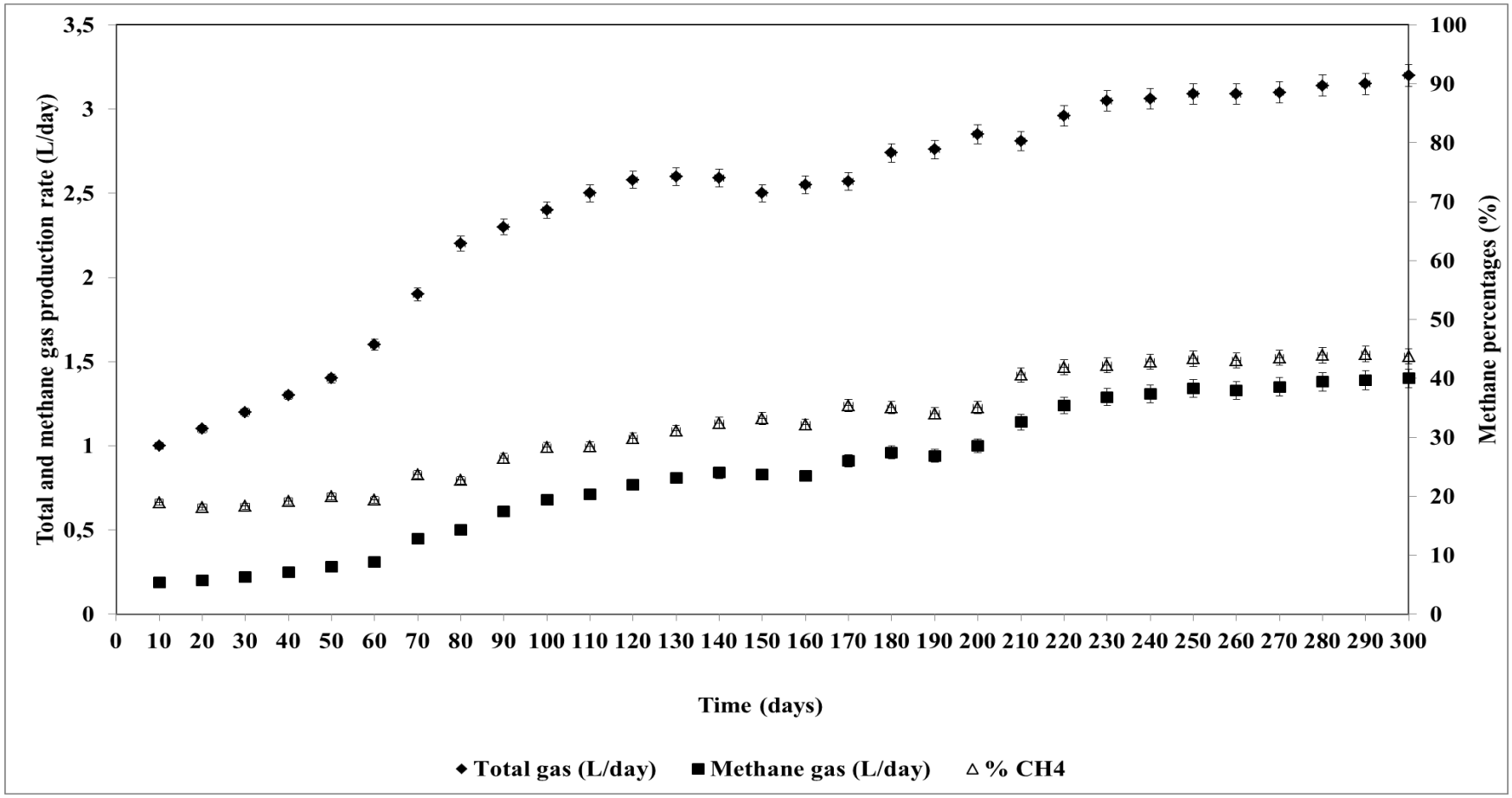

Figure 6.16 Total gas, methane gas and methane percentage during the start-up period in the anaerobic ITBR (HRT=2.75 days, SRT= 63 days and OLR=1.04 kg COD/m^3day)

6.4.1.3 Variation in Specific Methanogenic Activity (SMA) Through the Start-Up Period in the ITBR System

The SMA is an indicator of methanogenic activity of biomass in the anaerobic ITBR. Figure 6.17 illustrates the plot of the SMA of the biomass taking from bottom of the sampling point of the anaerobic ITBR. Throughout the start-up period, the SMA increased from 0.25 g CH_4-COD/g VSS.day to 0.59 g CH_4-COD/g VSS.day at a HRT of 2.75 days, at a SRT of 63 days and an OLR of 1.04 kg COD/m^3day. This could be attributed to the resistance of anaerobic sludge to real petrochemical wastewater containing 292 ng/mL PAH in the end of the start up period.

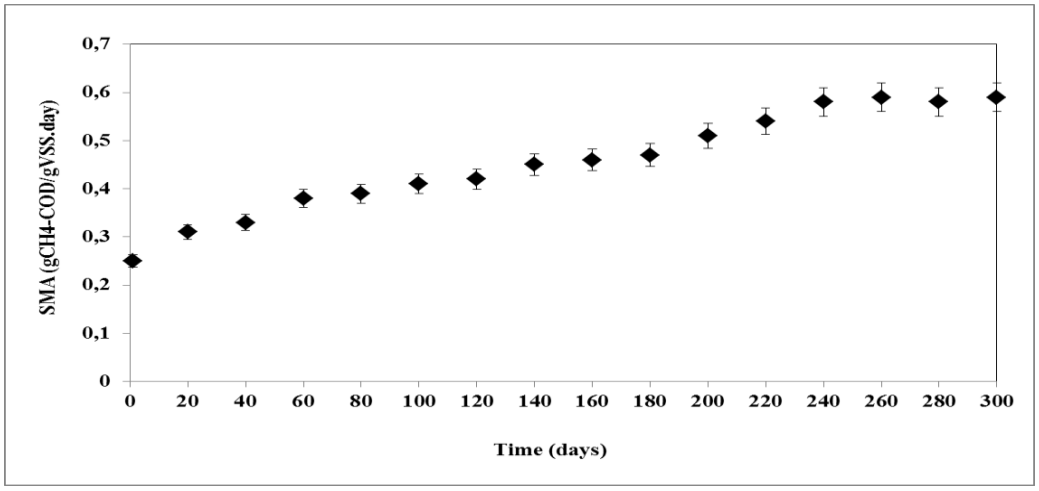

Figure 6.17 SMA values in the anaerobic ITBR system through the start-up period (HRT=2.75 days, SRT= 63 days and OLR=1.04 kg COD/m^3day)

This showed that the anaerobic ITBR system reached steady-state conditions. In this study the SMA values found in the anaerobic ITBR were higher than those studies performed by Fang et al. (2006) in an anaerobic sludge blanket reactor treating aromatic compound from chemical industry wastewater (SMA of 0.09 g CH_4 COD/gVSS.day) and by Veeresh et al. (2005) in an upflow anaerobic sludge blanket treating petrochemical industry wastewater containing phenolic compounds (SMA value of 0.23 g CH_4 COD/gVSS.day). Subramanyam and Mishra (2008) reported 0.34 and 0.20 g CH_4 COD/gVSS.day SMA values in an upflow anaerobic sludge blanket treating 1 g/L and 2 g/L catechol respectively, from petroleum wastewater at a HRT of 0.33 days.

6.4.2 Effects of Increasing RD Concentrations on COD_{dis}, Total PAHs and Individual PAH Removal Efficiencies in the Anaerobic ITBR

6.4.2.1 Effects of Increasing RD Concentrations on COD_{dis}, Total PAHs Removal Efficiencies in the Anaerobic ITBR

The anaerobic ITBR was inoculated with anaerobic sludge in order to determine the performance of the reactor in the presence of raw petrochemical wastewater containing the 15 PAHs (ACT, FLN, PHE, ANT, CRB, FL, PY, BaA, CHR, BbF, BkF, BaP, IcdP, DahA, and BghiP). The HRT was kept constant at 2.75 days throughout continuous (between days 300 and 620) period, at increasing RD concentrations at a HRT of 2.75 days, at a SRT of 63 days, OLR of 1.04 g COD_{dis}/m^3.day and OLR of 106.18 ng PAH/mL.day.

In order to determine the optimum RD dose for the maximum removals of COD_{dis} and PAHs, 15 mg/L, 50 mg/L, 75 mg/L, 100 mg/L and 150 mg/L RD were administered to the feed of the anaerobic ITBR system. An anaerobic ITBR reactor was operated only with real petrochemical wastewater and named as RD without RD biosurfactant. The COD_{dis} and total PAHs concentrations in the influent of the petrochemical industry wastewater were around 2850 mg/L and 292 ng/mL, respectively and then decreased to 150-830 mg/L and 29.29-71.29 ng/mL in the effluent of the anaerobic ITBR system after 320 days of operation period depending on increasing RD concentrations (Figures 6.18 and 6.19). These tables showed the influent, effluent COD_{dis} and total PAHs and their removal efficiencies with operation (between days 300 and 620) time at increasing biosurfactant concentrations in the anaerobic ITBR system at a HRT of 2.75 days, at a SRT of 63 days, OLR of 1.04 g COD_{dis}/m^3.day and OLR of 106.18 ng PAH/mL.day.

The COD_{dis} removal efficiencies in ITBR systems containing no RD, 15, 50, 75, 100, 150 mg/L RDs were 72%, 78%, 83%, 95%, 84%, 74%, on days 360, 426, 490, 554 and 620, respectively.

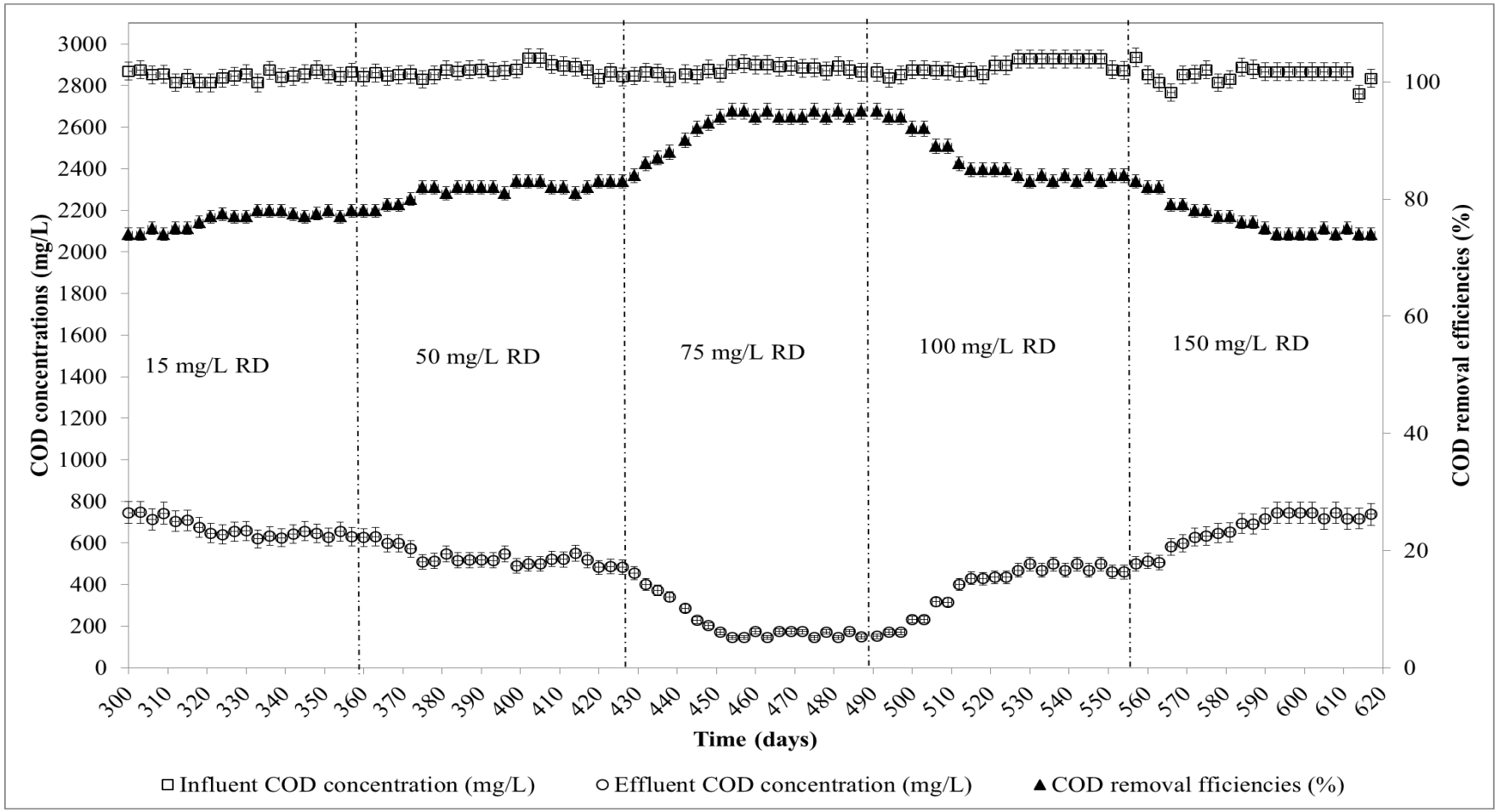

Figure 6.18 Variations of COD_{dis} concentrations (mg/L) and removal efficiencies (%) in the continuous operation periods in the ITBR system containing 15, 50, 75, 100 and 150 mg/L RD concentrations (influent COD_{dis} concentration=2760-2935 mg/L; effluent COD=150-830 mg/L)

As the RD concentrations were increased from 15 to 50 and 75 mg/L the total PAH removals increased from 80% to 86% and 89% respectively (Figure 6.19). The yields of PAH decreased from 89% to 86% and 78%, as the RD concentration was increased from 75 to 100 and 150 mg/L, respectively, in the anaerobic ITBR system. The maximum PAH removals with 75 mg/L RD could be attributed to the increase in solubility of the PAHs. These PAHs were probably utilized together with COD_{dis} equivalent organics as carbon and energy sources by the anaerobic *archeae* organisms in the anaerobic batch reactor as reported by Yu et al., (2007).

At high RD concentrations (100 and 150 mg/L) the PAH removals were low in the anaerobic ITBR system (Figure 6.19). The further addition of the RD concentration (100 and 150 mg/L) could have detrimental effects on anaerobic bacteria in the anaerobic ITBR system. This may be due to the toxic effect of the high biosurfactant concentrations on the biomass and due to the low rate of mass transport between biosurfactant micelles and death bacteria in the aqueous phase of the anaerobic batch reactor (Yu et al., 2007; Sartoros et al., 2005). The maximum COD_{dis} and total PAH removal efficiencies were obtained at a 75 mg/L RD concentration in the anaerobic ITBR at a HRT of 2.75 days, at a SRT of 63 day at an OLR of 1.04 kg COD_{dis}/m^3.day. Potential advantages of optimum RD concentrations include their unusual structural diversity that may lead to unique properties, the possibility of cost-effective production, biodegradability, effectiveness at extreme temperature and pH. These properties make RD a promising choice for applications in enhancing PAH degradation from petrochemical wastewaters (Mulligan, 2005).

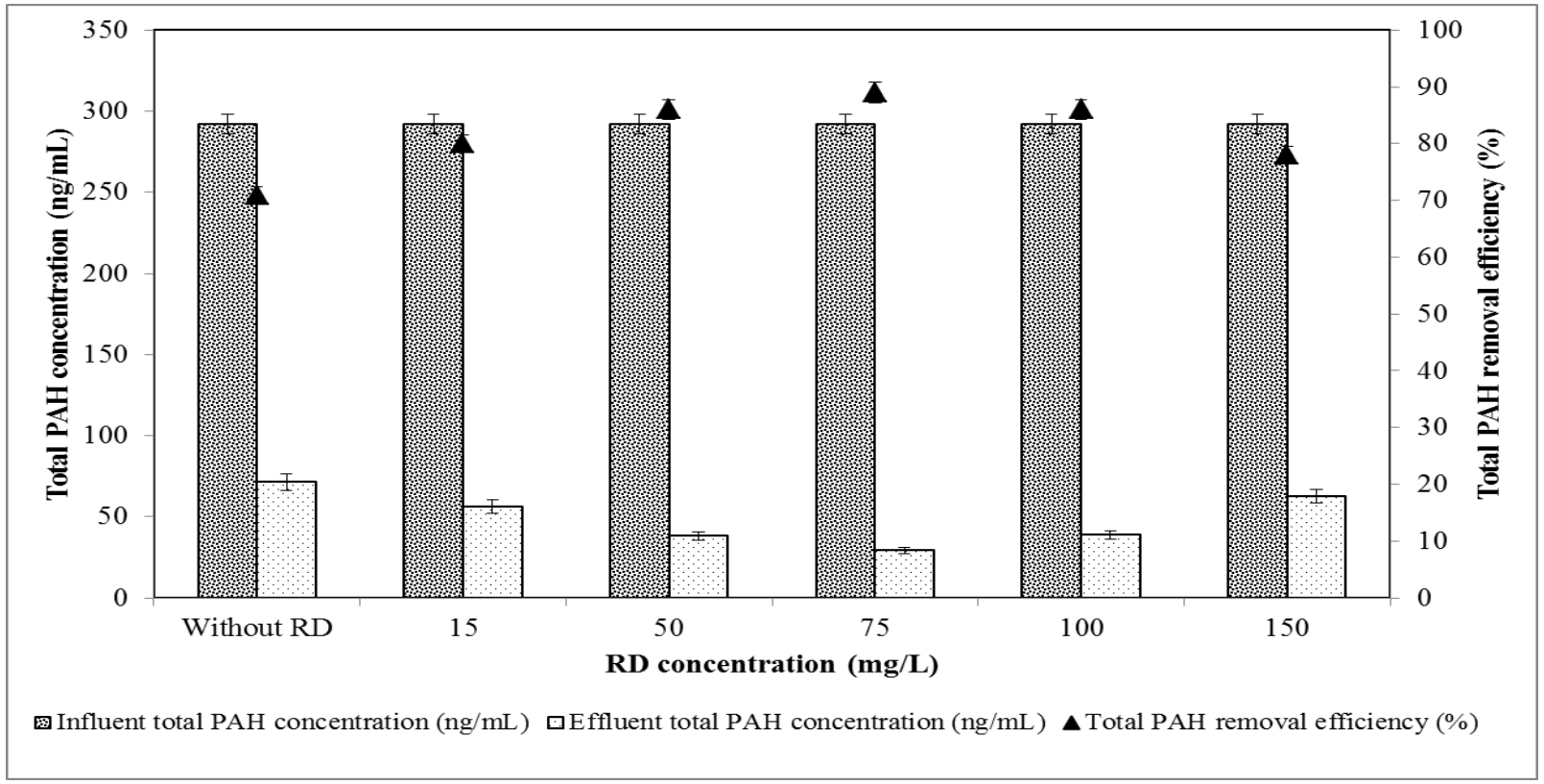

Figure 6.19 PAH removal efficiencies in the anaerobic ITBR system at increasing RD concentrations (influent PAH concentration=292 ng/mL, HRT=2.75 days, SRT=63 days, OLR= 106.18 ng PAH/mL.day)

6.4.2.2 Effects of Increasing RD Biosurfactant on the Individual PAH Removals in the Anaerobic ITBR System

When the RD concentration was 15 mg/L in the anaerobic ITBR reactor the ACT, PHE, and ANT PAHs yields increased from 81%, 65%, 79%, to 87%, 76%, 81%, respectively. The removal efficiencies of PY, BaA and BaP PAHs also increased from 61%, 58%, 63% to 67%, 60%, 66%, respectively (Table 6.33). The administration of 50 mg/L RD increased the 3 and 4 benzene rings PAHs removals from 81%, 54%, 79%, 61% to 91%, 87%, 87%, 74% for ACT, FLN, ANT, PY, respectively. The removal efficiencies of 5 and 6 benzene rings PAHs also increased from 61%, 58%, 63% to 67%, 60%, 66%, for CHR, BaP and BghiP, respectively (Table 6.33). As the RD concentrations were increased to 75 mg/L the individual PAHs removals increased from 43-81% to 55-95%, respectively (Table 6.33). The presence of 75 mg/L RD increased the less hydrophobic PAHs removals with 3 benzene rings (ACT, FLN, PHE, ANT and CRB), 4 benzene rings (FL, PY, BaA and CHR), 5 benzene rings (BbF, BkF and BaP) and 6 benzene rings (IcdP, DahA and BghiP) from 54-81%, 58-64%, 44-63%, 43-52% up to 83-95%, 77-84%, 55-74%, 55-65%, respectively. As the RD concentrations were increased from 75 to 100 mg/L the ACT, FLN and PHE PAH yields decreased from 95%, 88%, 92%, to 92%, 85%, 90%, respectively. The removal efficiencies of BaA, CHR, BaP and DahA PAHs also decreased from 79%, 84%, 74%, 55% to 74%, 81%, 71%, 51% respectively (Table 6.33). The presence of 150 mg/L RD, decreased the removal efficiencies of PHE, CRB, FL PAHs from 81%, 74%, 79% to 73%, 61%, 63%, respectively. Similarly, the removal efficiencies of CHR, BaP, BghiP PAHs also decreased from 81%, 71%, 51% to 61%, 63%, 54%, respectively, when the RD concentrations increased from 100 mg/L to 150 mg/L in the anaerobic ITBR system (Table 6.33).

Table 6.33 Influent and effluent PAH concentration and removal efficiencies in anaerobic ITBR at increasing RD concentrations

RLL_C		Without RD		15 mg/L		50 mg/L		75 mg/L		100 mg/L		150 mg/L	
	WW_{inf}	WW_{eff}	R_{eff}	WW_{eff}	R_{eff}	WW_{eff}	R_{eff}	WW_{eff}	R_{eff}	WW_{eff}	R_{eff}	WW_{eff}	R_{eff}
ACT	144.84±2.21	27.52±0.68	81±1.12	18.83±0.87	87±1.06	13.04±0.37	91±2.04	7.24±0.38	95±1.84	11.59±0.68	92±1.51	21.73±1.21	85±1.31
FLN	40.54±2.75	18.54±0.24	54±1.01	9.73±0.21	76±1.61	5.27±0.11	87±1.67	4.86±0.21	88±1.21	6.08±0.28	85±1.34	9.73±0.44	76±1.84
PHE	75.43±1.84	26.40±0.64	65±1.31	18.10±0.31	76±0.67	12.07±0.29	84±1.18	11.31±0.42	85±1.39	14.33±0.64	81±1.84	20.37±1.02	73±1.06
ANT	4.16±0.12	0.87±0.02	79±1.16	0.79±0.01	81±1.33	0.54±0.01	87±1.91	0.33±0.18	92±1.67	0.42±0.019	90±0.99	0.96±0.031	77±0.90
CRB	2.90±0.07	0.75±0.03	74±1.34	0.73±0.01	75±1.94	0.70±0.01	76±0.97	0.49±0.01	83±1.45	0.58±0.021	80±1.09	0.73±0.033	75±1.28
FL	10.94±0.06	4.05±0.16	63±1.34	3.83±0.03	65±1.75	3.17±0.11	71±1.06	2.52±0.09	77±1.94	2.84±0.11	74±0.64	4.27±0.14	61±1.64
PY	12.82±0.09	5.00±0.12	61±0.89	4.23±0.03	67±1.64	3.33±0.10	74±1.34	2.44±0.08	81±2.01	2.69±0.12	79±1.84	4.74±0.18	63±1.43
BaA	0.05±0.001	0.02±0.002	58±0.68	0.02±0.001	60±0.99	0.02±0.005	69±1.18	0.01±0.003	79±1.67	0.01±0.005	74±1.32	0.02±0.001	59±0.80
CHR	0.17±0.03	0.06±0.002	64±1.06	0.06±0.002	66±1.39	0.05±0.002	73±1.97	0.03±0.002	84±1.09	0.03±0.001	81±1.67	0.07±0.003	61±0.75
BbF	0.04±0.001	0.02±0.003	51±1.36	0.02±0.001	53±0.67	0.02±0.003	50±0.80	0.02±0.002	59±0.90	0.02±0.001	54±1.05	0.02±0.001	49±1.27
BkF	0.05±0.006	0.03±0.001	44±1.82	0.03±0.002	47±1.11	0.02±0.005	49±1.06	0.02±0.004	55±1.46	0.02±0.002	53±1.04	0.03±0.001	46±1.11
BaP	0.016±0.004	0.006±0.0002	63±1.19	0.005±0.0001	66±1.97	0.005±0.0002	69±1.39	0.004±0.0002	74±1.57	0.005±0.0004	71±0.85	0.006±0.0003	63±1.57
IcdP	0.010±0.002	0.006±0.0002	43±0.97	0.005±0.0001	49±1.30	0.005±0.0002	51±1.76	0.004±0.0002	59±1.67	0.005±0.0002	54±1.94	0.006±0.0002	44±1.63
DahA	0.014±0.003	0.008±0.0002	45±1.06	0.007±0.0001	48±1.51	0.007±0.0003	49±0.69	0.006±0.0004	55±1.04	0.007±0.0003	51±1.30	0.008±0.0004	45±0.95
BghiP	0.035±0.001	0.017±0.0001	52±1.31	0.016±0.0001	54±1.69	0.014±0.0003	59±1.34	0.012±0.0005	65±0.97	0.013±0.0006	63±1.18	0.016±0.003	54±1.02
Total	292.00±1.41	71.29±1.78	71±1.58	56.40±1.89	80±1.56	38.26±0.90	86±1.84	29.29±0.77	89±1.91	38.64±1.29	86±1.75	62.69±1.01	78±1.34

WW_{inf}: PAHs concentration of influent wastewater (ng/mL), WW_{eff}: PAHs concentration of effluent wastewater in anaerobic ITBR system, R_{eff}: PAHs removal efficiencies (%), RLL_C: RD concentration (mg/L) (HRT of 2.75 days, at a SRT of 63 days, OLR of 106.18 ngPAH/mL.day)

The maximum PAH removal efficiencies found as 89% at a 75 mg/L RD concentration in the anaerobic ITBR system. It was observed that the more hydrophobic PAHs with high molecular weights can be effectively biodegraded as high as less hydrophobic PAHs.

The high PAHs removals with 75 mg/L RD could be attributed to the improved solubility of the PAHs and the reduced interfacial tension of wastewater, which subsequently increased the substrate flux from aqueous medium to bacterial cells (Pathak at al., 2009). Tsai et al. (2009) reported % 55 FLN and 72% PHE removals in an anaerobic contact reactor without biosurfactant at a SRT of 21 days. In our study, FLN and PHE removal efficiencies in the anaerobic ITBR were 88% and 85%, respectively at 75 mg/L RD concentration at a COD_{dis} loading rate of 1.04 kg/m^3day. This showed that the RD improved the PAH yields. In a study performed by Grishchenkov et al. (2000), 23% removal was obtained for FL PAHs within 50 days of operation in an anaerobic bioreactor. The FL removal yield result obtained in this study is higher than those obtained by Grishchenkov et al. (2000). The results found in our study are consistent with the findings of Haritash and Kaushik (2009). High PAH yields in the presence of biosurfactant could be attributed to the combining effects of anaerobic granule bacteria which are resistant to PAHs, to the optimum RD concentration and to the anaerobic ITBR configuration which is a high rate reactor and resistant to the toxic organics. In this study it was found that PAHs treatment with 75 mg/L RD was beneficial for the degradation of all ring PAHs with a total PAHs removal yield of 89% in comparison to 71% in the RD-free case (See Table 6.33). The results of this study showed that 75 mg/L RD increased the removal efficiency of both less **(**3 and 4 ring PAHs) and more (5 and 6 ring PAHs) hydrophobic PAHs compounds.

PAHs removals did not decrease from high-ring PAHs to low-ring PAHs contrarily to the studies performed by Chang et al. (2003) and Haritash and Kaushik (2009). Zhao et al. (2011) found that 400 mg/L RD increased the PAH yield from 82.2 to 92.7 at an influent PHE concentration of 250 mg/L at a SRT of 30 days in an anaerobic contact reactor. The differences in PAH yields could be attributed to the

PAH and biomass concentrations, to the reactor configuration, to the wastewater characterization and to the some environmental conditions such as redox potential and temperature. It was observed that the anaerobic PAH yields found in recent literatures were lower than that our data: Guieysse et al. (2000) investigated the removals of ACT, PHE and PY PAHs using an anaerobic packet bed reactor. In this study, ACT, PHE and PY PAHs were removed with 77%, 79% and 80% yields, respectively, at a HRT of 10 hours. In a study performed by Benabdallah El-Hadj et al. (2007), 43% and 31% NAP and PY removals were obtained at a HRT of 20 days in an anaerobic reactor. In our study, PY removal efficiencies increased from %61 to 81% at a SRT of 63 days with 75 mg/L RD. The PY removal yield result obtained in this study is higher than those obtained by Benabdallah El-Hadj et al. (2007).

6.2.2.2.1 Effect of increasing RD concentrations on the gas production and methane percentage in the anaerobic ITBR system. Biogas production was monitored through the operation of the anaerobic ITBR system, particularly for detection the methanogenic activity. The effect of PAHs on daily total gas, methane gas production and methane percentages with increasing RD are illustrated in Figure 6.20. The maximum total gas, methane gas and methane percentage were measured as 2.4 L/day, 1.1 L/day and 50%, respectively, at a RD concentration of 15 mg/L on days between 300 and 363. The maximum total gas, methane gas and methane percentage were obtained as 2.8 L/day, 1.6 L/day and 57%, respectively, at a RD concentration of 50 mg/L on days between 366 and 426. The maximum total gas, methane gas and methane percentage were measured as 3.1 L/day, 2.1 L/day and 67%, respectively, at a RD concentration of 75 mg/L on days between 429 and 491 (See Figure 6.20). The daily total gas, methane gas productions and methane percentage decreased after these RD concentrations. On days between 494 and 554, the maximum total gas, methane gas and methane percentage were determined as 2.6 L/day, 1.5 L/day and 58%, respectively, at a RD concentration of 100 mg/L. The maximum total gas, methane gas and methane percentage were found as 2.3 L/day, 1.2 L/day and 52%, respectively, at a RD concentration of 150 mg/L on days between 557 and 617 (See Figure 6.20).

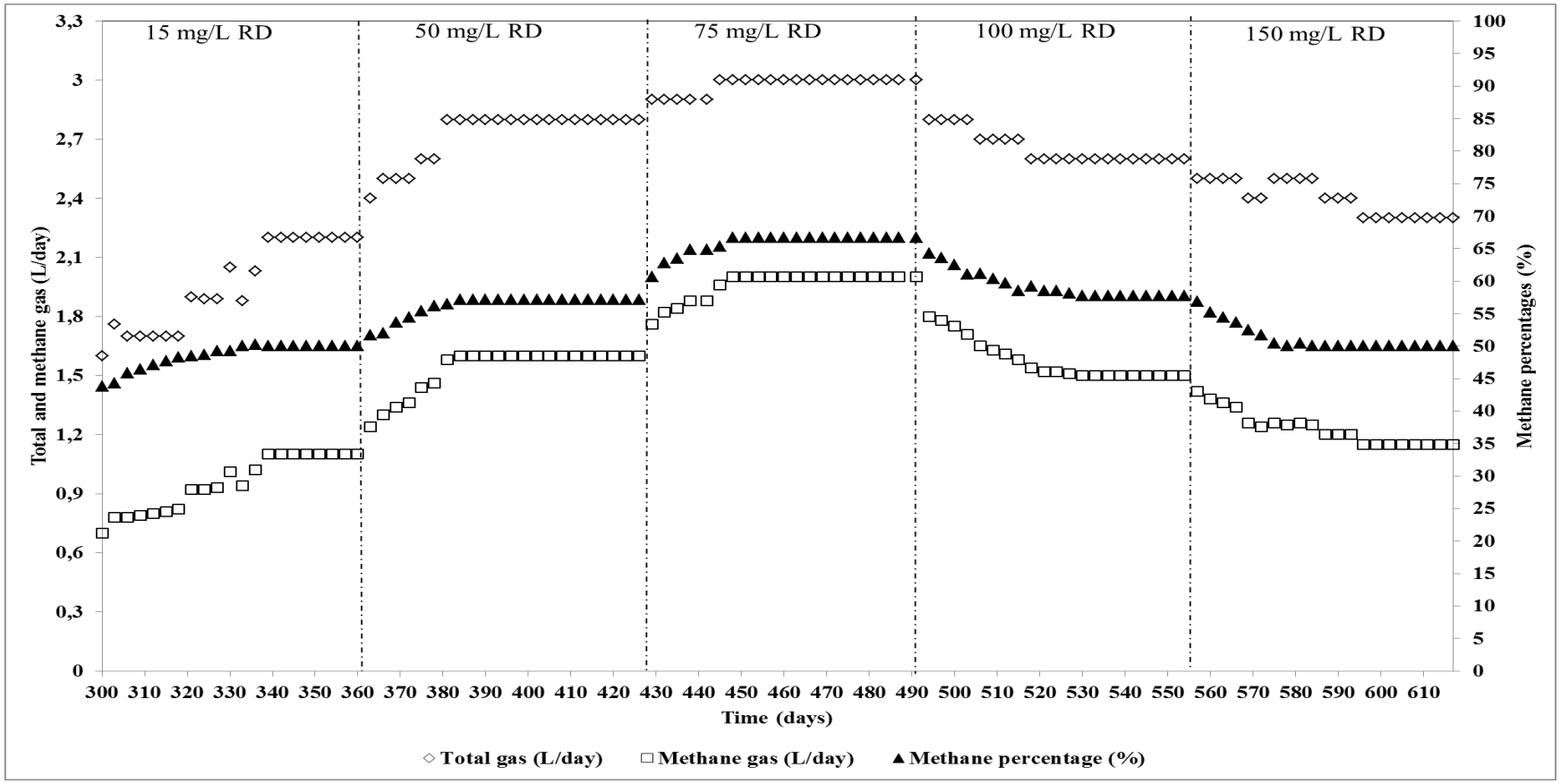

Figure 6.20 Variation of methane gas and methane percentages in the continuous operation periods in the anaerobic ITBR at increasing (15 mg/L, 50 mg/L, 75 mg/L, 100 mg/L and 150 mg/L) RD concentrations

6.4.2.2.2 Variation of Methane Yields Versus Increasing RD Concentrations in the Anaerobic ITBR System. Figure 6.21 shows the variations of methane yields versus increasing RD. It was observed that the methane yield increased partly with increasing RD. The methane yield decreased after concentrations of 75 mg/L RD in the anaerobic ITBR system.

The methane yield, expressed as $m^3CH_4/kg.COD_{rem}$, increased from 0.27 up to 0.34 m^3CH_4/kg COD with the increasing of RD concentration from 15 to 75 mg/L (See Figure 6.21). The methane yield was 0.34 m^3 CH_4/kg COD removed at a RD concentration of 75 mg/L. The methane yield decreased from 0.30 to 0.28 m^3 CH_4/kg COD_{rem} as the RD concentration increased from 100 mg/L to 150 mg/L.

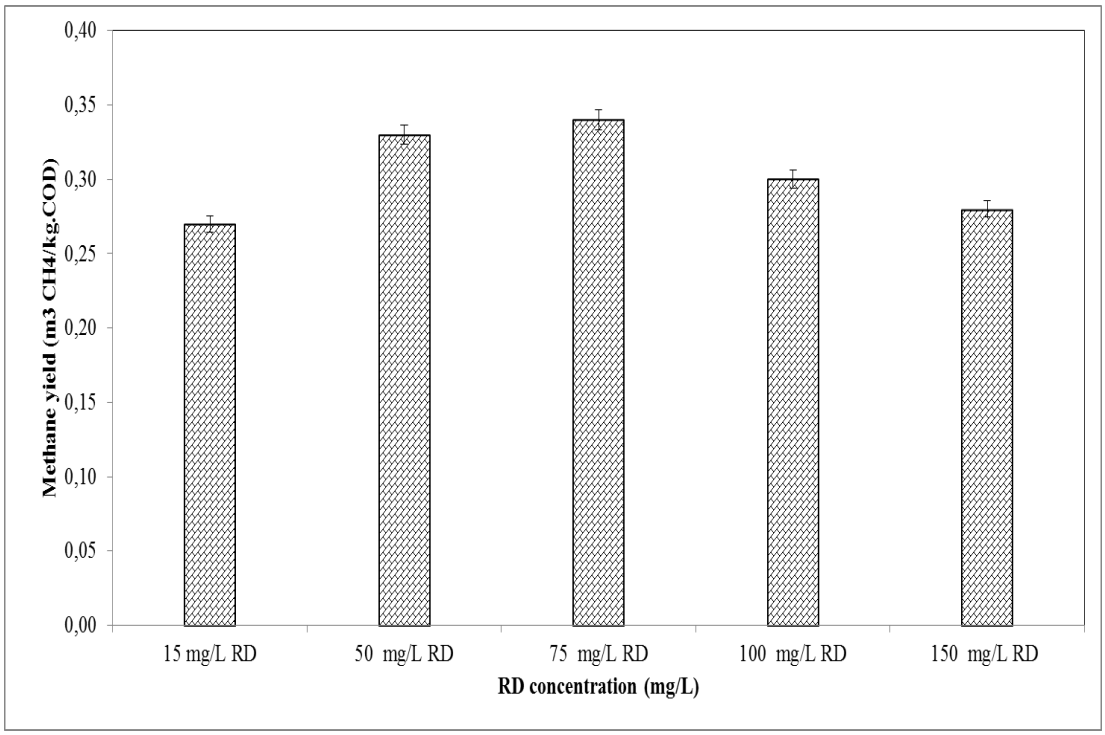

Figure 6.21 Variations of methane yields versus increasing RD concentration in the anaerobic ITBR

The maximum methane yield was measured as 0.34 m^3CH_4/kg COD_{rem} which is near to the theoretical methane value calculated and is also in agreement with the methane yield reported by De La Rubia (2006) in an industrial wastewater containing hydrocarbons. Patel and Madamwar (2002) found that the highest methane yield was 0.45 m^3 CH_4/kg COD_{rem} in an anaerobic fixed film reactor treating petrochemical

wastewater at an OLR of 21.7 kg COD/m^3.day at 37 ^oC.

Sowmeyan and Swaminathan (2008) studied the performance of an inverse anaerobic fluidized bed reactor to treat the high strength organic wastewaters. The methane gas production and the methane percentage were 5.59 L CH_4/day, 44.9% respectively, after 70 days of operation period with the increasing of loading rate from 10 to 35 kg COD/m^3 day**.**

In our study, 67% methane percentage and 3.1 L/day methane production was measured at influent COD and PAH concentrations of 2950 and 292 mg/L, respectively at a 75 mg/L RD concentration at an OLR of 1.04 g COD/m^3.day and 106.18 ng PAH/mL.day in an anaerobic ITBR system. The data obtained in the aforementioned studies are low in comparison to the methane percentage found in our study. This could be attributed to the long start-up period and to the resistance of anaerobic culture used in our study which is not sensitive to the toxic PAHs and to the reactor configuration and to the operational conditions.

Buffiere et al. (2000) investigated the biodegradation of oil/hydrocarbon industry wastewaters using an anaerobic ITBR. 55-58% methane percentage was obtained at an OLR of 15 kg COD/m^3 day after 90 days of operation. The data obtained in our study, are higher than the aforementioned study. In our study 67% methane percentage was obtained at a RD concentration of 75 mg/L at OLRs of 1.04 g COD/m^3.day and 106.18 ng PAH/mL.day indicating that a significant part of the PAHs together COD was converted to methane and used as a carbon and energy source by methanogens, as reported by Arnaiz et al. (2007).

6.4.2.2.3 Effects of Increasing RD Concentration on pH, Total Volatile Fatty Acid (TVFA), Bicarbonate Alkalinity (Bic.Alk.) and TVFA/Bic.Alk. Ratio Variations in the Anaerobic ITBR. pH values have an important role, which may affect the activity of the microorganisms in the anaerobic processes. The increase in the pH may be due to the accumulation of Bic. Alk. or decrease the pH could be due to the formation of TVFA. Optimum pH for anaerobic activity is in the range between 6.6 and 7.6

(Speece, 1996). When the rate of pH value exceeds the rate of break-down to methane, a process unbalance results in which the pH decreases, gas production falls off, and the CO_2 content of the gas increases. Therefore, pH control is essential to ensure a high rate of methane production in the anaerobic processes. Bic. alk. is commonly used to raise the pH of an anaerobic system when there is a process imbalance. Caution must be taken, since excess application of Bic.alk. will result in precipitation of calcium carbonate. It is desirable to have a Bic. Alk. in the range of 2 to 5 g/L in order to provide a buffer capacity to handle volatile acids with a minimal decrease in pH (Eckenfelder, 1989).

Figures 6.22 and 6.23 show the variations in total volatile fatty acid (TVFA), bicarbonate alkalinity (Bic. Alk.), pH, and TVFA/Bic. Alk. ratios throughout operation time at increasing RD concentrations in an anaerobic ITBR reactor containing 292 ng/mL total PAHs concentration at OLRs of 1.04 kg COD_{dis}/m^3 day and 106.18 ng PAH/mL.day.

The pH values varied between 7.0 and 7.7 in the effluent of anaerobic ITBR system while the Bic. Alk. and TVFA concentrations decreased from 1875 mg/L, 321 mg/L to 980 mg/L, 64 mg/L with increasing of RD from 15 to 75 mg/L, respectively.

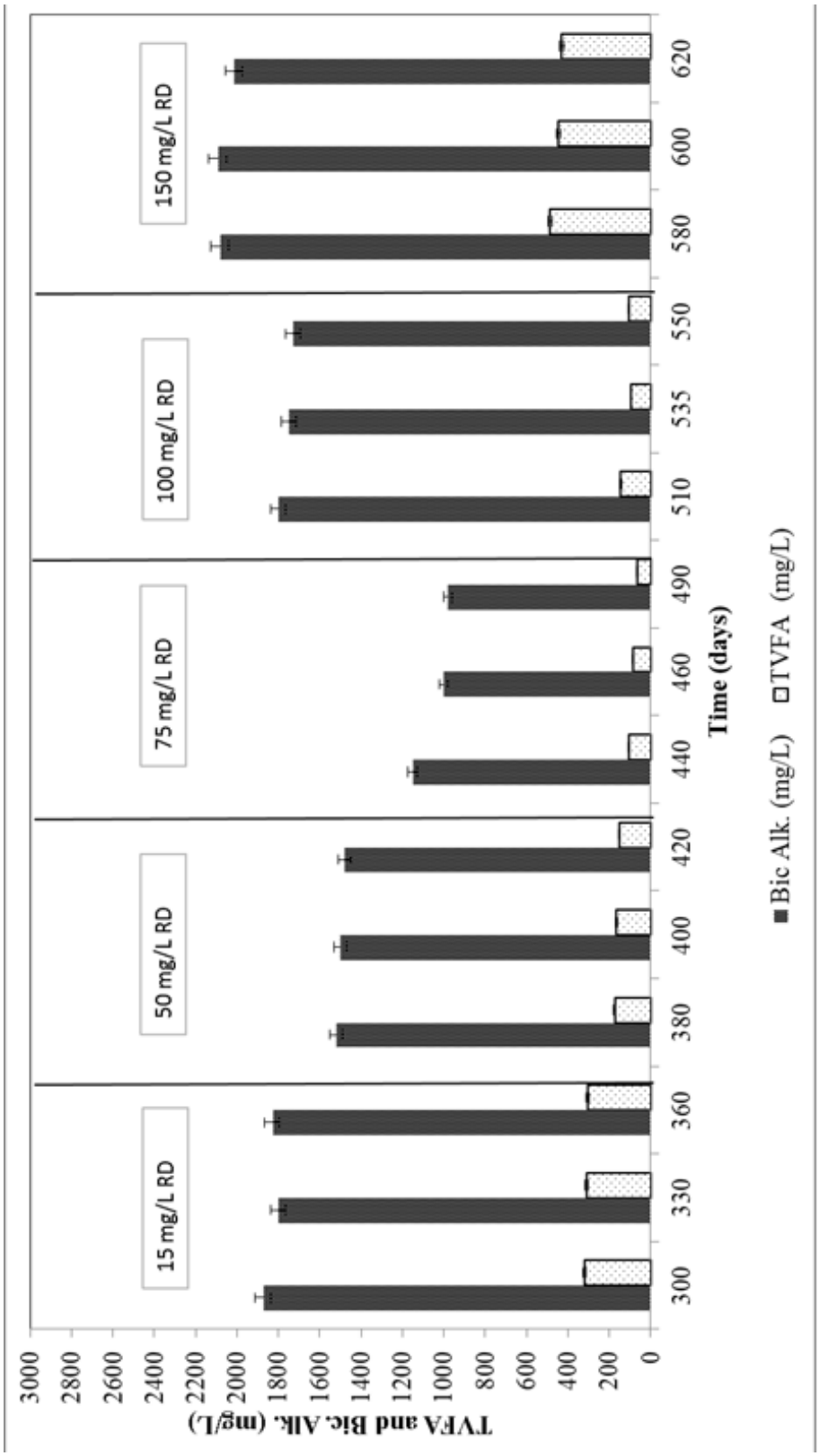

Figure 6.22 Variation of Bic. Alk. and TVFA, in the effluent of the anaerobic ITBR at increasing RD concentrations

The maximum Bic. Alk. and the maximum TVFA obtained as 2015 and 430 mg/L, respectively, in the effluent of anaerobic ITBR system at a concentration of 150 mg/L RD in the effluent of ITBR system. The minimum Bic. Alk. and TVFA measured as 980 and 64 mg/L, respectively, in the effluent of ITBR system at a concentration of 75 mg/L RD (See Figure 6.22).

The Bic.Alk. concentrations were lower in the effluent of the anaerobic ITBR system at a 75 mg/L RD than the others RD concentration due to decline of pH. This indicates the utilization of alkalinity to buffer the TVFA and CO_2 produced from the anaerobic co-metabolism of PAHs.

The TVFA/Bic. Alk. ratios were between 0.03 and 0.11 at all RD concentrations (15, 50, 75, 100 and 150 mg/L RD) indicating the moderately stability of the anaerobic ITBR system (See Figure 6.23). This ratio changed between 0.17 and 0.18 at a 15 mg/L RD, between 0.10 and 0.11 at 50 mg/L RD, between 0.07 and 0.09 at 75 mg/L RD, between 0.06 and 0.08 at a 100 mg/L RD and between 0.21 and 0.23 at a 150 mg/L RD in the effluent of ITBR (See Figure 6.23).

TVFA/Bic.Alk. ratio gives necessary information to determine the stability of in the anaerobic reactor. If the TVFA/Bic.Alk. ratio is lower than 0.4, the reactor is stable. When the TVFA/Bic.Alk. ratio is lower than 0.8, the reactor system is moderately stable or unstable **(**Behling et al., 1997).

Anaerobic reactor system was stable as reported by Busu et al (2010) since the TVFA/Bic.Alk. ratios in the effluent were lower than 0.3. It was found that the TVFA concentrations were lower than the studies performed by Arnaiz et al. (2007) in the operation period. These results indicated that anaerobic ITBR system was stable at increasing RD concentration because the TVFA/Bic.Alk. ratios in the effluents were lower than 0.3.

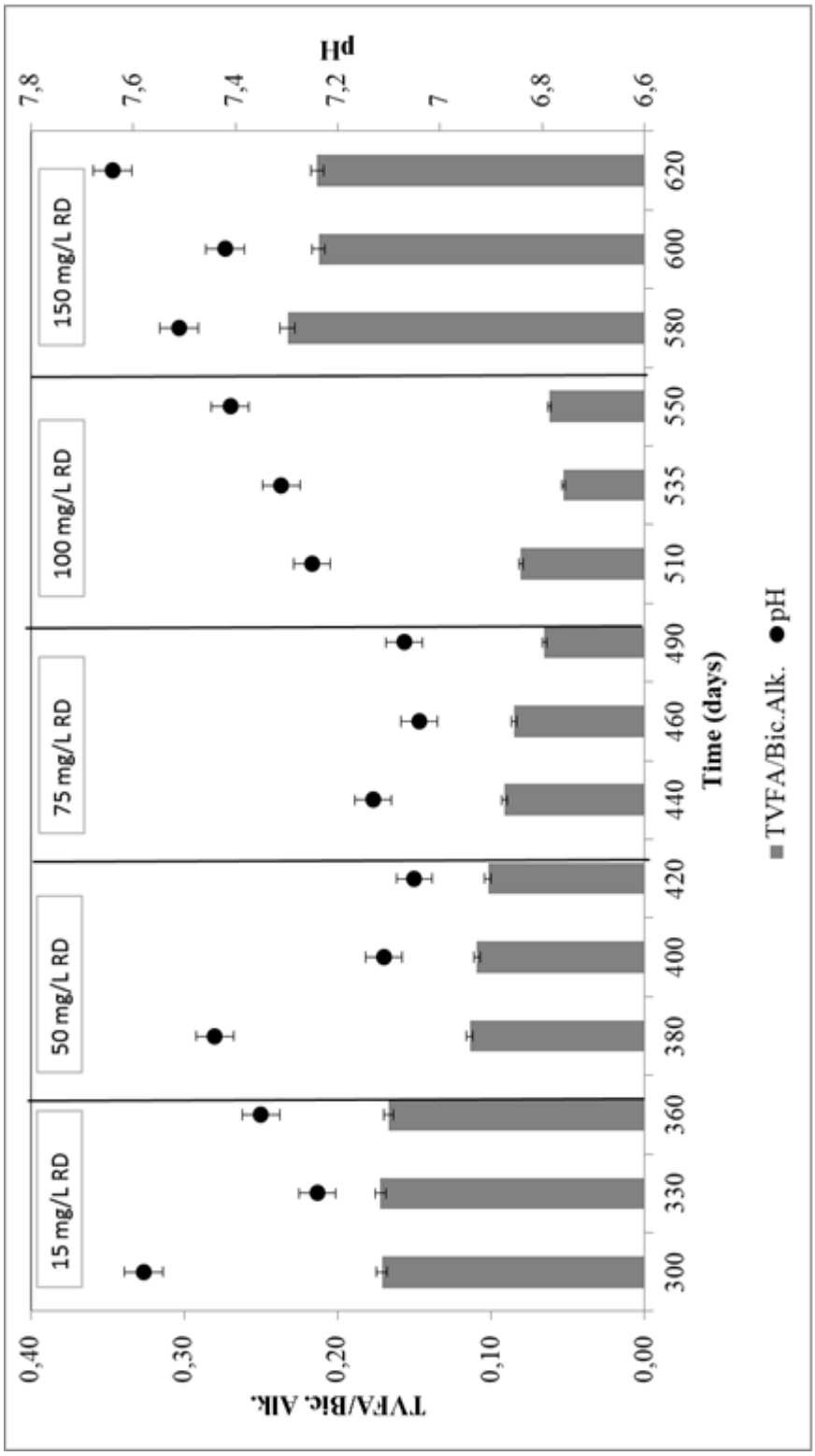

Figure 6.23 Variation of TVFA/Bic.Alk. and pH in the effluent of the anaerobic ITBR at increasing RD concentrations

6.4.3 Effects of HRTs on COD_{dis} and Total PAH Removal Efficiencies in the Sequential Anaerobic ITBR/Aerobic CSTR System Containing 75 mg/L RD

6.4.3.1 Effects of HRTs on COD_{dis} and Total PAH Removal Efficiencies in the Anaerobic ITBR System Containing 75 mg/L RD from the Sequential System

In previous studies, in order to detect the effects of different RD concentrations (15-50-75-100-150 mg/L) on the COD_{dis} and total PAH removal efficiencies studies were performed in the anaerobic ITBR system. It was found that 75 mg/L RD is the optimum dose for maximum total PAHs and COD_{dis} removal from the petrochemical industry wastewater. In this section, the effect of increasing HRTs (1.38-1.83-2.75-5.50 and 11 days) on the COD_{dis} and total PAHs removal efficiencies were studied in the presence of 75 mg/L RD in the ITBR. The operational conditions for this study are summarized in Table 5.11 in the section Material and Methods.

The variations of COD_{dis} and total PAH removal efficiencies versus increasing HRTs in the anaerobic ITBR system containing 75 mg/L RD are shown in Figure 6.24. 95% COD_{dis} and 89% total PAH removal efficiencies were obtained at a HRT of 2.75 days at an influent total PAH concentration of 292 ng/mL in the anaerobic ITBR system. The COD_{dis} and total PAH removal efficiencies increased from 94% to 95% and from 88% to 89%, respectively, as the HRTs were increased from 1.38 days to 2.75 days.

The maximum COD_{dis} and total PAH (E=94-95% and E=88-89%) removal efficiencies were observed at HRTs varying between 1.38 and 2.75 days (See Figure 6.24). The minimum COD_{dis} and total PAH removal efficiencies were 79% and 69%, respectively, at a HRT of 11.00 days.

In the ITBR reactor the PAH and COD_{dis} in the real petrochemical wastewater were biodegraded anaerobically as follows: in the first step the COD_{dis} and total hydrophobic PAHs were hydrolyzed to more degradable COD subcategories and partially to less hydrophobic (more hydrophilic) PAHs by anaerobic acidophilic

bacteria. In the second phase the COD_{dis} and PAHs began to transform to less biodegradable compounds by the acidogenic bacteria throughout acidogenesis together with low methane productions. In the last phase (methanogenesis) the COD_{dis} and PAHs were mainly biodegraded to H_2O and CO_2 with low concentration of H_2S. In this phase the methane productions increased excessively.

Bernal-Martinez et al. (2005) found 48% total PAHs removal efficiency at a HRT of 40 days at an OLR of 1 kg COD/m^3day in the anaerobic batch reactor treating industrial wastewater. Michaud et al. (2002) reported maximum COD removal efficiencies (E=75-80%) in an anaerobic inverse bed reactor at HRTs varying between 1.5 and 6.4 days at OLRs increasing from 1.10 to 6 g COD/L.day. Kennes et al. (1995) investigated the performance of an anaerobic upflow sludge blanked reactor (UASB) treating petroleum wastewater at HRTs varying between 0.37 and 0.80 days. In this study, COD removal efficiency was 99% at a HRT of 0.7 days in the system.

Veeresh et al. (2005) reported that the optimum HRT for the treatment of oil production wastewater in UASB reactor was 0.6 day, corresponding to an OLR of 2 kg COD/m^3 day, where COD removal efficiency was over 80%. Barret al. (2010) reported 70% PAHs removal efficiency at a HRT of 20 days treating synthetic industry wastewater in an anaerobic rector. Rockne and Strand et al. (1998) studied the anaerobic biodegradation of the PAHs, such as NAP and PHE in an anaerobic fluidized bed reactor (FBR). 17% NAP and 96% PHE removal efficiencies were observed in the system. Tsai et al. (2009) found 88% FLN and 65% PHE PAHs removal efficiencies at an incubation period of 21 days in an anaerobic batch reactor.

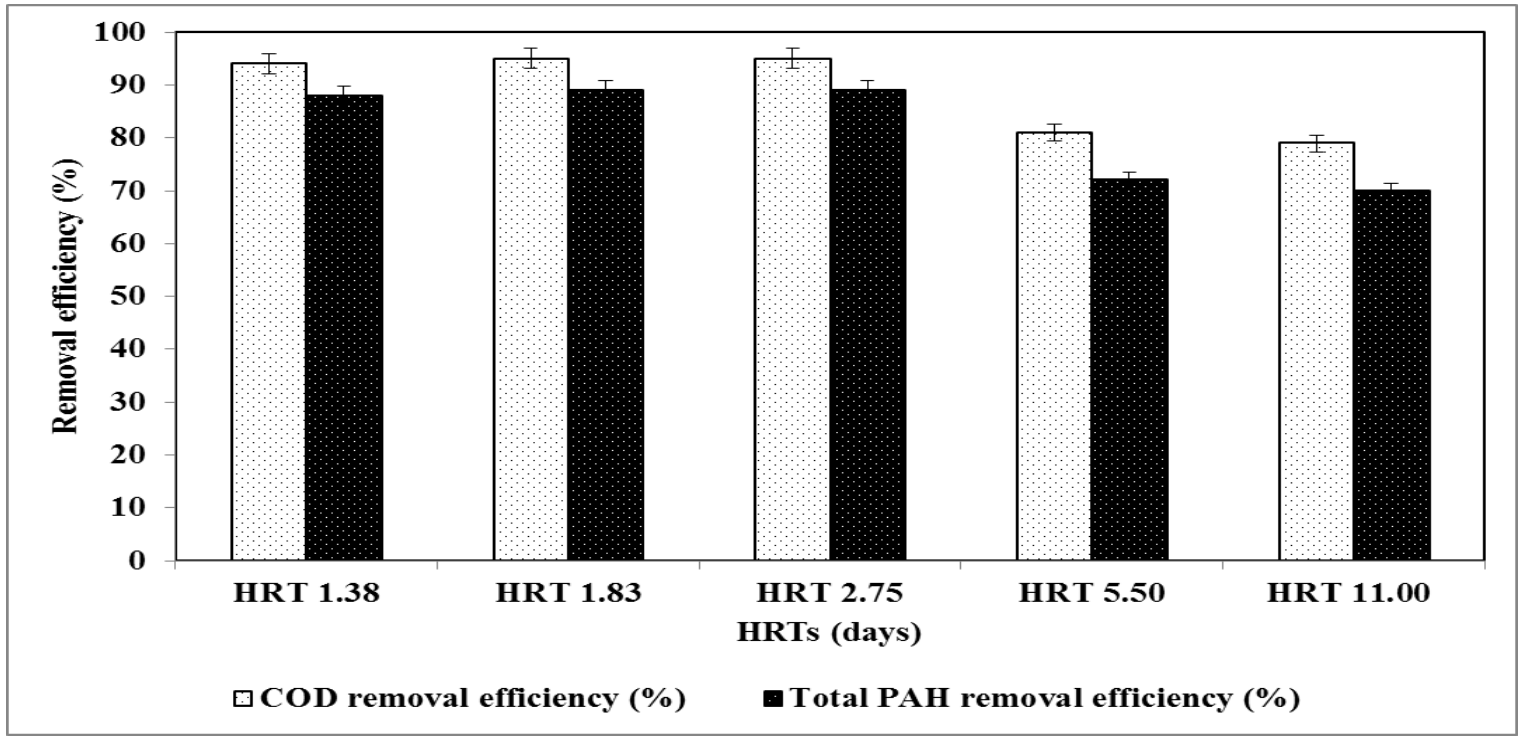

Figure 6.24 The variations of COD_{dis} and total PAH removal efficiencies versus increasing HRTs in the anaerobic ITBR system containing 75 mg/L RD (SRT=63 days, OLR=0.26-2.07 kg COD/m^3.day; OLR=26.35-212.36 ng PAH/mL.day)

6.4.3.1.1 Effect of HRTs on the Total and the Methane Gas Productions in the Anaerobic ITBR System Containing 75 mg/L RD. The variations of the total, methane gas productions and methane gas percentage in the anaerobic ITBR containing 75 mg/L are shown in Figure 6.25 for all HRTs studied. The total gas productions were obtained as 3.10, 3.20 and 3.10 L/day at HRT of 1.38 to 1.83 and 2.75 days, respectively. As the HRTs were increased from 2.75 to 5.50 and 11 days, the total gas productions decreased from 3.10 to 2.50 and to 2.40 L/day in the anaerobic ITBR containing 75 mg/L RD. The daily methane gas productions and methane percentages were approximately 2.00, 2.10, 2.05 L/day and 64%, 65% and 66%, at HRTs of 1.38, 1.83 and 2.75 days, respectively. After these HRTs, the daily total gas, methane gas productions and methane percentages decreased. When the HRTs were increased from 2.75 to 5.50 and to 11 days, the methane productions decreased from 2.05 to 1.30 and to 0.90 L/day. The optimum HRT for the maximum total gas, methane gas productions and methane percentage found as 3.10 and 2.10 L/d and 66%, respectively at a HRT of 2.75 days in the anaerobic ITBR system.

In this study, the methane yield (m^3 CH_4/kg COD) can be a useful parameter to assess the performance of the anaerobic ITBR system. As the treatment of wastewater is directly related to the amount of methane produced, the amount of methane generated per kg of COD_{dis} stabilized is taken to be an indicator of total PAH and COD_{dis} stabilization degree (Cresson et al., 2008). Figure 6.26 shows the variations of methane yields versus HRTs. It was observed that the methane yields increased from 0.18 to 0.35 m^3CH_4/kg COD, when the HRT were increased from 1.38 days to 2.75 days.

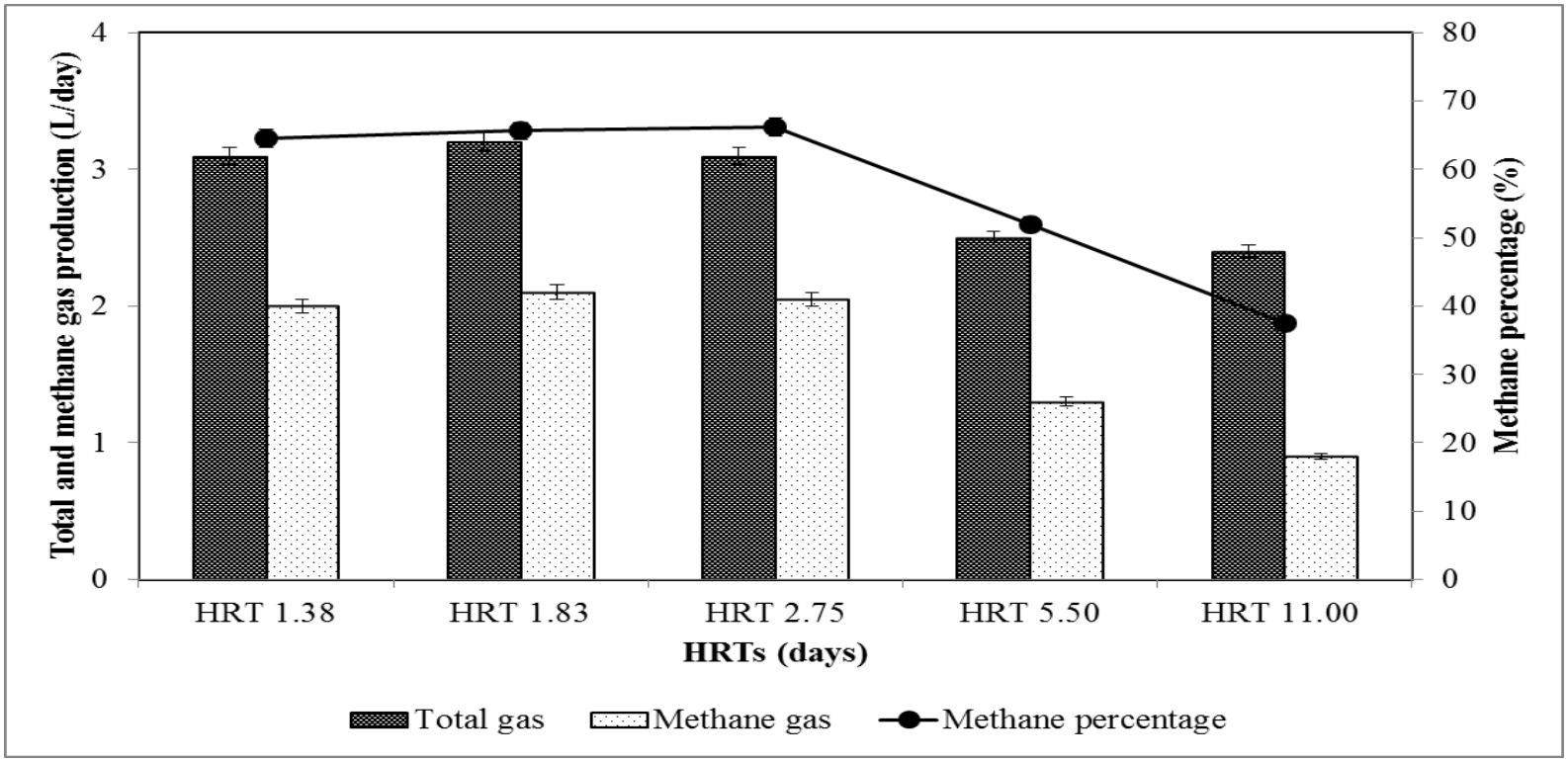

Figure 6.25 The variations of total, methane gas production and methane percentage versus increasing HRTs in the anaerobic ITBR containing 75 mg/L RD (SRT=63 days, OLR=0.26-2.07 kg COD/m^3.day; OLR=26.35-212.36 ng PAH/mL.day)

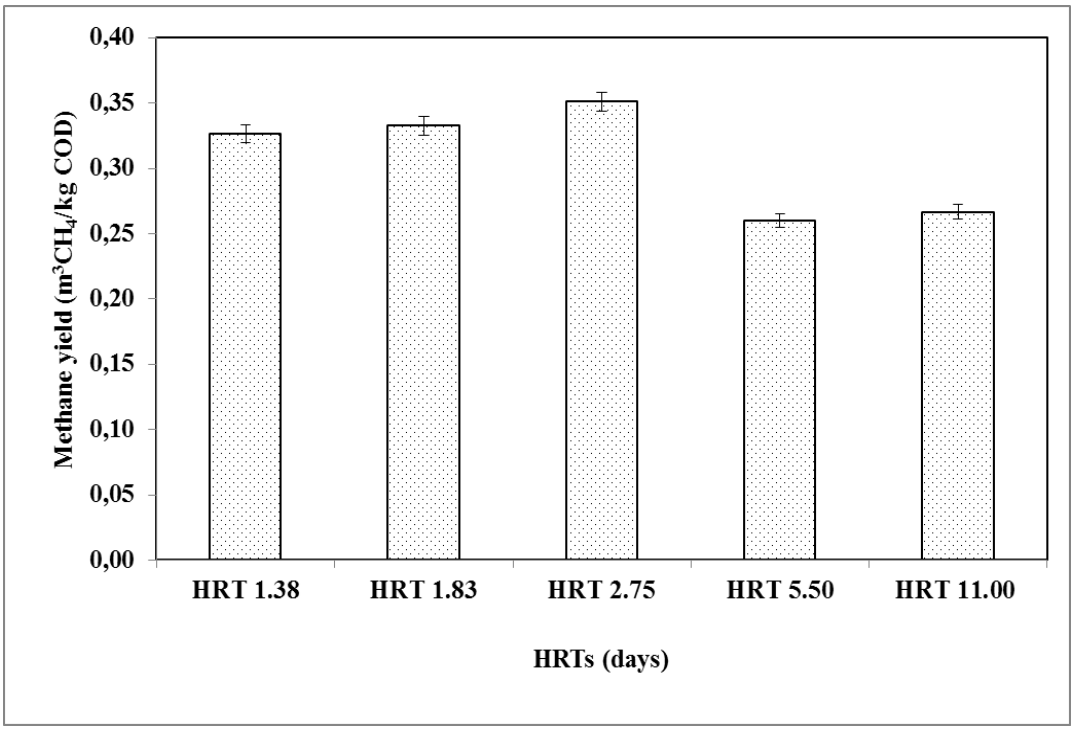

Figure 6.26 Variations of methane yields versus increasing HRTs in the anaerobic ITBR system containing 75 mg/L RD (SRT=63 days, OLR=0.26-2.07 kg COD/m^3.day; OLR=26.35-212.36 ng PAH/mL.day)

The methane yields were obtained as 0.33, 0.33 and 0.35 m^3 CH_4/kg COD at HRTs of 1.38, 1.83 and 2.75 days, respectively. As the HRT were increased from 5.50 days to 11.00 days, the methane yields decreased from 0.35 to 0.27 m^3 CH_4/kg COD in the ITBR system containing 75 mg/L RD. A lower methane yield value (0.20 m^3 CH_4/kg COD) was obtained in the anaerobic treatment of chemical industry wastewater at a HRT of 1.98 days (Ince et al., 2000). The lower methane yields in the studies mentioned above could be due to the configuration of the anaerobic reactor, to the type of anaerobic microorganism to the operational conditions. Cresson et al. (2007) found the 0.05 to 0.31 m^3 CH_4/kg COD methane yield at an OLR of 8 g COD/L.day during first 20 days, in anaerobic ITBR treating wine based wastewater. These results are in accordance with the observations of Michaud et al. (2003). The maximum methane yield was measured as 0.37 m^3 CH_4/kg COD which is near to the theoretical methane value calculated and is also in agreement with the methane yield reported by Sowmeyan et al. (2008).

6.4.3.1.2 Effects of HRTs on pH, Total Volatile Fatty Acid (TVFA), Bicarbonate Alkalinity (Bic.Alk.) and TVFA/Bic.Alk. ratio Variations in the Anaerobic ITBR Containing 75 mg/L RD. Figure 6.27 shows the pH, TVFA and Bic.Alk. in the effluent of anaerobic ITBR at increasing HRTs. The pH values in the effluent of the anaerobic ITBR varied between 7.1-7.7. These values were between optimum pH values as reported by Speece (1996).

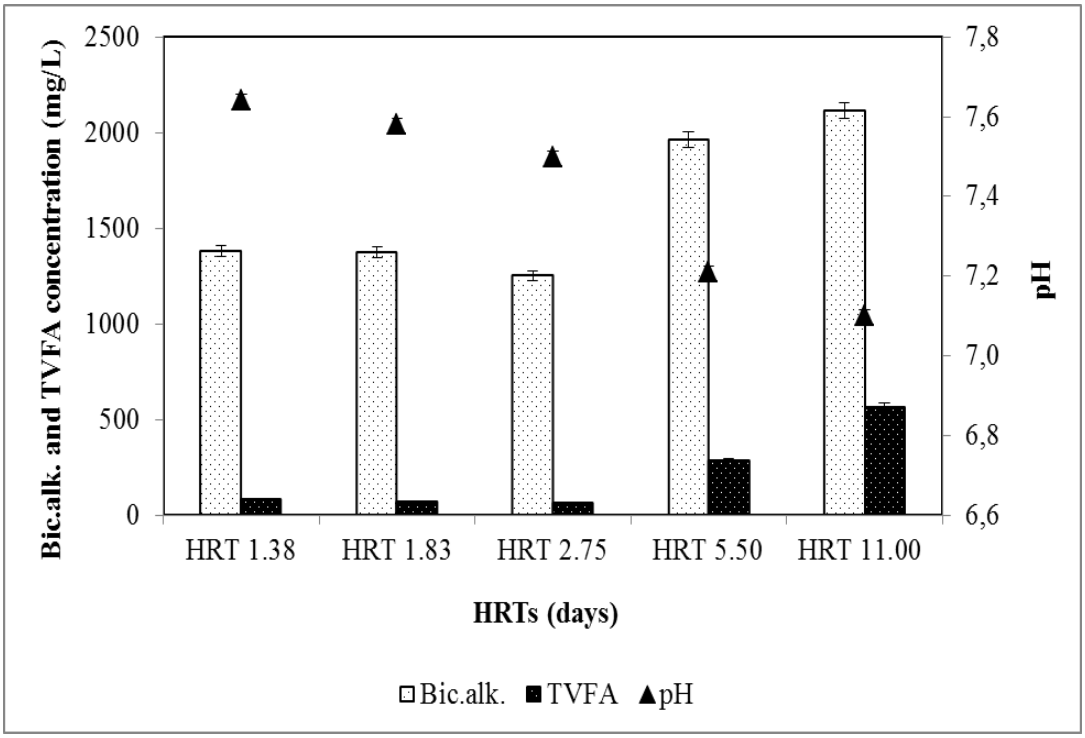

Figure 6.27 The variations of TVFA, Bic.Alk. and pH versus increasing HRTs in the anaerobic ITBR containing 75 mg/L RD (SRT=63 days, OLR=0.26-2.07 kg COD/m^3.day; OLR=26.35-212.36 ng PAH/mL.day)

TVFA concentration varied between 64 and 564 mg/L in the effluent of anaerobic ITBR at all HRTs. The TVFA concentrations found as 78, 71 and 64 mg/L at HRT of 1.38, 1.83 and 2.75 days in effluent of anaerobic ITBR system. After these HRTs, TVFA concentration increased to 285 and 564 mg/L at HRT of 5.50 and 11.00 days (See Figure 6.27). This shows that the produced TVFA could not be converted to methane and accumulation of acids occurred at high HRTs.

Alkalinity is one of the most central concepts because it controls the pH and thus is a measure the capacity of an aquatic system to buffer the pH in the presence of acids (Speece, 1996). Therefore a sufficient bicarbonate alkalinity must be present to neutralize wastewater. If the H_2CO_3 and TVFA concentrations exceed the available alkalinity, the reactor will sour, severely inhibiting the methanogens (Speece, 1996). The Bic.Alk. were measured between 1250 and 2115 mg/L in the effluent of anaerobic ITBR system at increasing HRTs. Bic.Alk. concentrations in the effluent wastewater were found as 1380, 1375, 1250 mg/L, respectively, when the HRTs were increased from 1.38 to 1.83 and to 2.75 days. Bic. Alk. concentrations were measured as 1964 and to 2115 mg/L at HRTs of 5.50 and to 11 days. The miumum Bic. Alk. obtained as 1250 mg/L at a HRT of 2.75 days. This indicates the utilization of alkalinity to buffer the TVFA and CO_2 produced from the anaerobic co-metabolism of PAHs in the system. The reason for low Bic.Alk. concentrations compared to the highest could be explained by the accumulation of TVFA at HRT of 5.50 and 11.00 days in the anaerobic ITBR system.

In anaerobic reactor system TVFA/Bic.Alk. ratio gives necessary information to determine the stability of the anaerobic reactor. Increases in TVFA/B.Alk. ratio in the anaerobic ITBR system effluent samples indicate the activity of acid producing bacteria and TVFA accumulation. If the TVFA/B.Alk. ratio is lower than 0.80 the reactor system is moderately stable or unstable as reported by Behling et al. (1997). Figure 6.28 shows the TVFA/Bic.Alk. ratios in the effluent of anaerobic ITBR system. TVFA/Bic.Alk. ratio was found as approximately 0.05 at HRTs of 1.38, 1.83 and 2.75 days while this ratio were calculated 0.15 and 0.27 at HRTs 5.50 and 11.00 days, respectively. TVFA/Bic.Alk. ratios were found below 0.30 at all HRTs in the effluent of the anaerobic ITBR system, which indicates the stability of system.

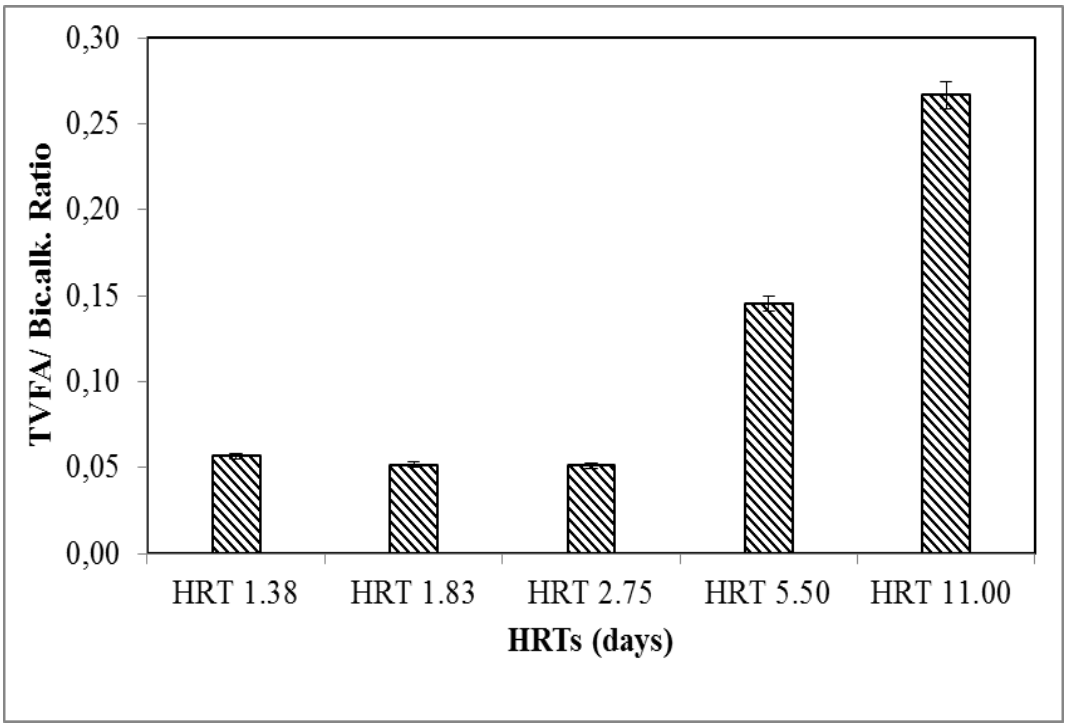

Figure 6.28 The variations of TVFA/Bic.Alk. versus increasing HRTs in the anaerobic ITBR system containing 75 mg/L RD (SRT=63 days, OLR=0.26-2.07 kg COD/m^3.day; OLR=26.35-212.36 ng PAH/mL.day)

6.4.3.2 Effects of HRTs on the COD_{dis} and PAH Removal Efficiencies in Aerobic CSTR from the Sequential System

Table 6.34 shows the effect of decreasing HRT on the COD_{dis} and PAHs removals in the CSTR reactor. The COD_{dis} concentrations were 17, 14, 14,124 and 150 mg/L at HRTs of 2.5, 3.3, 5, 10 and 20 days, respectively in the effluent of the CSTR. The COD_{dis} removal efficiencies were around 90% for HRTs of 2.5, 3.3 and 5 days, respectively in the aerobic CSTR. The COD_{dis} yields were 77% and 75% for HRTs of 5.5 and 11 days, respectively. The COD_{dis} yields decreased from 90% to 75% when the HRTs were increased from 5 to 20 days, respectively (see Table 6.34).

As shown in Table 6.34 the total PAHs concentrations were increased from 6.30 to 4.81 ng/mL as the HRTs were increased from 2.5 to 3.3 days respectively in the effluent of the CSTR. The total PAHs removal efficiencies were around 85% at HRTs of 3.3 and 5 days, respectively in the aerobic CSTR (see Table 6.34). The total PAHs yields were 70% and 67% for HRTs of 10 and 20 days, respectively. The total

PAHs removal efficiencies decreased from 85% to 67% at HRTs of 2.75 and 11 days, respectively. The COD_{dis} and total PAHs removal efficiencies decreased at high HRTs in the aerobic CSTR system.

Table 6.34 COD_{dis} and total PAHs yields in the CSTR at five different HRTs (initial COD concentration= 2850 mg/L, PAH concentration=292 ng/mL)

Parameters	**HRT (days)**				
	2.5	3.3	5	10	20
COD_{dis} concentration in influent (mg/L)	171	142	142	541	598
COD_{dis} concentration in effluent (mg/L)	17	14	14	124	150
COD_{dis} removal efficiency (%)	90	90	90	77	75
Total PAHs concentration in influent (mg/L)	35.04	32.12	32.12	81.76	87.60
Total PAHs concentration in effluent (ng/mL)	6.30	4.81	4.81	24.52	28.90
Total PAHs removal efficiency (%)	82	85	85	70	67

6.4.3.3 Performance of sequential anaerobic ITBR /aerobic CSTR system

Figure 6.29 shows the removal efficiencies of COD_{dis}, total PAHs at five studied HRTs in the sequential anaerobic ITBR/aerobic CSTR system. The COD_{dis} and total PAHs removals in the sequential anaerobic ITBR/aerobic CSTR system were ≥ 98% at total HRTs of 3.88, 5.13 and 7.75 days. The maximum COD_{dis} and total PAHs yields were 99% and 98%, respectively, for the HRTs given above while the minimum COD_{dis} and total PAHs yields efficiencies were 95% and 90%, respectively, at a HRT of 31 days, in the sequential anaerobic ITBR/aerobic CSTR system.

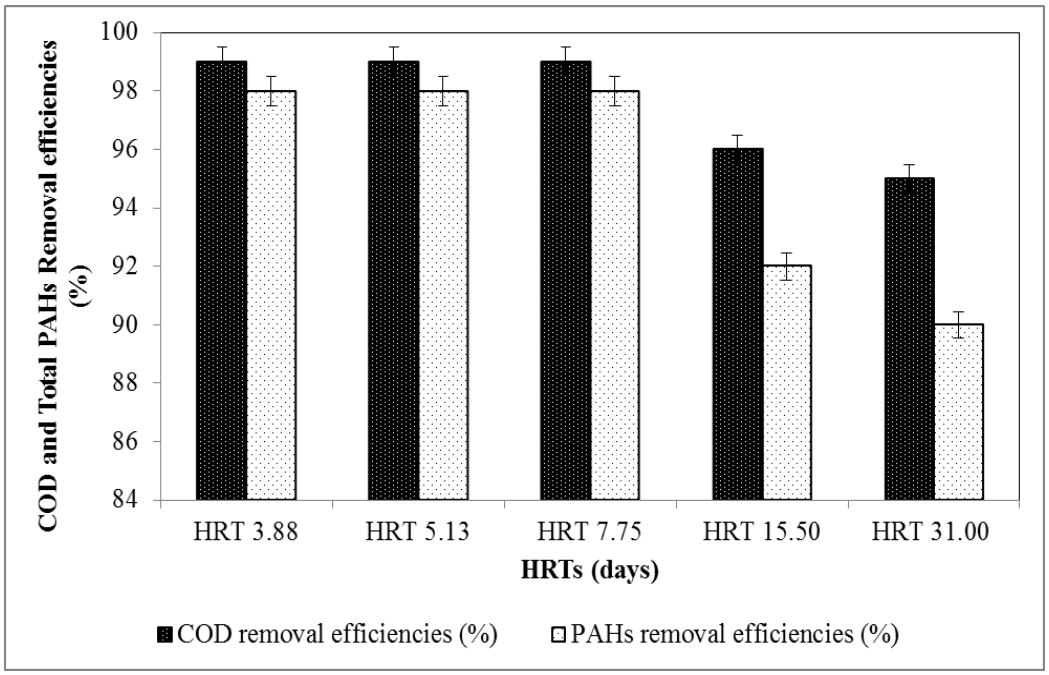

Figure 6.29 COD_{dis} and total PAHs removal efficiencies in the sequential anaerobic ITBR /aerobic CSTR system with increasing HRTs (total OLR=1.039 g COD_{dis}/m^3.day and total OLR=113.19 ngPAH/mL.day)

The maximum COD_{dis} and total PAHs yield were 99.8% and 99.5% respectively in the sequential anaerobic ITBR/aerobic CSTR system in the presence of 75 mg/L RD at a Total SRT 88 days, at a total HRT of 7.75 days, at a total OLR of 0.583 g COD_{dis}/m^3.day and at a total OLR of 107.22 ngPAH/mL.day. The PAHs and COD_{dis} in the real petrochemical industry wastewater were mainly removed in the anaerobic of the sequential anaerobic/aerobic system. From 292 ng/mL initial total PAHs concentration of 256.96 ng/mL PAH were biodegraded (88% PAH yield) in the anaerobic ITBR reactor. Since the effluent of the ITBR reactor was used as feed of the CSTR the PAH concentration (35.04 ng/mL) remaining from the anaerobic reactor was removed with a yield of 96% in the CSTR ending with a PAH concentration of 1.40 ng/mL in the effluent of CSTR. From 2850 mg/L initial COD_{dis} 2679 mg/L was biodegraded in the anaerobic ITBR reactor similarly to the PAH. The effluent of ITBR containing 171 mg/L COD_{dis} was removed in the CSTR with a yield of 97% resulting in COD_{dis} effluent concentration of 5.13 mg/L.

The contribution of aerobic reactor to the removal of PAH in the sequential system was the biodegradation of the PAHs in the effluent of the anaerobic reactor (35.04 ng/mL) and to mineralize the PAH metabolites produced from the ITBR. Although the COD concentration (171 mg/L) in the effluent of the ITBR is between the discharge limits for COD given by the Turkish Water Pollution and Control Regulation (2004) the aerobic reactor provides the ultimate mineralization of PAHs and of carbonaceous organic metabolites.

6.4.4 Biofilm Development on the Carrier Material with and without 75 mg/L RD in the Anaerobic ITBR System

At the end of study (after 300 days of operation the ITBR) the diameter of the support material excessive increased due to attachment of the biomass around Omega U- Spheres W particles (See Figures 6.30 and 6.31 and Table 6.35). After 300 days of continuous operation the color of the particles obviously turned from almost light grey to intense gray, black and brown. Microscopic observations confirmed the presence of biomass on the carrier, either as local outgrowth colonies, or as uniform covering of the particles. A large number of particles were covered regularly by a thick biofilm with a constant thickness of 480 and 270 μm in the ITBR containing 75 mg/L RD and in the ITBR without RD, respectively. The high biofilm thickness could be attributed to the spherical shapes of the Omega U- Spheres W particles and to the circulation of gas from to lower stage of ITBR to upper stage of the ITBR resulting in turbulence between biomass surrounding carrier particle and wastewater.

The amount of biomass accumulated on the carrier increased significantly to reach values above 43.8 g/L, while the biomass concentration in the liquid phase was 2.60 g/L after 300 days of operation period in the anaerobic ITBR system in the presence of 75 mg/L RD at a HRT of 2.75 days (Table 6.35). The amount of biomass accumulated on the carrier was measured as 30.2 g/L in the absence of RD after 300 days of operation period. It was found that the VSS concentration in the ITBR containing RD increased significantly compared to RD free anaerobic ITBR system.

The average biofilm thicknesses were 675 and 465 μm in the ITBR with RD and without RD, respectively at the end of the operation period (See Table 6.35 and Figures 6.30 and 6.31). The high biofilm thickness in the reactor containing RD could be attributed unifying and binding properties of biosurfactant to the bacterial excretes and extracellular microbial products resulting in more compact biofilm formation in the anaerobic ITBR system.

Table 6.35 Effect of RD on the biofilm thickness, on the carrier attachment in the anaerobic ITBR system with and without 75 mg/L RD (initial COD_{dis} = 2850 mg/L, initial PAH=292 ng/mL, HRT=2.75 days, SRT =63 days, operation time =300 days)

	With 75 mg/L RD	Without RD
Particle diameter (μm) before operation	195	195
Coverage particle diameter (μm) after 300 days of operation	675	465
Biofilm thickness before operation (μm)	0	0
Average biofilm thickness (μm) after 300 days of operation period	480	270
Initial VSS in liquid (g/L)	16.4	16.4
VSS in mixed liquor after 300 days of operation (g/L)	3.6	2.4
VSS (biomass) accumulated on the carrier (g/L) after 300 days of operation	43.8	30.2
Total VSS concentration (in mixed liquor +on the carrier surface) after 300 days of operation	47.4	32.6
Attached volatile solids (AVS) on the carrier ($g_{VSS}/g_{carrier}$)	0.23	0.16

The amount of attached volatile solids (AVS) per gram of carrier could be measured once a reasonable biomass amount was established on the particles (Chen and Chen, 2000; Sowmeyan and Swaminathan, 2008). The AVS amounts on the carrier were measured as 0.23 $g_{AVS}/g_{carrier}$ and 0.16 $g_{AVS}/g_{carrier}$ in the ITBR with 75 mg/L RD and without RD. This means that the anaerobic ITBR was excessively capable of high biomass attachment and accumulation, thus it could be inferred to the high the total PAHs and COD_{dis} performances.

The results study showed that the performance of the anaerobic ITBR can be attributed to the high attached VSS concentration surrounding U-Sheper particles. The contribution of VSS concentration in the mixed liquor was found to be partially significant in the treatment of petrochemical wastewater containing the PAHs and the COD_{dis}.

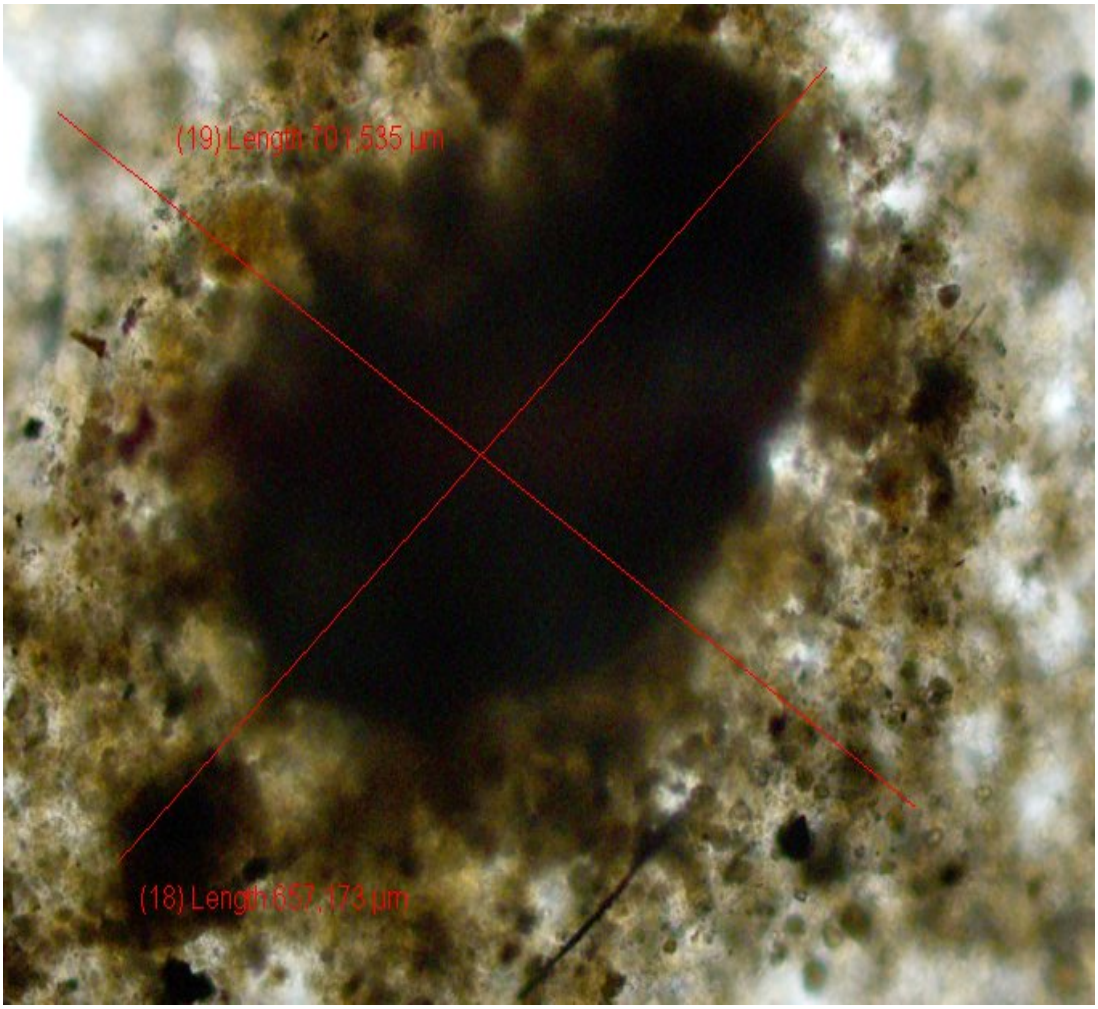

Figure 6.30 Microscopic observation of carrier at the end of operation period in the anaerobic ITBR system at 75 mg/L RD (magnification 20X10, average diameter: 675µm)

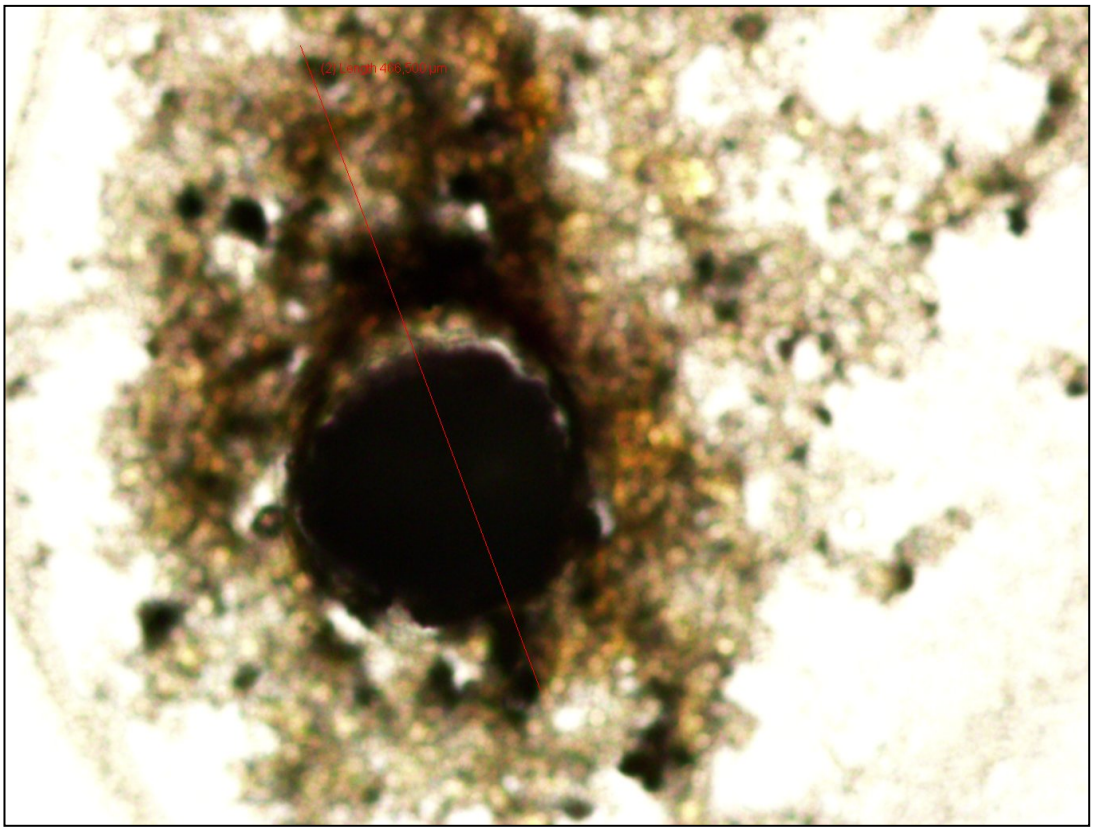

Figure 6.31 Microscopic observation of carrier at the end of operation period in the anaerobic ITBR system without RD (magnification 20X10, average diameter: 465µm)

Garcia-Calderon et al (1998) found an AVS value of 0.2 gVSS/g carrier in an inverse fluidized bed reactor after 160 days of operation using pearlite as biomass carrier. Although the AVS values exhibited similarities, the COD yields in this study performed by Garcia-Calderon et al (1998) was recorded as 55% which is very low compared to the our COD_{dis} yields (94%).

6.5 Determination of Kinetic Constants for Aerobic CSTR and Anaerobic ITBR System

6.5.1 Biodegradation Kinetics in the CSTR System

In order to determine the most suitable biokinetic model in the CSTR, some kinetic models such as Monod, zero, half, first, and second order substrate removal kinetics were applied to the experimental results obtained from the continuous operation. The kinetic constant of the CSTR treating COD_{dis} and PAHs were evaluated according to the experimental data at five SRTs (5-10-20-25-40 days). The interpretations of the models and the kinetic constants were performed in this step.

6.5.1.1 *Monod Kinetic Model for COD_{dis} and PAHs Removals with 15 mg/L RD and without RD*

The biodegradation kinetics of COD_{dis} and total PAHs were investigated. Therefore, Monod, kinetic was applied to the experimental data to determine the type of PAH and COD_{dis} biodegradation kinetics in the presence and absence of biosurfactant (15 mg/L RD).

6.5.1.1.1 Monod Kinetic Model for COD_{dis} Removal with 15 mg/L RD and without RD. Figs. 6.32 and 6.33 were plotted from the Eq. (5.30) and Eq. (5.31) (See chapter 5.13.1) to determine the values of half saturation concentration (K_s) (mg/L), maximum rate of substrate utilization (k), the growth yield coefficient (Y) (mgVSS/mgCOD), endogenous decay coefficient (k_d) (1/day) and maximum specific substrate utilization rate (μ_{max}) (1/day) in Monod kinetic model for COD_{dis} metabolization.

K_s and k determined from the slope and intercept of the straight line as shown in Figure 6.32 as 56.34 mgCOD_{dis}/L and 0.17 1/day, respectively for COD_{dis} in the presence of 15 mg/L RD.

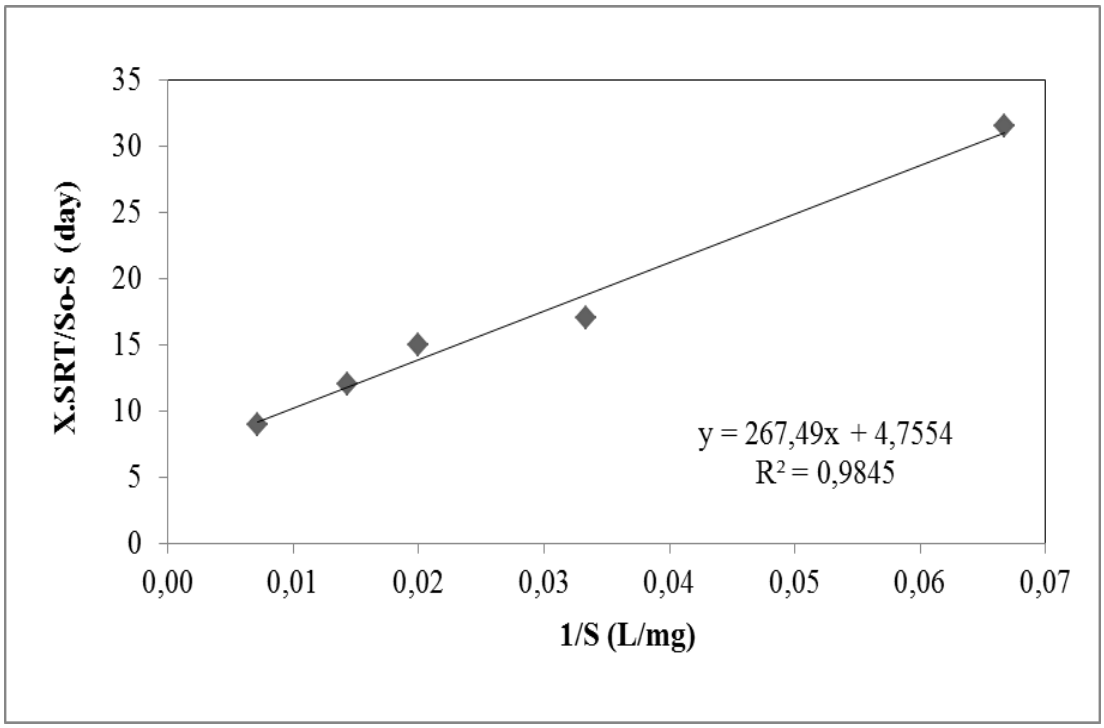

Figure 6.32 Determination of half saturation constant (K_s) and maximum rate of substrate utilization (k) for COD_{dis} at 15 mg/L RD

Y and k_d values were calculated from the slope and the intercept of the straight line as illustrated in Figure 6.33 for COD_{dis}. The value of Y and k_d were determined from Figure 6.33 as 0.86 mgVSS/mg COD_{dis} and 0.015 1/day and respectively. μ_{max} was calculated as 0.18 1/day by multiplying Y by k_d at 15 mg/L RD. In the RD free case, K_s and k determined from slope and intercept of the straight line as illustrated in Figure 6.34. These kinetic constants were calculated as 110.59 mgCOD_{dis}/L and 0.19 1/day, respectively.

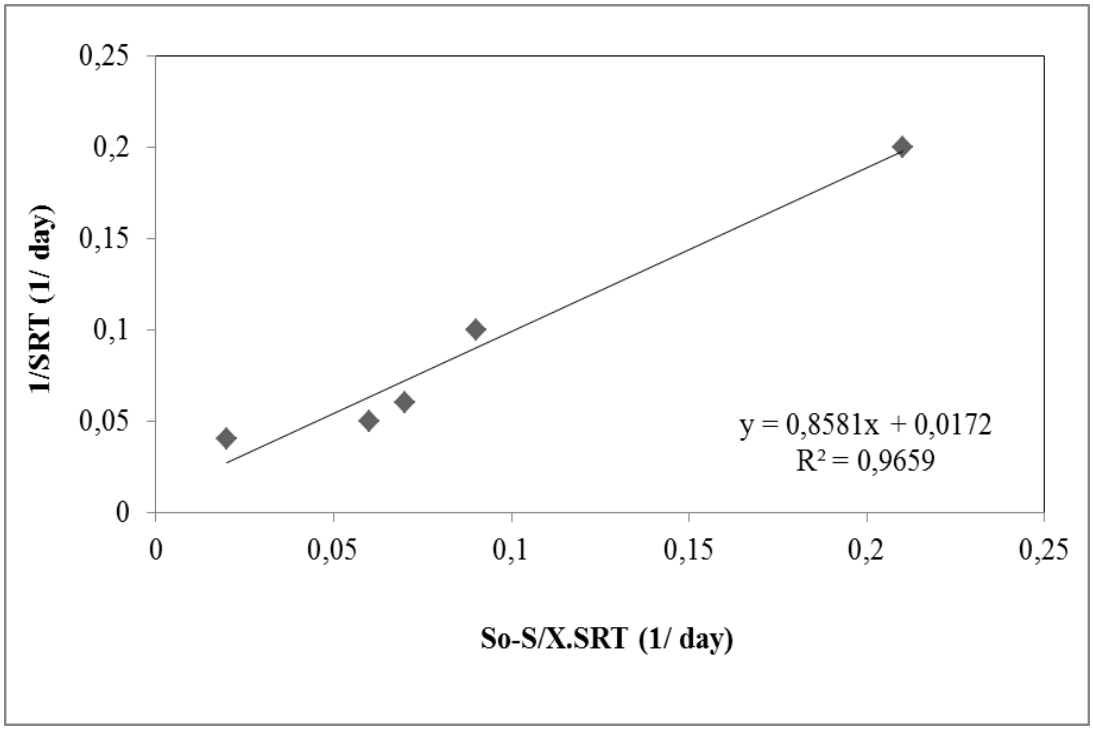

Figure 6.33 Determination of yield coefficient (Y), maximum specific substrate utilization rate (μ_{max}), death rate constant (k_d) for COD_{dis} at 15 mg/L RD

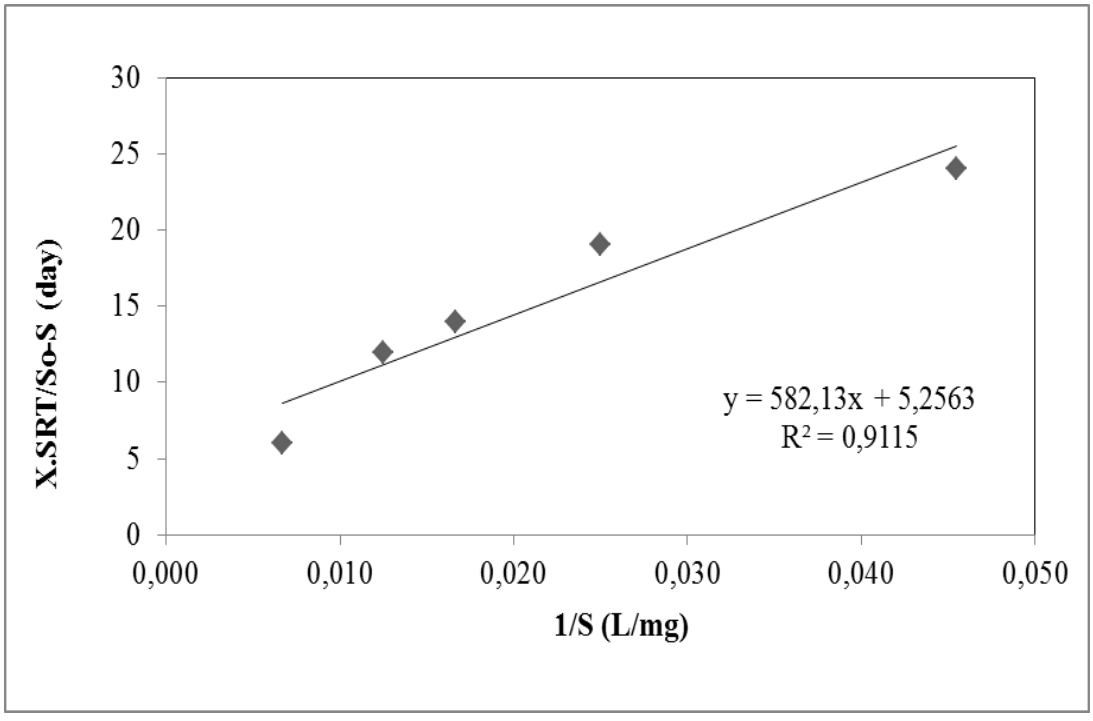

Figure 6.34 Determination of half saturation constant (K_s) and maximum rate of substrate utilization (k) for COD_{dis} without RD

Y and k_d values were calculated from the slope and the intercept of the straight line and are illustrated in Figure 6.35 for COD_{dis} in the absence of RD. The value of Y and k_d were determined as 0.45 mgVSS/mgCOD_{dis} and 0.017 1/day and respectively. μ_{max} was calculated as 0.10 1/day by multiplying Y by k_d.

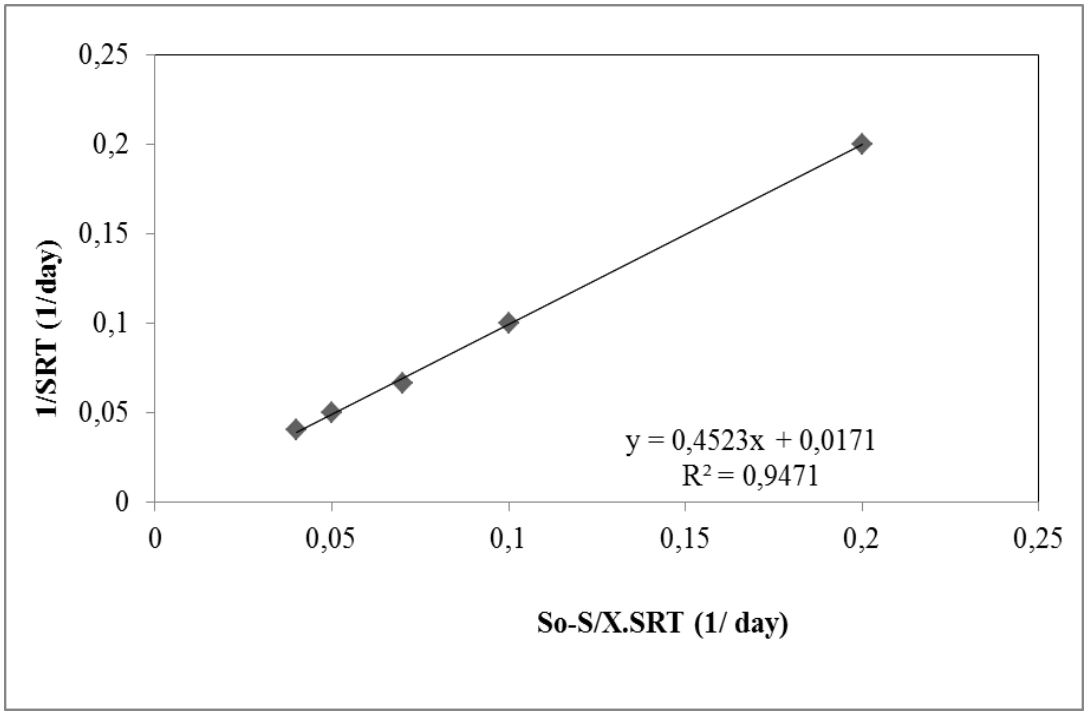

Figure 6.35 Determination of yield coefficient (Y), maximum specific substrate utilization rate (μ_{max}), death rate constant (k_d) for COD_{dis} without RD

6.5.1.1.2 Monod Kinetic Model for PAHs Removal with 15 mg/L RD and without RD. K_s and k values calculated from the slope and the intercept of the straight line illustrated in Fig 6.36 for PAHs at 15 mg/L RD in the CSTR system. K_s and k values were as 0.0013 mg PAHs/L and 0.22 1/day, respectively, through the degradation of PAHs in the CSTR while μ_{max} was 0.14 1/day at 15 mg/L RD. Y and k_d values calculated from the slope and the intercept of the straight line as illustrated in Figure 6.37 for PAHs at 15 mg/L RD in the CSTR system. Y was determined as 0.64 mg VSS/mg PAHs while k_d was determined as 0.003 1/day at 15 mg/L RD (Figure 6.37).

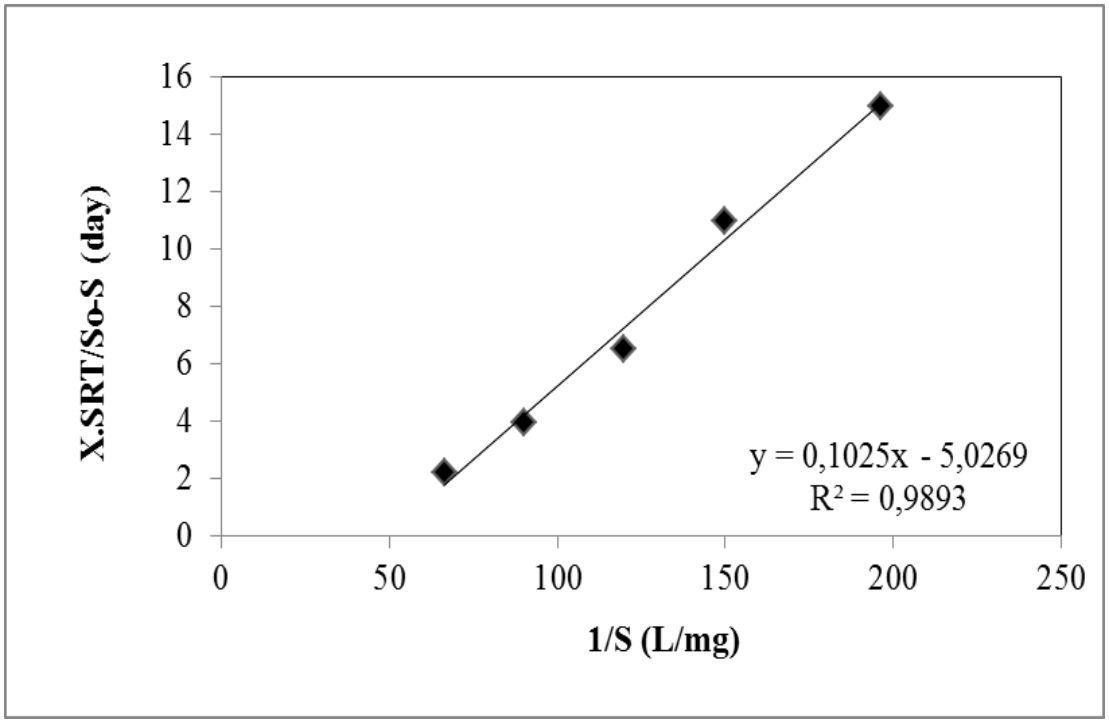

Figure 6.36 Determination of half saturation constant (K_s) and maximum rate of substrate utilization (k) for PAHs at 15 mg/L RD

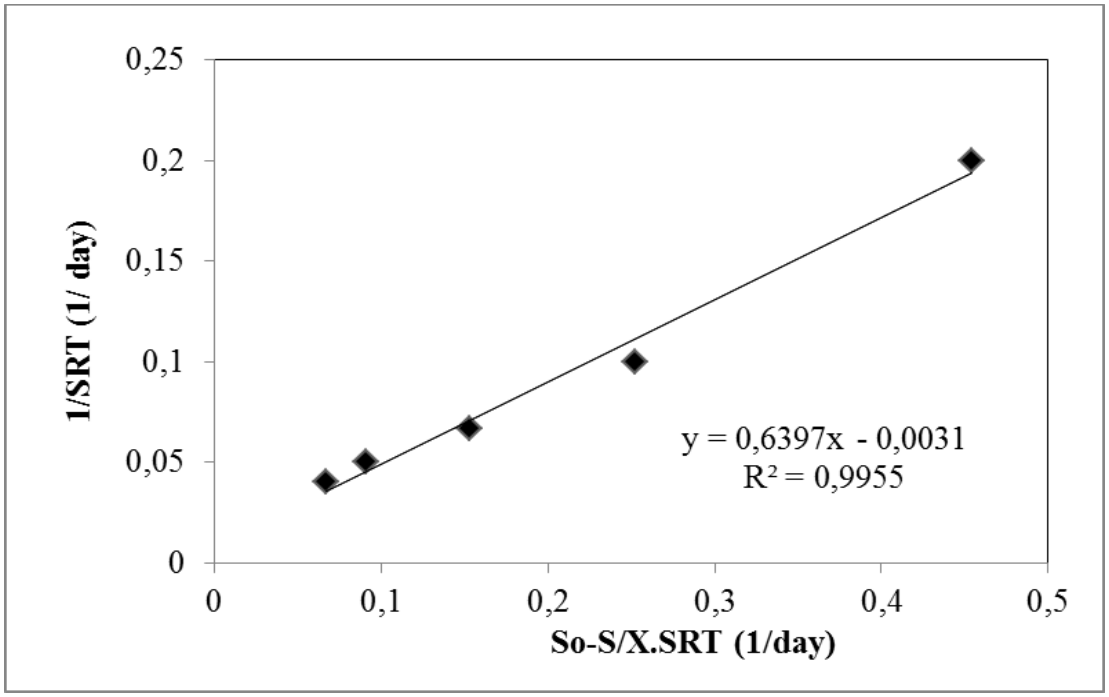

Figure 6.37 Determination of yield coefficient (Y), maximum specific substrate utilization rate (μ_{max}), death rate constant (k_d), for PAHs at 15 mg/L RD

K_s and k values were calculated from the slope and the intercept of the straight line illustrated in Figure 6.38 for PAHs in the absence of RD. K_s and k values were calculated as 0.0042 mgPAHs/L and 0.06 1/day, respectively, through the degradation of PAHs in the CSTR while μ_{max} was 0.02 1/day.

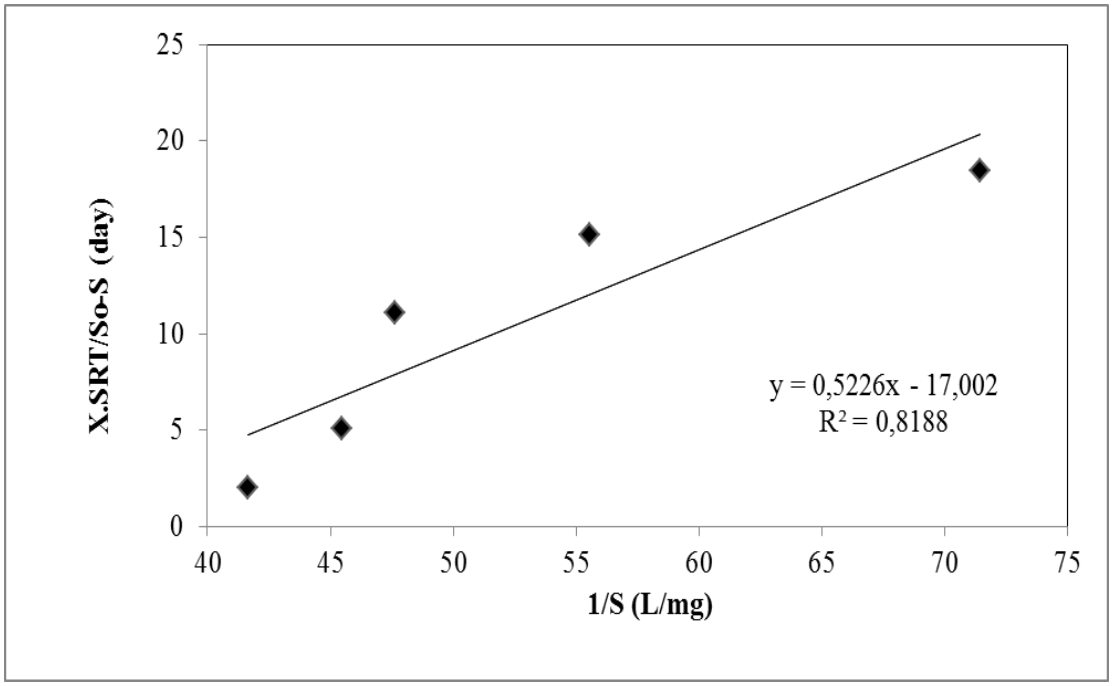

Figure 6.38 Determination of half saturation constant (K_s) and maximum rate of substrate utilization (k) for PAHs without RD

Y and k_d values were calculated from the slope and the intercept of the straight line illustrated in Figure 6.39 for PAHs. Y was determined as 0.38 mgVSS/mgPAHs and k_d was calculated as 0.015 1/day.

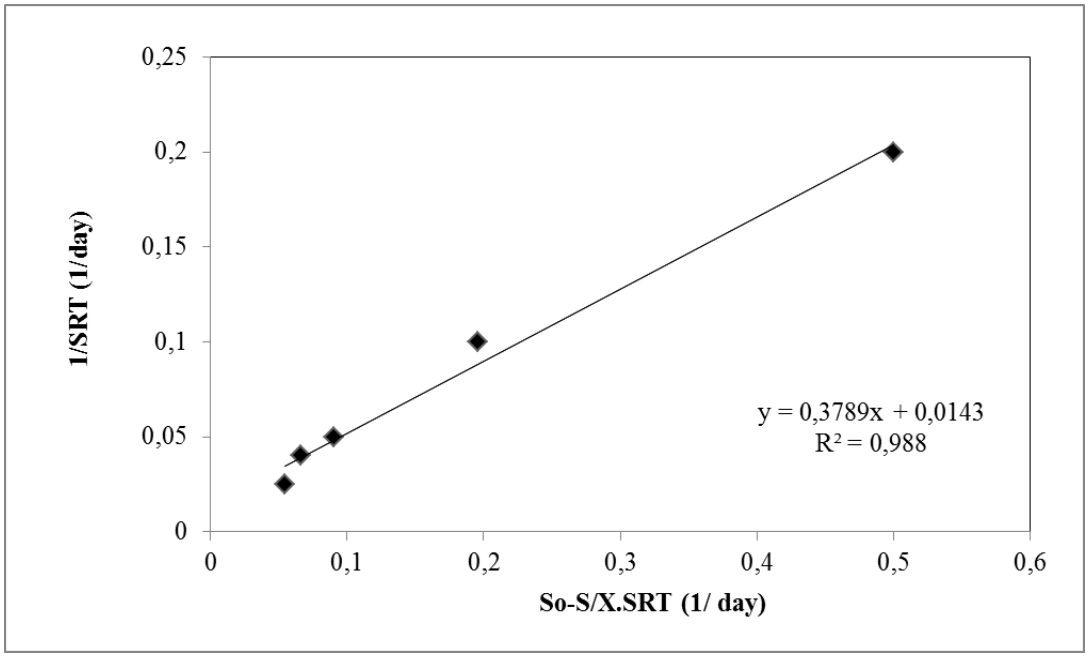

Figure 6.39 Determination of yield coefficient (Y), maximum specific substrate utilization rate (μ_{max}), death rate constant (k_d) for PAHs without RD

6.5.1.2 Zero Order Substrate Biodegradation Model for COD_{dis} and PAHs Removals with 15 mg/L RD and without RD at Increasing SRTs

6.5.1.2.1 Zero Order Substrate Biodegradation Model for COD_{dis} Removal with 15 mg/L RD and without RD. The value of zero order kinetic constant (k_0) (mg COD_{dis}/L.day) was obtained from the slope of the line by plotting "S" versus operation time (day) in Eq. (5.34). S is COD_{dis} concentrations in the effluent (mgCOD_{dis}/L). Figure 6.40 shows the plot between S and operation time (day). k_0 was calculated as 107.48 mg/L.day with regression coefficient of 0.87 (y=-107.48x+1118.8) for COD_{dis} at a SRT of 25 days with 15 mg/L RD. Figure 6.41 shows the plot between S and operation time (day) at a SRT of 25 days. k_0 was calculated as 52.2860 mg/L.day with regression coefficient of 0.74 (y=52.286x+101.86) for COD_{dis} without RD at a SRT of 25 days.

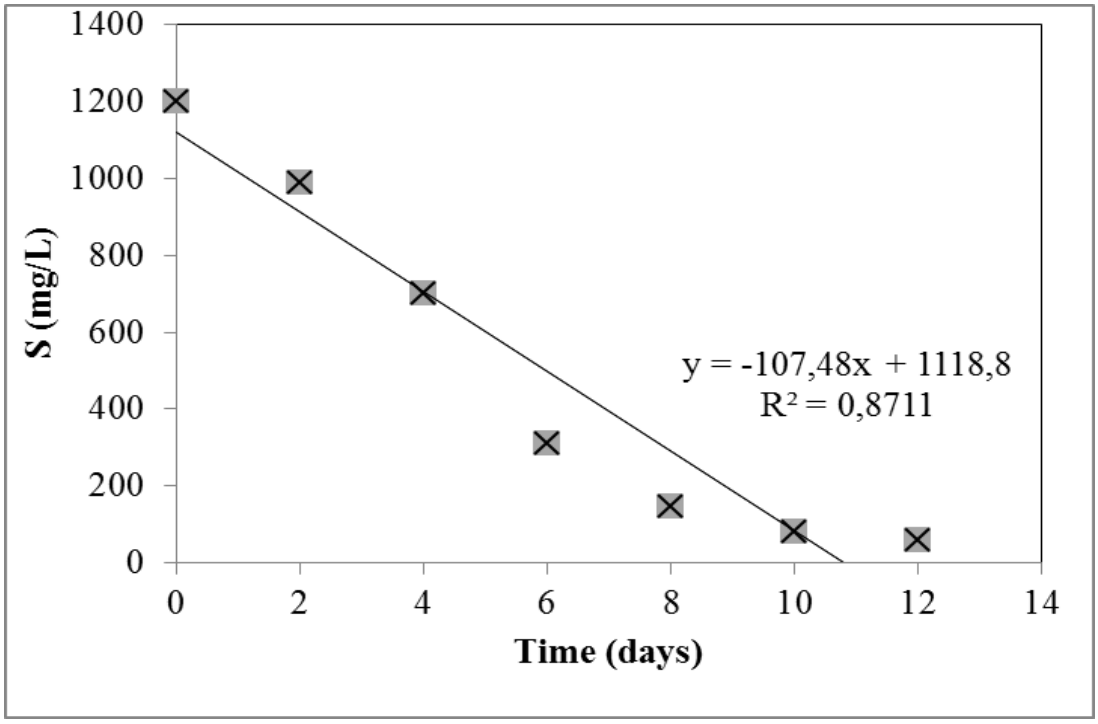

Figure 6.40 The model plot of zero order substrate removal for COD_{dis} at a SRT of 25 days with 15 mg/L RD

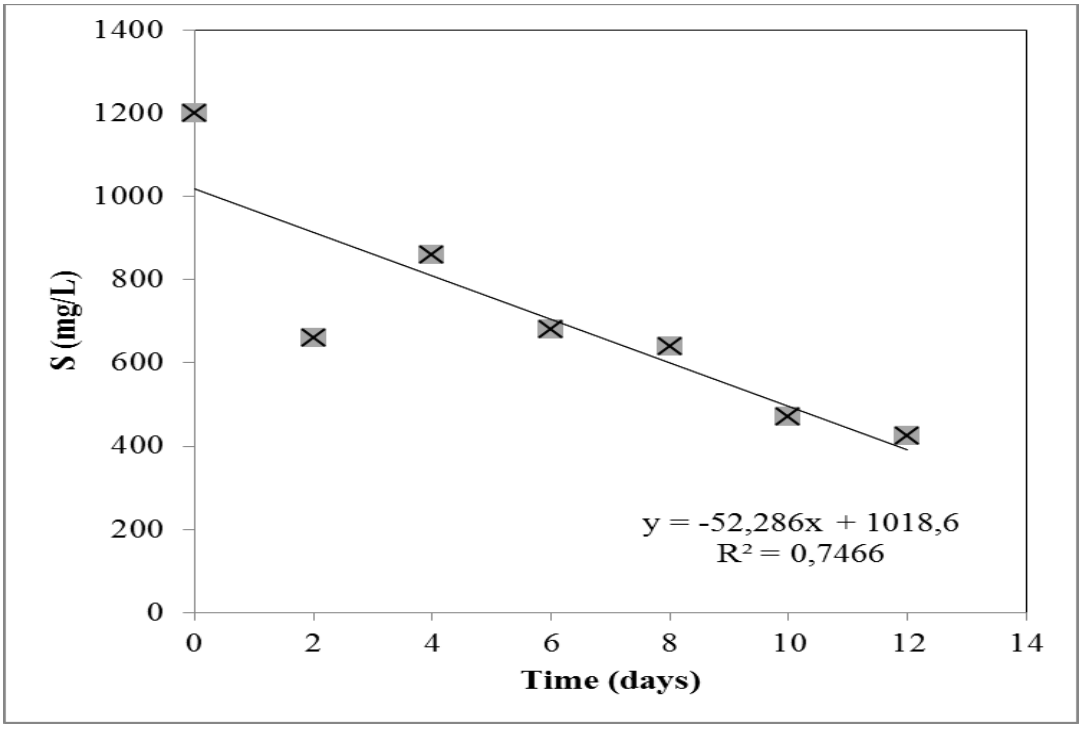

Figure6.41 The model plot of zero order substrate removal for COD_{dis} at a SRT of 25 days without RD

6.5.1.2.2 Zero Order Substrate Biodegradation Model for PAHs Removal with 15 mg/L RD and without RD. The value of zero order kinetic constant (k_0) (ng PAHs/mL.day) was obtained from the slope of the line by plotting "S" versus operation time (day) in Eq. (5.34). S is the PAHs concentrations in the effluent (ngPAHs/mL). Figure 6.42 shows the plot between S and operation time (day). k_0 was calculated as 4.9634 ng/mL.day with regression coefficient of 0.87 (y=-4.9634x+61.305) for PAHs at a SRT of 25 days with 15 mg/L RD.

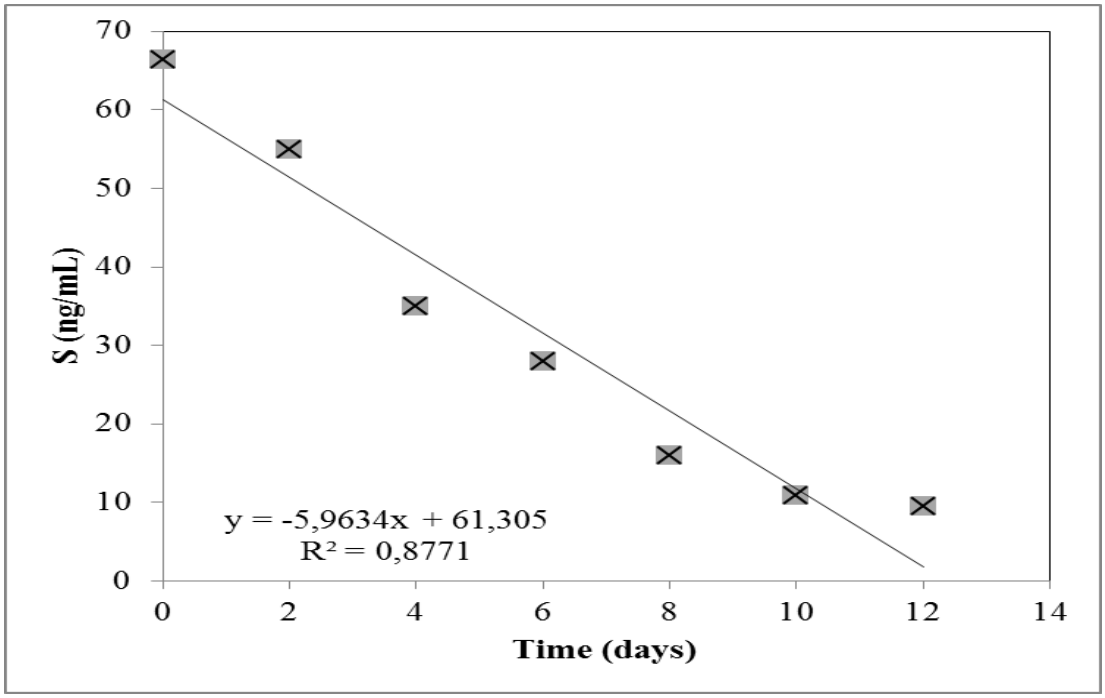

Figure 6.42 The model plot of zero order substrate removal for PAHs at a SRT of 25 days with 15 mg/L RD

Figure 6.43 shows the plot between S and operation time (day) at a SRT of 25 days. k_0 was calculated as 5.1264 ng/mL.day with regression coefficient of 0.79 (y=-5.14264x+68.667) for PAHs without RD at a SRT of 25 days.

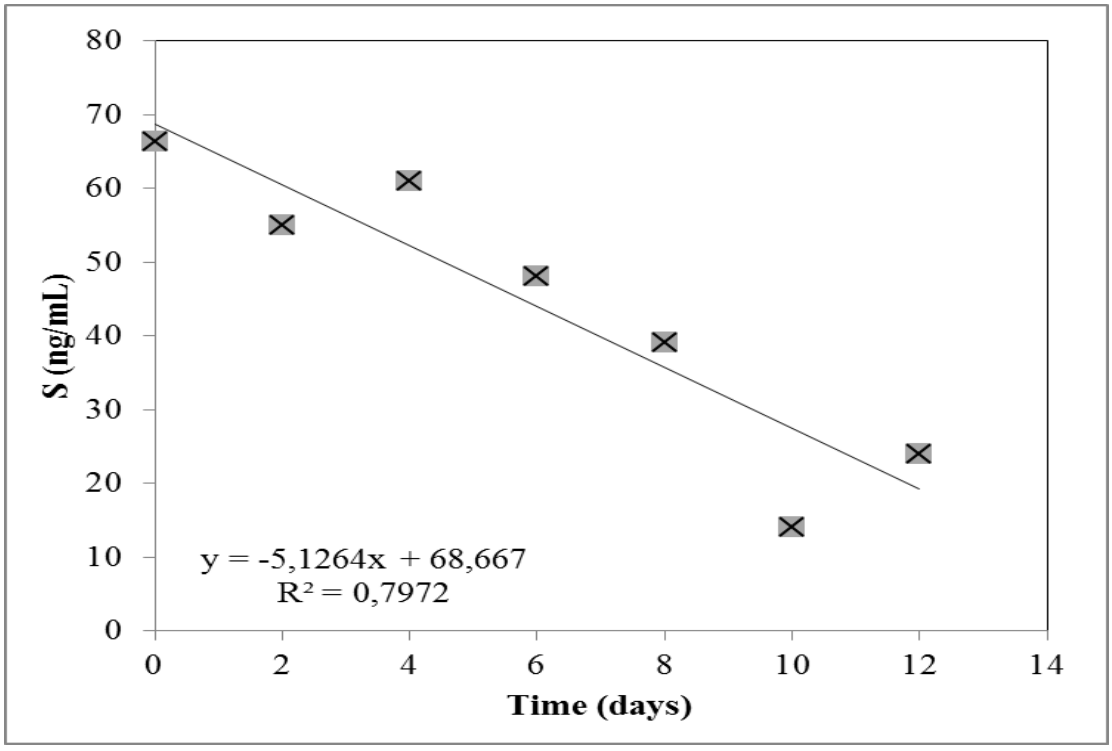

Figure 6.43 The model plot of zero order substrate removal for PAHs at a SRT of 25 days without RD at a SRT of 25 days

6.5.1.3 Half Order Substrate Removal Model for COD_{dis} and PAHs Removals with 15 mg/L RD and without RD at Increasing SRTs

6.5.1.3.1 Half Order Substrate Removal Model for COD_{dis} Removal with 15 mg/L RD and without RD. Half order kinetic constant ($k_{1/2}$) (1/day) was obtained from the slope of the line by plotting "1/√S" versus operation time (day) in Eq (5.36). S is COD_{dis} concentrations in the effluent ($mgCOD_{dis}/L$). Figure 6.44 shows the plot between "1/√S" and operation time (day). S is COD_{dis} concentrations in the effluent ($mgCOD_{dis}/L$). $k_{1/2}$ was calculated as 0.0091 1/day with correlation coefficient of 0.86, (y=0.0091x-0.1037) for COD_{dis} with 15 mg/L at a SRT of 25 day with 15 mg/L RD. Figure 6.45 shows the plot between "1/√S" and operation time (day). S is COD_{dis} concentrations in the effluent ($mgCOD_{dis}/L$). $k_{1/2}$ was calculated as 0.0014 1/day with correlation coefficient of 0.82, (y=0.0014x+0.0308) for COD_{dis} without RD at a SRT of 25 days.

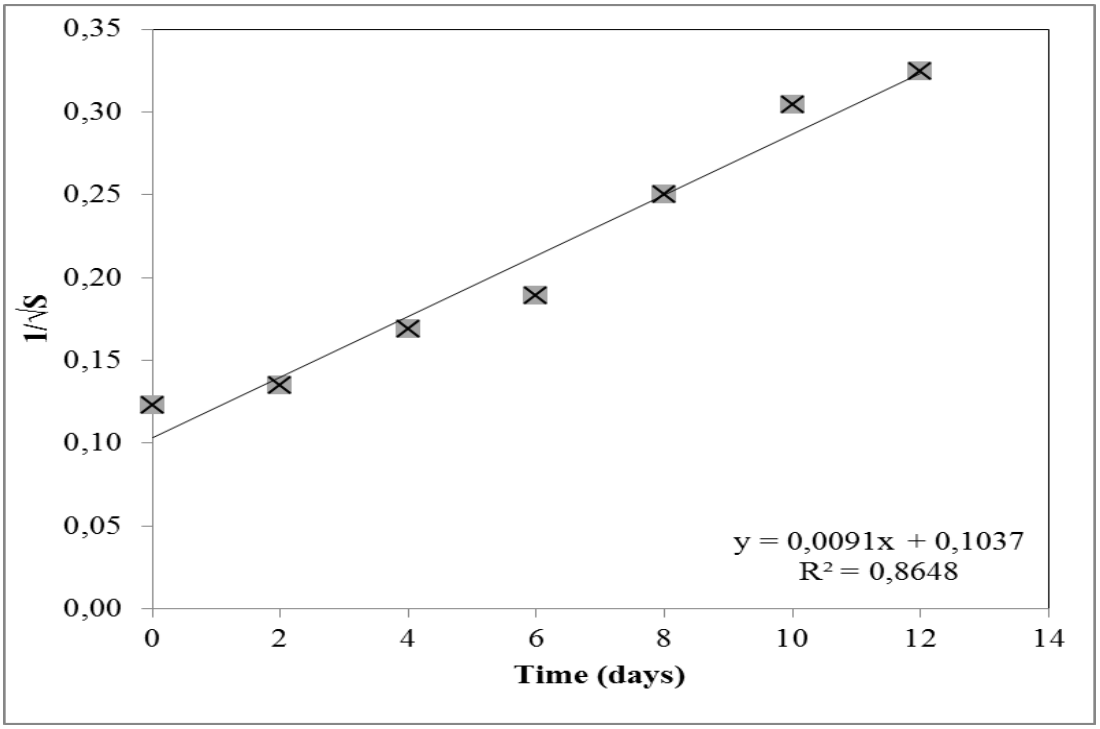

Figure 6.44 Determination of half order substrate removal for COD_{dis} with 15 mg/L RD at a SRT of 25 days.

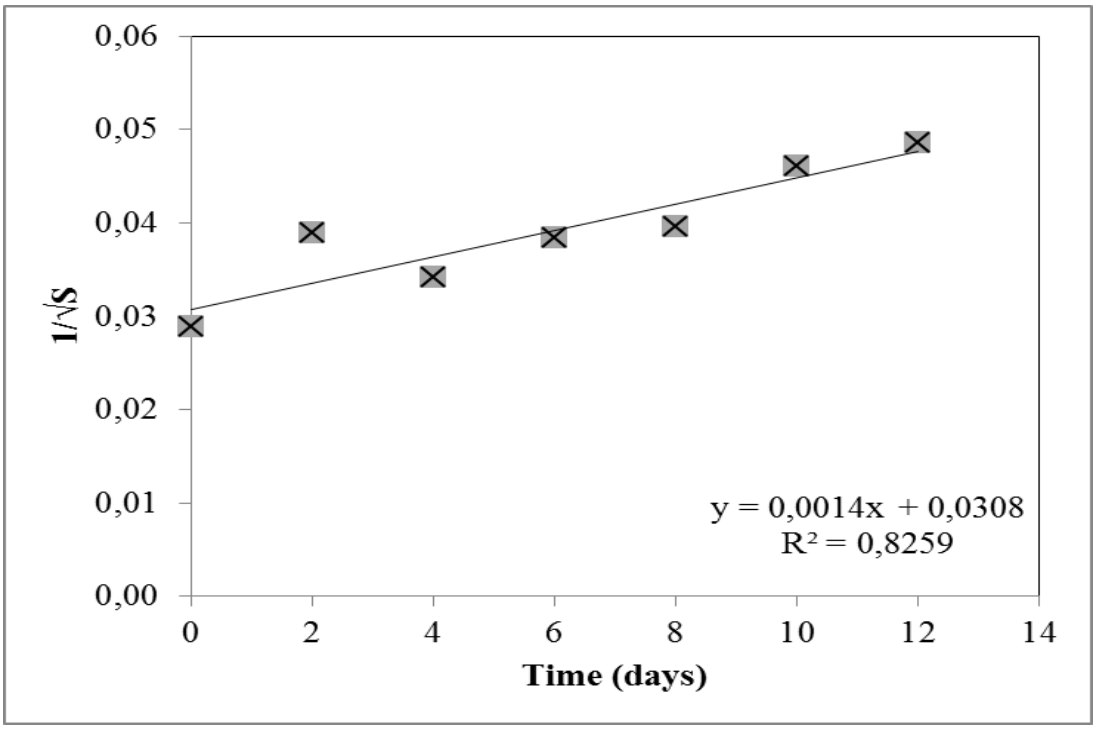

Figure 6.45 Determination of half order substrate removal for COD_{dis} without RD at a SRT of 25 days.

6.5.1.3.2 Half Order Substrate Removal Model for PAHs Removal with 15 mg/L RD and without RD. Half order kinetic constant ($k_{1/2}$) (1/day) was obtained from the slope of the line by plotting "1/√S" versus operation time (day) in Eq (5.36). S is PAHs concentrations in the effluent (ngPAHs/mL). Figure 6.46 shows the plot between "1/√S" and operation time (day). S is PAHs concentrations in the effluent (ngPAHs/mL). $k_{1/2}$ was calculated as 0.0183 1/day with correlation coefficient of 0.88, (y=0.0183x+0.1037) for PAHs with 15 mg/L at a SRT of 25 days.

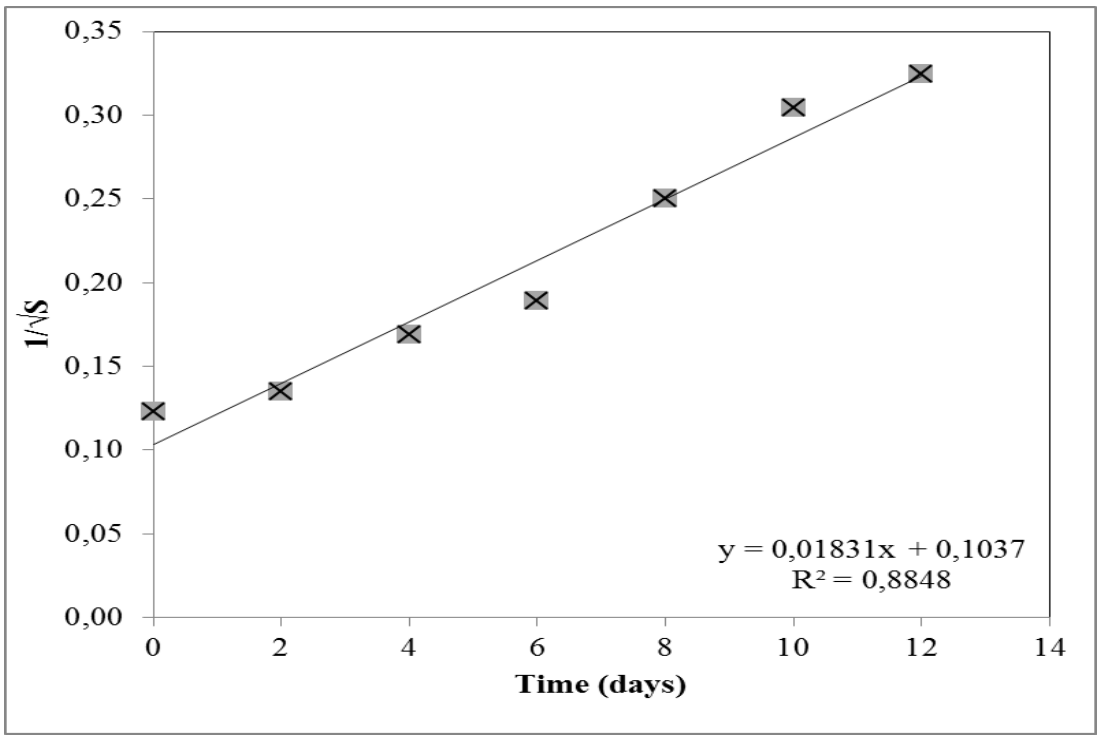

Figure 6.46 Determination of half order substrate removal for COD_{dis} with 15 mg/L at a SRT of 25 days.

Figure 6.47 shows the plot between "1/√S" and operation time (day). S is PAHs concentrations in the effluent (ngPAHs/mL). $k_{1/2}$ was calculated as 0.0156 1/day with correlation coefficient of 0.74, (y=0.0156x+0.108) for PAHs without RD at a SRT of 25 days.

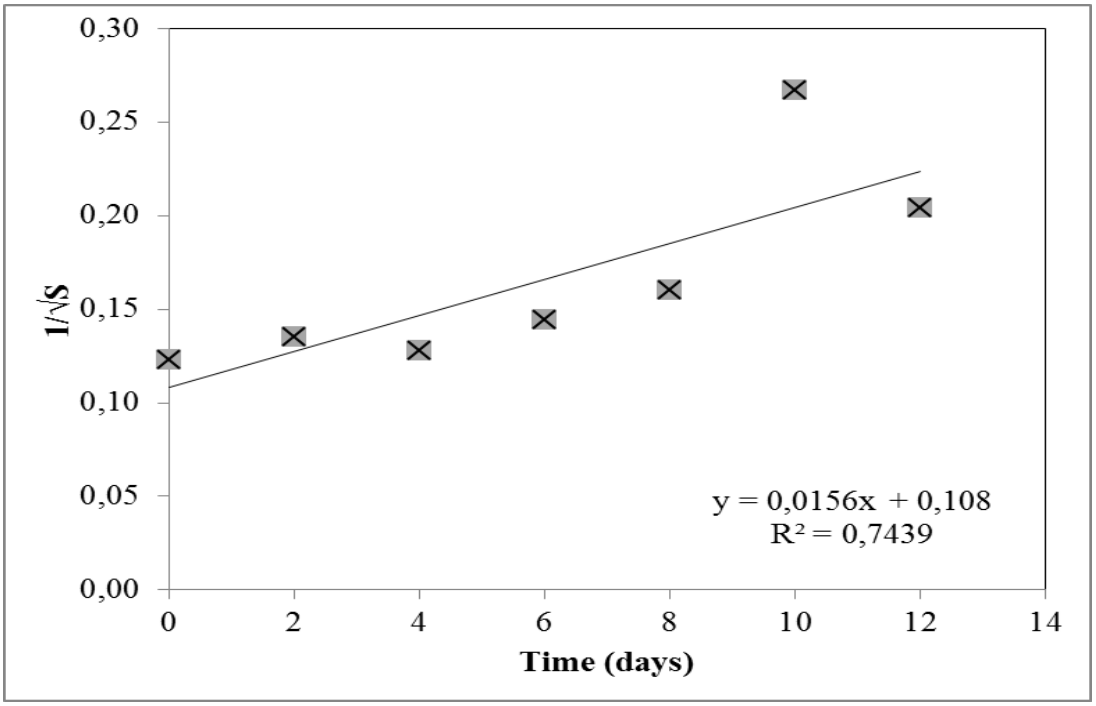

Figure 6.47 Detemination of half order substrate removal for COD_{dis} without RD at a SRT of 25 days.

6.5.1.4 First Order Substrate Biodegradation Model for COD_{dis} and PAHs Removals with 15 mg/L RD and without RD at Increasing SRTs

6.5.1.4.1 First Order Substrate Biodegradation Model for COD_{dis} Removal with 15 mg/L RD and without RD. First order kinetic constant (k_1) (1/day) was obtained from the slope of the line by plotting "lnS" versus operation time (day) in Eq (5.38). Figure 6.48 shows the plot between "lnS" and operation time (day) with 15 mg/L at a SRT of 25 days. S is COD_{dis} concentrations in the effluent ($mgCOD_{dis}/L$). k_1 was calculated as 0.3103 1/day with correlation coefficient of 0.92, (y=-0.3103x+7.4523) for COD_{dis} with15 mg/L RD.

Figure 6.49 shows the plot between "lnS" and operation time (day) without RD at a SRT of 25 days. k_1 was calculated as 0.0731 1/day with correlation coefficient of 0.81, (y=-0.07313x+6.9424) for COD_{dis} without RD at a SRT of 25 days.

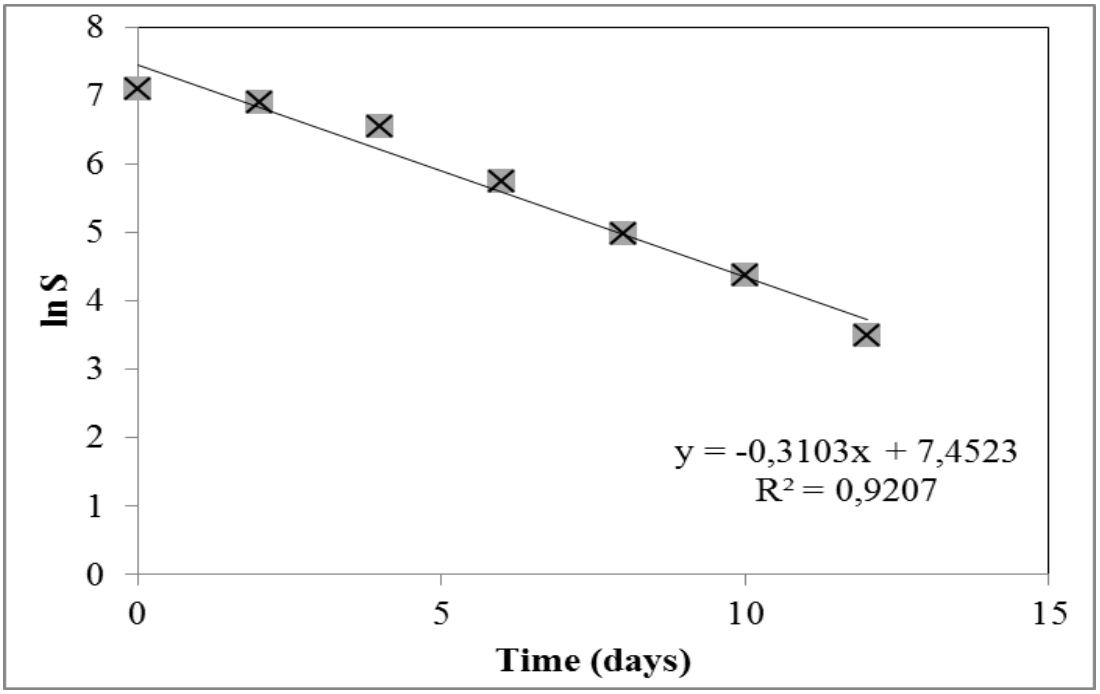

Figure 6.48 Determination of first order substrate removal for COD_{dis} with 15 mg/L RD at a SRT of 25 days

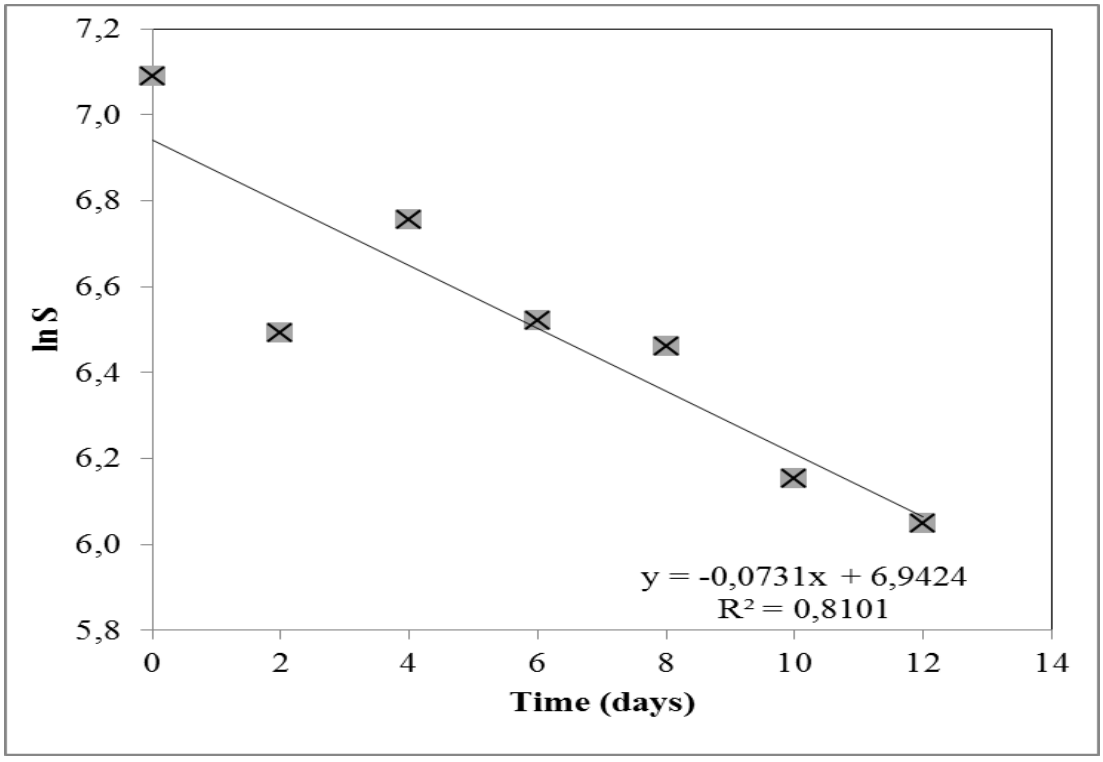

Figure 6.49 Determination of first order substrate removal for COD_{dis} without RD at a SRT of 25 days

6.5.1.4.2 First Order Substrate Biodegradation Model for PAHs Removal with 15 mg/L RD and without RD. First order kinetic constant (k_1) (1/day) was obtained from the slope of the line by plotting "lnS" versus operation time (day) in Eq (5.38). Figure 6.50 shows the plot between "lnS" and operation time (day) with 15 mg/L at a SRT of 25 days. S is PAHs concentrations in the effluent (ng PAHs/mL). k_1 was calculated as 0.1762 1/day with correlation coefficient of 0.92, (y=-0.1762x+4.7206) for PAHs with 15 mg/L RD at a SRT of 25 days.

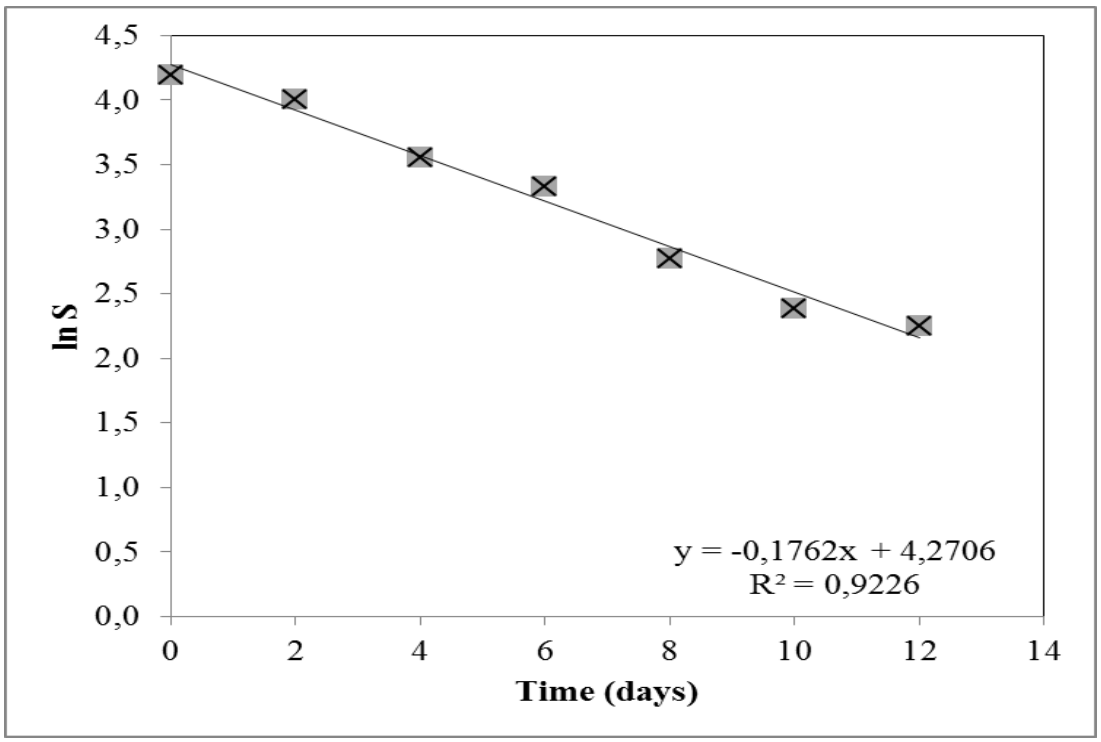

Figure 6.50 Determination of first order substrate removal for PAHs with 15 mg/L RD at a SRT of 25 days

Figure 6.51 shows the plot between "lnS" and operation time (day) without RD at a SRT of 25 days. k_1 was calculated as 0.1615 1/day with correlation coefficient of 0.78, (y=-0.1615x+4.3345) for PAHs without RD at a SRT of 25 days.

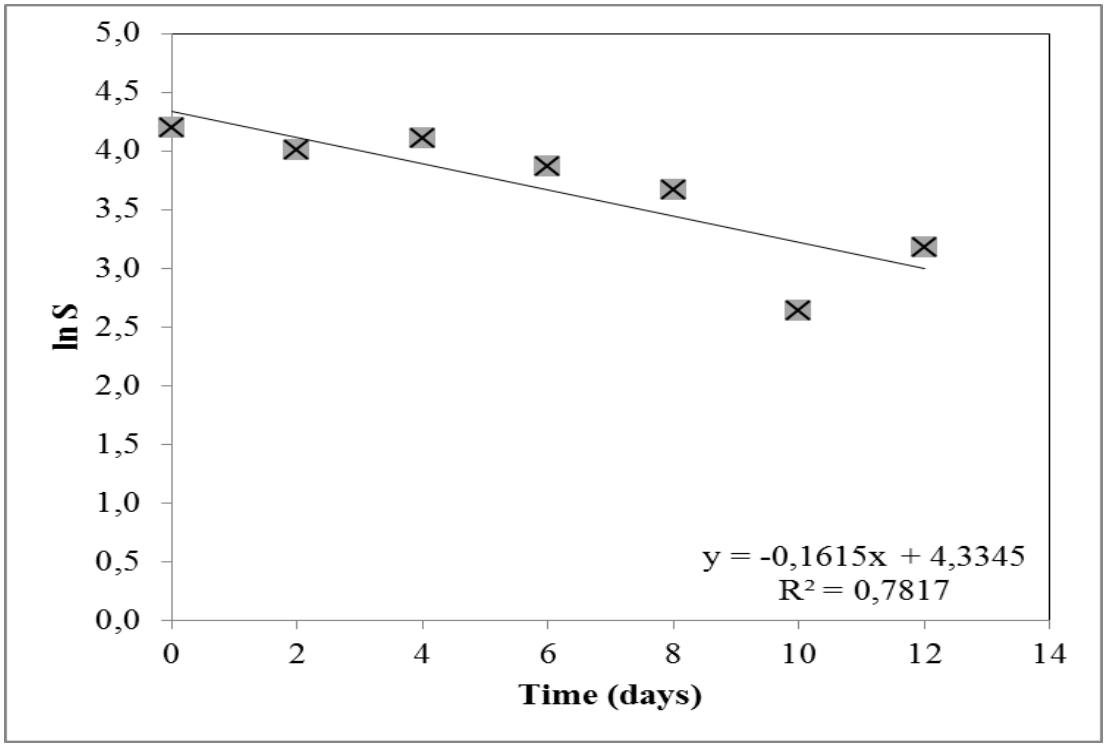

Figure 6.51 Determination of first order substrate removal for PAHs without RD at a SRT of 25 days

6.5.1.5 Second Order Substrate Biodegradation Model for COD_{dis} and PAHs Removals with 15 mg/L RD and without RD at Increasing SRTs

6.5.1.5.1 Second Order Substrate Biodegradation Model for COD_{dis} Removal with 15 mg/L RD and without RD at Increasing SRTs. In the second order substrate removal kinetic model (k_2) (1/day) was obtained from the slope of the line by plotting "1/S" versus operation time (day) in Eq (5.41). Figure 6.52 shows the plot between 1/S and operation time (day). S is COD_{dis} concentrations in the effluent ($mgCOD_{dis}/L$) with 15 mg/L RD at a SRT of 25 days. k_2 for COD_{dis} was calculated as 0.0014 L/mg.day with regression coefficient of 0.84 (y=0.0014x-0.0017) with 15 mg/L RD at a SRT of 25 days.

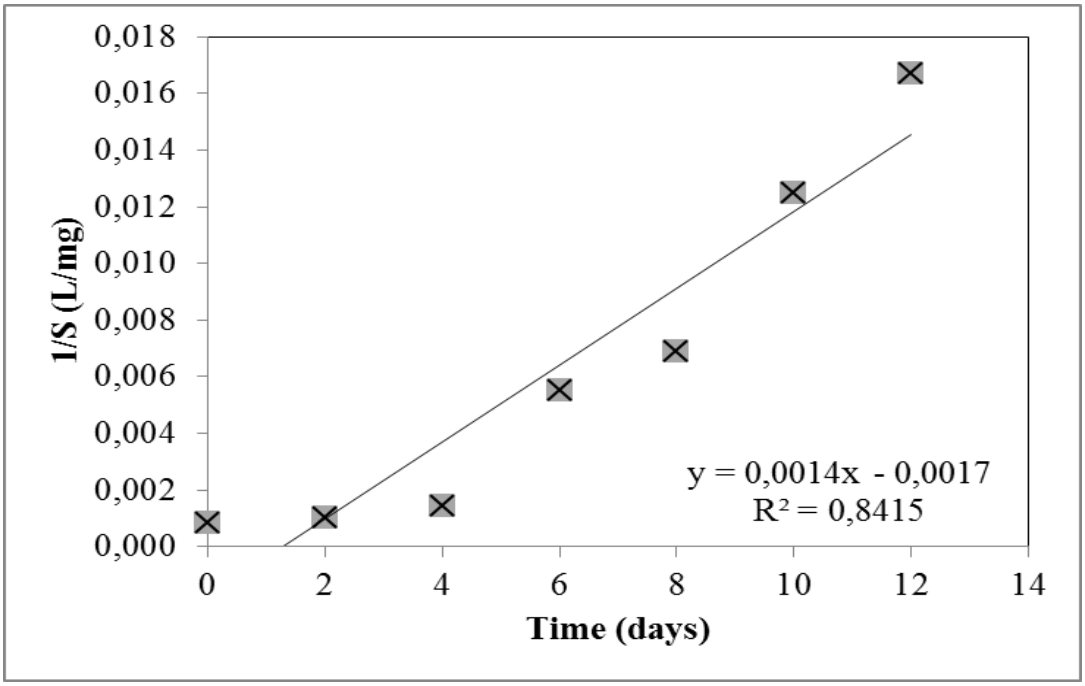

Figure 6.52 Determination of second order substrate removal for COD_{dis} with 15 mg/L RD at a SRT of 25 days

Figure 6.53 shows the plot between 1/S and operation time (day). k_2 for COD_{dis} was calculated as 0.0001 L/mg.day with regression coefficient of 0.79 (y=0.0001x+0.0009) without RD at a SRT of 25 days.

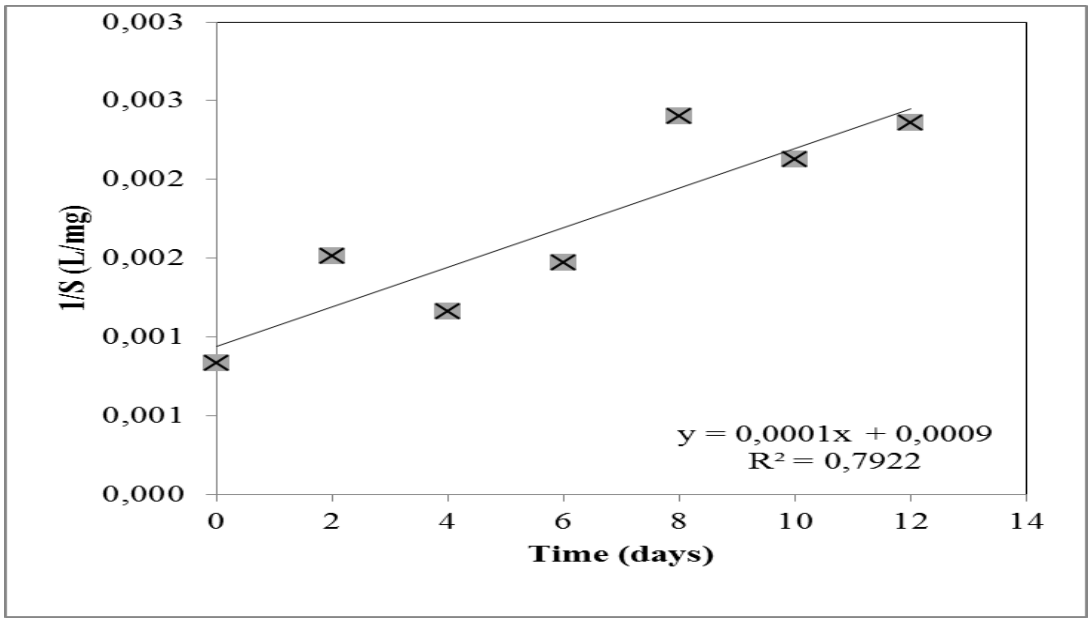

Figure 6.53 Determination of second order substrate removal for COD_{dis} without RD at a SRT of 25 days

6.5.1.5.2 Second Order Substrate Biodegradation Model for PAHs Removal with 15 mg/L RD and without RD at Increasing SRTs. In the second order substrate removal kinetic model (k_2) (1/day) was obtained from the slope of the line by plotting "1/S" versus operation time (day) in Eq (4.41). Figure 6.54 shows the plot between 1/S and operation time (day). S is PAHs concentrations in the effluent (ngPAHs/mL) with 15 mg/L RD at a SRT of 25 days. k_2 for PAHs was calculated as 0.0081 mL/ng.day with regression coefficient of 0.88 (y=0.0081x+0.0026) with 15 mg/L RD at a SRT of 25 days.

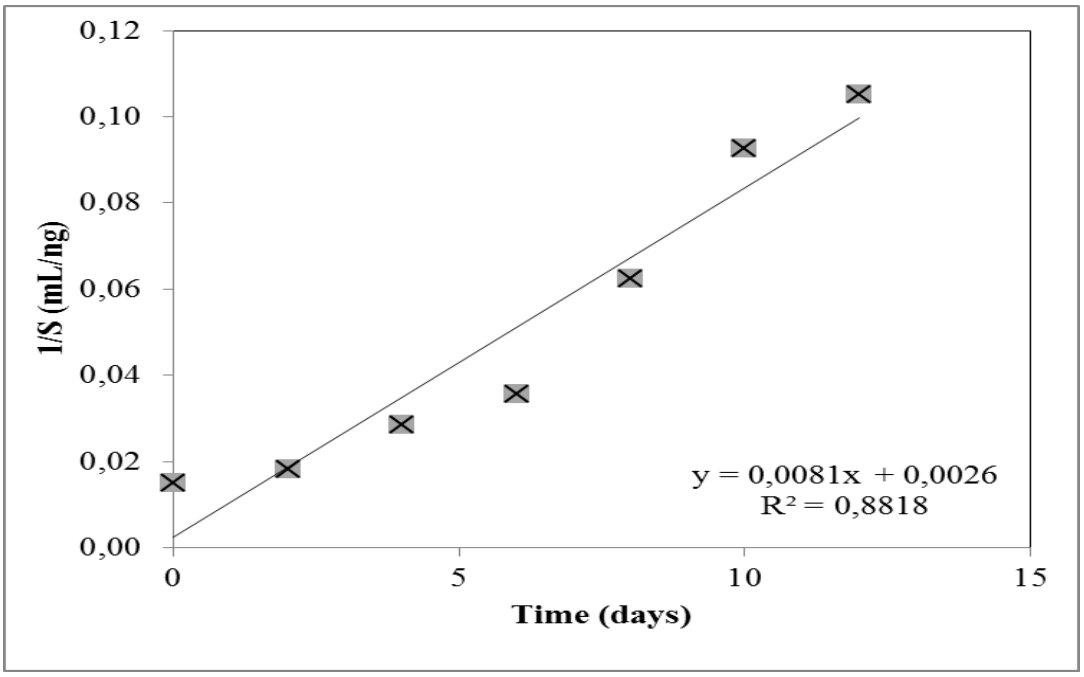

Figure 6.54 Determination of second order substrate removal for PAHs with 15 mg/L RD at a SRT of 25 days

Figure 6.55 shows the plot between 1/S and operation time (day). k_2 for PAHs was calculated as 0.0081 mL/ng.day with regression coefficient of 0.88 (y=0.0081x-0.0062) without RD at a SRT of 25 days.

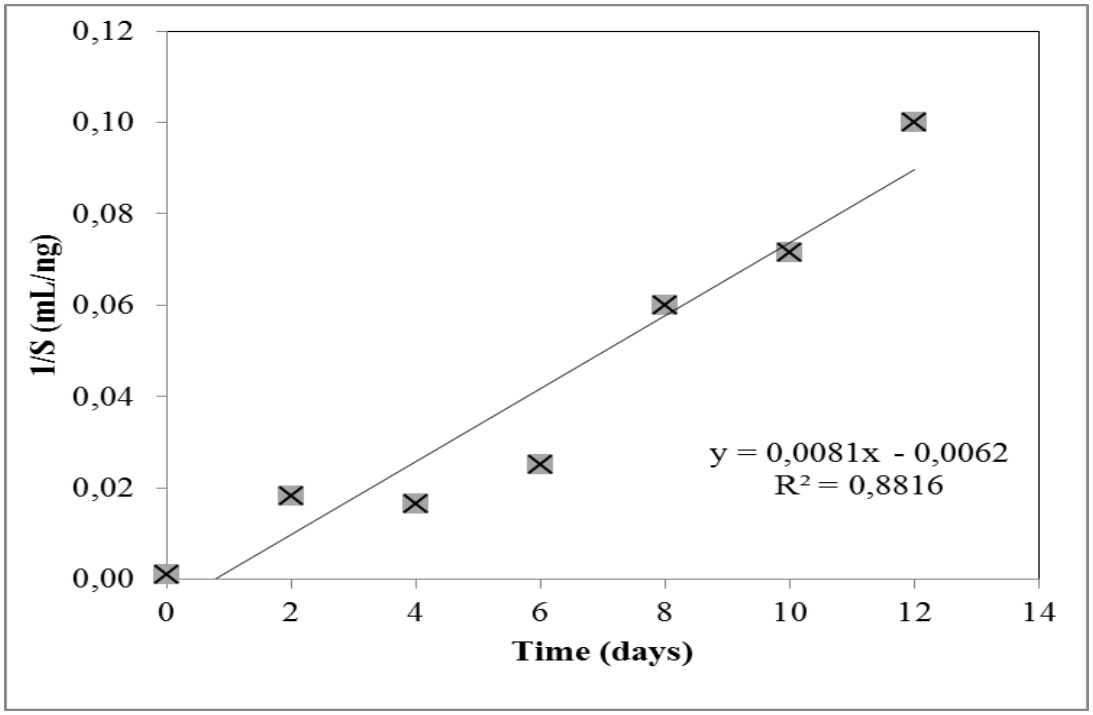

Figure 6.55 Determination of second order substrate removal for PAHs without RD at a SRT of 25 days

6.5.1.6 Evaluation of Monod, Zero, Half, First and Second Order Substrate Biodegradation Models for COD_{dis} and PAHs Removals at Increasing SRTs with 15 mg/L and without RD in the CSTR System

In this study, in order to determine the kinetic constants five different kinetic models (Monod, zero order, half order, first order and second order biodegradation kinetic model) were used for the aerobic CSTR system based on degradation of COD_{dis} and PAHs at five different SRTs through 50 days of operation with 15 mg/L RD and without RD under steady-state conditions. Comparison of the kinetic constants according to Monod models for COD_{dis} and PAHs with/without RD biosurfactant in the CSTR system are summarized in Tables 6.36 and 6.37.

The yield efficiencies (Y) values increased from 0.45 to 0.86 mg VSS/mgCOD and from 0.38 to 0.64 mgVSS/mg PAHs for COD_{dis} and PAHs, respectively, with addition of 15 mg/L RD in Monod kinetic model. The half saturation constant (K_s) values were obtained as 56.34 mg/L and 0.0013 mg/L for COD_{dis} and PAHs,

respectively, in Monod model with 15 mg/L RD. K_s values were obtained as 110.59 mg/L and 0.0042 mg/L for COD_{dis} and PAHs, respectively, in Monod model without RD in the CSTR system. The lower K_s value indicates the affinity of aerobic microorganisms to the substrate. Therefore, it can be said that no COD_{dis} and PAHs accumulation was observed in the CSTR. Therefore, COD_{dis} (E=96%) and PAHs (E=94%) removal efficiencies were high in the aerobic CSTR at the optimum SRT of 25 days.

Maximum specific growth rates (μ_{max}) were obtained as 0.17 1/day and 0.14 1/day for COD_{dis} and PAHs, respectively with 15 mg/L RD in the aerobic CSTR. The death rate coefficient (k_d) values were obtained as 0.015 and 0.003 1/day for COD_{dis} and PAHs, respectively, at 15 mg/L RD. In presence of 15 mg/L RD, μ_{max} values for COD_{dis} and PAHs decreased in Monod model. μ_{max} were found as 0.10 1/day and 0.02 1/day for COD_{dis} and PAHs without RD in the aerobic CSTR. The k_d values were obtained as 0.017 and 0.015 1/day for COD_{dis} and PAHs, respectively, without RD. Therefore, COD_{dis} (E=79%) and PAHs (E=69%) removal efficiencies were low without RD in the aerobic CSTR at a SRT of 25 days. The k values were higher in the presence 15 mg/L RD (k=0.21 and k=0.22 for COD_{dis} and PAHs respectively) in comparison to the RD free (k=0.19 and k=0.06 for COD_{dis} and PAHs, respectively) aerobic CSTR reactor for COD_{dis} and PAHs.

K_s and k_d values were lower both COD_{dis} and PAHs at 15 mg/L RD in Monod model. Y and μ_{max} kinetic constants were higher in comparison to without RD containing reactor both COD_{dis} and PAHs in Monod model.

Table 6.36 Comparison of the kinetic constants according to Monod models for COD_{dis} with/without RD biosurfactant in the CSTR system (SRT= 5, 10, 20, 25 and 40 days, HRT= 5 days, influent COD_{dis}= 1200 mg/L, influent PAHs= 65.32 ng/mL, with and without 15 mg/L RD)

Kinetic model	Kinetic constants	COD_{dis} removal (15 mg/L RD)		COD_{dis} removal (without RD)	
		Values	Regression coefficients (R^2)	Values	Regression coefficients (R^2)
Monod	Y (mg VSS/mg)	0.86	0.97	0.45	0.95
	k_d (1/day)	0.015	0.97	0.017	0.95
	μ max (1/day)	0.17	0.97	0.10	0.95
	k (1/day)	0.21	0.98	0.19	0.91
	K_s (mg/L)	56.34	0.98	110.59	0.91

Table 6.37 Comparison of the kinetic constants according to Monod models for PAHs with/without RD biosurfactant in the CSTR system (SRT= 5, 10, 20, 25 and 40 days, HRT= 5 days, influent COD_{dis}= 1200 mg/L, influent PAHs= 65.32 ng/mL, with and without 15 mg/L RD)

Kinetic model	Kinetic constants	PAHs removal (15 mg/L RD)		PAHs removal (without RD)	
		Values	Regression coefficients (R^2)	Values	Regression coefficients (R^2)
Monod	Y (mg VSS/mg)	0.64	0.99	0.38	0.99
	k_d (1/day)	0.003	0.99	0.015	0.99
	μ max (1/day)	0.14	0.99	0.02	0.99
	k (1/day)	0.22	0.99	0.06	0.82
	K_s (mg/L)	0.0013	0.99	0.0042	0.82

The comparison of regression coefficients (R^2) relevant to zero, half, first and second order rate constants k_0 (mg COD_{dis}/L.day; ngPAHs/L.day), $k_{1/2}$ (L/mgCOD_{dis}.day; mL/ng PAHs.day), k_1 (1/day) and k_2 (L/mgCOD.day; mL/ngPAHs.day) values were given in Tables 6.38- 6.41 with/without RD for COD_{dis} and PAHs at increasing SRTs.

The zero order regression coefficients of R^2 for COD_{dis} varied between 0.79 and 0.87 while the k_0 values varied between 96.786 and 107.48 mg COD_{dis}/L.day with 15 mg/L RD as the SRTs varied between 5 and 40 days. The half order regression coefficients of R^2 for COD_{dis} varied between 0.79 and 0.86 while the $k_{1/2}$ kinetic constant values varied between 0.0042 and 0.0091 L/mg COD_{dis} day with 15 mg/L RD as the SRTs varied between 5 and 40 days. The first order regression coefficients of R^2 for COD_{dis} varied between 0.88 and 0.92 while k_1 kinetic constants values varied between 0.1794 and 0.3103 1/day with 15 mg/L RD as the SRTs varied between 5 and 40 days. The second order regression coefficients of R^2 for COD_{dis} varied between 0.80 and 0.87 while the k_2 kinetic constants values varied between 0.0004 and 0.0014 L/mg COD_{dis} day with 15 mg/L RD as the SRTs varied between 5 and 40 days (Table 6.38).

Table 6.38 Zero, half, first and second order reaction kinetic constant in CSTR tests during COD_{dis} degradation for different SRTs with 15 mg/L RD (COD_{dis} = 1200 mg/L, PAHs=65.32 ng/mL, HRT=5 days, SRT=5-10-20-25-40 days)

		COD_{dis}				
Kinetics	Constant	SRT 5 days	SRT 10 days	SRT 20 days	SRT 25 days	SRT 40 days
Zero order	k_0 (mg /L.day)	100.71	102.14	105.00	107.48	96.786
	R^2	0.81	0.82	0.84	0.87	0.79
Half order	$k_{1/2}$ (L/ mg.day)	0.0045	0.0059	0.0052	0.0091	0.0042
	R^2	0.81	0.83	0.84	0.86	0.79
First order	k_1 (1/day)	0.1871	0.2204	0.1993	0.3103	0.1794
	R^2	0.90	0.91	0.91	0.92	0.88
Second order	k_2 (L/mg.day)	0.0005	0.0007	0.0006	0.0014	0.0004
	R^2	0.80	0.85	0.84	0.87	0.80

The zero order regression coefficients of R^2 for COD_{dis} varied between 0.67 and 0.74 while the k_0 values varied between 46.429 and 52.286 mg COD_{dis} /L.day without RD as the SRT varied between 5 and 40 days. The half order regression coefficients of R^2 for COD_{dis} varied between 0.66 and 0.82 while the $k_{1/2}$ values varied between 0.001 and 0.0014 L/mg COD_{dis} day without RD as the SRTs varied between 5 and 40 days. The first order regression coefficients of R^2 for COD_{dis} varied between 0.66 and 0.81 while the k_1 values varied between 0.0389 and 0.0731 1/day without RD as the SRTs varied between 5 and 40 days. The second order regression coefficients of R^2 for COD_{dis} varied between 0.65 and 0.79 while the k_2 values varied between 0.00007 and 0.0001 L/mg COD_{dis} without RD as the SRT varied between 5 and 40 days (Table 6.39).

In presence of 15 mg/L RD, zero, half, first and second order reaction kinetic constants (k_0, $k_{1/2}$, k_1, k_2) and regression coefficients (R^2) were higher than RD no-added in CSTR during COD_{dis} degradation as the SRTs varied between 5 and 40 days. In other words the kinetic constants and R^2 increased with added 15 mg/L RD.

The zero order regression coefficients of R^2 for PAHs varied between 0.80 and 0.87 while the k_0 values varied between 4.9634 and 5.9634 ng PAHs /mL.day with 15 mg/L RD as the SRT varied between 5 and 40 days. The half order regression coefficients of R^2 for PAHs varied between 0.77 and 0.88 while the $k_{1/2}$ values varied between 0.0096 and 0.0183 mL/ngPAHs.day with 15 mg/L RD as the SRTs varied between 5 and 40 days. The first order regression coefficients of R^2 for PAHs varied between 0.82 and 0.92 while the k_1 values varied between 0.1465 and 0.1762 1/day with 15 mg/L RD as the SRTs varied between 5 and 40 days. The second order regression coefficients of R^2 for PAHs varied between 0.80 and 0.88 while the k_2 values varied between 0.0051 and 0.0083 mL/ngPAHs.day with 15 mg/L RD as the SRT varied between 5 and 40 days (Table 6.40).

Table 6.39 Zero, half, first and second order reaction kinetic constant in CSTR tests during COD_{dis} degradation for different SRTs without RD (COD_{dis} = 1200 mg/L, PAHs=65.32 ng/mL, HRT=5 days, SRT=5-10-20-25-40 days)

		COD_{dis}				
Kinetics	Constant	SRT 5 days	SRT 10 days	SRT 20 days	SRT 25 days	SRT 40 days
Zero order	k_0 (mg /L.day)	46.429	51.25	51.875	52.286	51.536
	R^2	0.72	0.73	0.74	0.74	0.67
Half order	$k_{1/2}$ (L/ mg.day)	0.001	0.0012	0.0013	0.0014	0.001
	R^2	0.80	0.73	0.79	0.82	0.66
First order	k_1 (1/day)	0.0564	0.0672	0.0708	0.0731	0.0389
	R^2	0.71	0.73	0.79	0.81	0.66
Second order	k_2 (L/mg.day)	0.00007	0.00009	0.0001	0.0001	0.00007
	R^2	0.73	0.74	0.77	0.79	0.65

Table 6.40 Zero, half, first and second order reaction kinetic constant in CSTR tests during PAHs degradation for different SRTs with 15 mg/L RD (COD_{dis} = 1200 mg/L, PAHs=65.32 ng/mL, HRT=5 days, SRT=5-10-20-25-40 days)

		PAHs				
Kinetics	Constant	SRT 5 days	SRT 10 days	SRT 20 days	SRT 25 days	SRT 40 days
Zero order	k_0 (ng /mL.day)	5.7827	5.1114	5.5907	5.9634	5.0634
	R^2	0.80	0.82	0.86	0.87	0.80
Half order	$k_{1/2}$ (mL/ng.day)	0.0096	0.0141	0.0191	0.0183	0.0134
	R^2	0.77	0.88	0.88	0.88	0.82
First order	k_1 (1/day)	0.1125	0.1527	0.1687	0.1762	0.1465
	R^2	0.87	0.91	0.91	0.92	0.82
Second order	k_2 (mL/ng.day)	0.0051	0.0055	0.0081	0.0081	0.0051
	R^2	0.82	0.85	0.86	0.88	0.80

The zero order regression coefficients of R^2 for PAHs varied between 0.74 and 0.79 while the k_0 values varied between 3.7354 and 5.1264 ng PAHs /mL.day without RD as the SRTs varied between 5 and 40 days. The half order regression coefficients of R^2 for PAHs varied between 0.60 and 0.74 while the $k_{1/2}$ values varied between 0.0074 and 0.0156 mL/ngPAHs.day without RD as the SRTs varied between 5 and 40 days. The first order regression coefficients of R^2 for PAHs varied between 0.62 and 0.78 while the k_1 values varied between 0.0773 and 0.1615 1/day without RD as the SRTs varied between 5 and 40 days. The second order regression coefficients of R^2 for PAHs varied between 0.64 and 0.75 while the k_2 values varied between 0.0025 and 0.0071 mL/ngPAHs.day without RD as the SRTs varied between 5 and 40 days (Table 6.41).

The administration of 15 mg/L RD increased the zero, half, first and second order reaction kinetic constants (k_0, $k_{1/2}$, k_1 and k_2) and regression coefficients (R^2) during PAHs degradation as the SRTs varied between 5 and 40 days. In other words the kinetic constants and R^2 increased with added 15 mg/L RD.

As the SRT varied between 5 and 40 days, the k_0, $k_{1/2}$, k_1 and k_2 kinetic constants values and R^2 increased throughout COD_{dis} and PAHs biodegradations with 15 mg/L RD.In this study, the zero order kinetic constants (k_0) were extremely high and the coefficient regressions (R^2) of zero values were low for COD_{dis} and PAHs at all SRTs. Furthermore half ($k_{1/2}$) and second (k_2) order kinetic constants and the coefficient regressions (R^2) of zero and second values were low for COD_{dis} and PAHs at all SRTs with and without RD in the aerobic CSTR system. The first order kinetic constants (k_1) and the coefficient regressions (R^2) of first order were lower than Monod model for COD_{dis} and PAHs. The results showed that zero, half, first and second kinetic models were not appropriate for COD_{dis} and PAHs removals in the aerobic CSTR reactor with and without RD in the aerobic CSTR system. It was found that Monod kinetic model was more suitable than the zero, half, first and second order, having R^2 values of 0.98 and 0.99 for COD_{dis} and PAHs respectively, at a SRT of 25 days including 15 mg/L RD in the CSTR system.

Table 6.41 Zero, half, first and second order reaction kinetic constant in CSTR tests during PAHs degradation for different SRTs without RD (COD_{dis} = 1200 mg/L, PAHs=65.32 ng/mL, HRT=5 days, SRT=5-10-20-25-40 days)

		PAHs				
Kinetics	Constant	SRT 5 days	SRT 10 days	SRT 20 days	SRT 25 days	SRT 40 days
Zero order	k_0 (ng /mL.day)	3.7354	4.8436	5.2871	5.1264	4.0905
	R^2	0.75	0.76	0.77	0.79	0.74
Half order	$k_{1/2}$ (mL/ ng.day)	0.0083	0.0131	0.0148	0.0156	0.0077
	R^2	0.61	0.71	0.72	0.74	0.60
First order	k_1 (1/day)	0.097	0.1421	0.1507	0.1615	0.0773
	R^2	0.69	0.70	0.74	0.78	0.62
Second order	k_2 (mL/ng.day)	0.0051	0.0054	0.0064	0.0071	0.0025
	R^2	0.68	0.70	0.73	0.75	0.64

In this study for Monod model, the K_s (0.00013 mgPAHs/L) values obtained in this study was lower than then reported by Desai et al. (2008) (K_s=0.02 mg/L for FLE, and 0.08 mg/L for NAP) an aerobic reactor treating FLN and NAP PAHs. Fountoulakis et al. (2009) reported that k value found as 1.10 (1/day) for first order kinetic model, respectively in aerobic reactor treating PAHs. This result is lower than the k value obtained from the present study for first order kinetic model. k value obtained from the study performed by Lin at al. (2010) was 0.043 1/day throughout biodegradation of NAP PAH. In this study the K_s values are lower than those from another study treating aromatic hydrocarbon mixtures (20 mg/L) by pure and mixed bacterial cultures under aerobic conditions (Reardon et al., 2002). The k_1 value obtained from the kinetic study performed by Chen et al. (2008) was 0.1185 1/h in an up-flow aerobic batch reactor treating PHE at a HRT of 6 days. This result is significantly lower than the k value obtained from the present study.

Aerobic degradation of naphthalene was found to be best fitted with Monod kinetics model (MacRae and Hall 1998**;** Jajuee et al. 2007). The estimated kinetic parameter, maximum specific substrate utilization rate (μ_{max}) and half-saturation constant (K_s), varied greatly from 0.005 to 0.627 1/h and from 0.08 to 2.21 mg/L, respectively (Ahn et al. 1998). For other PAHs, a number of studies have shown that biodegradation kinetics fitted well with the first order kinetics. For instance, the biodegradation rate constant (k_1) of PHE, another low-molecular weight PAHs, were between 0.0088 and 1.13 1/day (Yuan et al. 2001; Maletic et al. 2009).

6.5.2 Biodegradation Kinetic Models in the Anaerobic ITBR System

6.5.2.1 Modified Stover Kincannon Kinetic Model for COD_{dis} and PAH Removals in the Anaerobic ITBR with 75 mg/L RD

Modified Stover Kincannon model suggests that the substrate removal rates are affected by the OLR entering to the reactor as described in Eq. (5.44) (See chapter 5.14.3.1).

6.5.2.1.1 Modified Stover Kincannon Kinetic Model for COD_{dis} Removal. Figure 6.56 shows the graph plotted between reciprocal of total removed organic loading rates (OLRs), "$[V/(Q.(S_i-S_e)]$", against to the reciprocal of OLRs, "$V/(Q.S_i)$" using Eq. (5.44) (See chapter 5.13.3.1). Since the plot of "$[V/(Q.(S_i-S_e)]$" versus "$V/(Q.S_i)$" was found to be linear, linear regressions were used to determine the intercept "$(1/R_{max})$" and the slope "(K_B/R_{max})". Q and V are the inflow rate (L/day) and the volume of the anaerobic reactor (L), respectively.

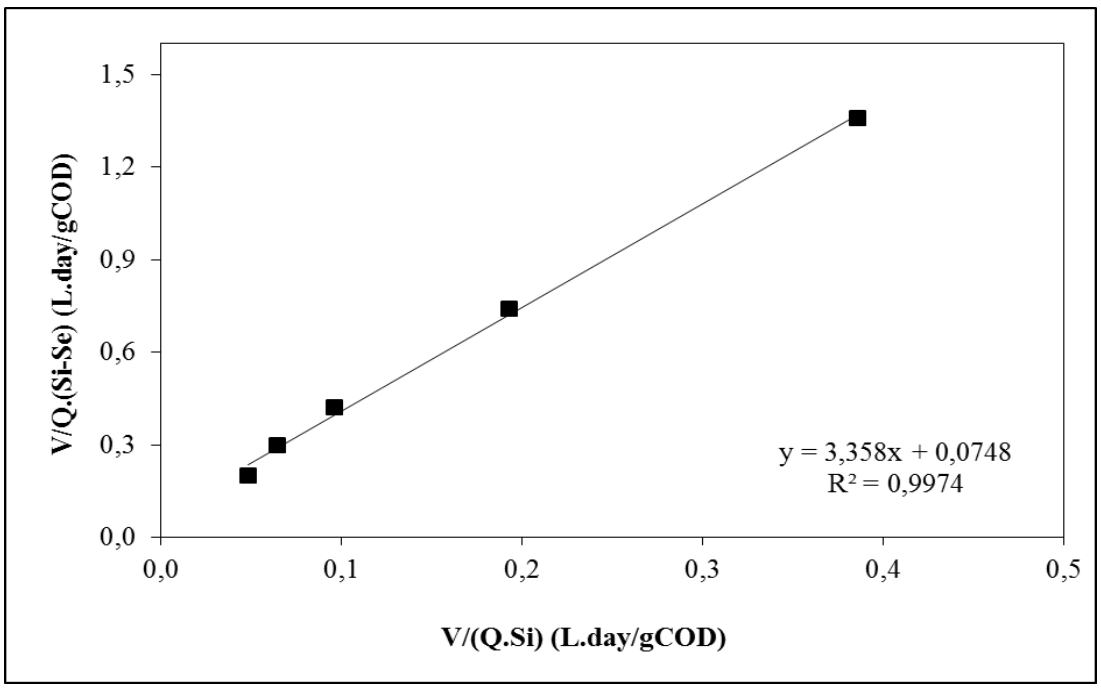

Figure 6.56 Determination of kinetic constants (R_{max} and K_B) in Modified Stover-Kincannon model for COD_{dis}

S_i and S_e are influent and effluent COD_{dis} ($gCOD_{dis}/L$) concentrations, respectively. Saturation value constant (K_B) and maximum utilization rate (R_{max}) for COD_{dis} were calculated from the line plotted on graph given in Figure 6.56. K_B and R_{max} were found as 44.91 $mgCOD_{dis}/L.day$ and 13.37 $mgCOD_{dis}/L.day$, respectively with high regression coefficient (R^2=0.99; y= 3.358x+0.0748) for COD_{dis}.

6.5.2.1.2 Modified Stover Kincannon Kinetic Model for PAHs Removal. Figure 6.57 shows the graph plotted between reciprocal of total removed organic loading rates (OLRs), "[V/(Q.(S_i-S_e)]", against to the reciprocal of OLRs, "V/(Q.S_i)" using Eq. (5.44) (See chapter 5.13.3.1). Since the plot of "[V/(Q.(S_i-S_e)]" versus "V/(Q.S_i)" was found to be linear, linear regressions were used to determine the intercept "(1/R_{max})" and the slope "(K_B/R_{max})". Q and V are the inflow rate (L/day) and the volume of the anaerobic reactor (L), respectively. S_i and S_e are influent and effluent PAH (ngPAHs/mL) concentrations, respectively. Saturation value constant (K_B) and maximum utilization rate (R_{max}) for PAHs were calculated from the line plotted on graph given in Figure 6.57. K_B and R_{max} values for PAHs were obtained as 20.12 ngPAH/mL.day and 12.28 ngPAH/mL.day, respectively, with high regression coefficient (R^2=0.99 y= 1.6584x+0.0834).

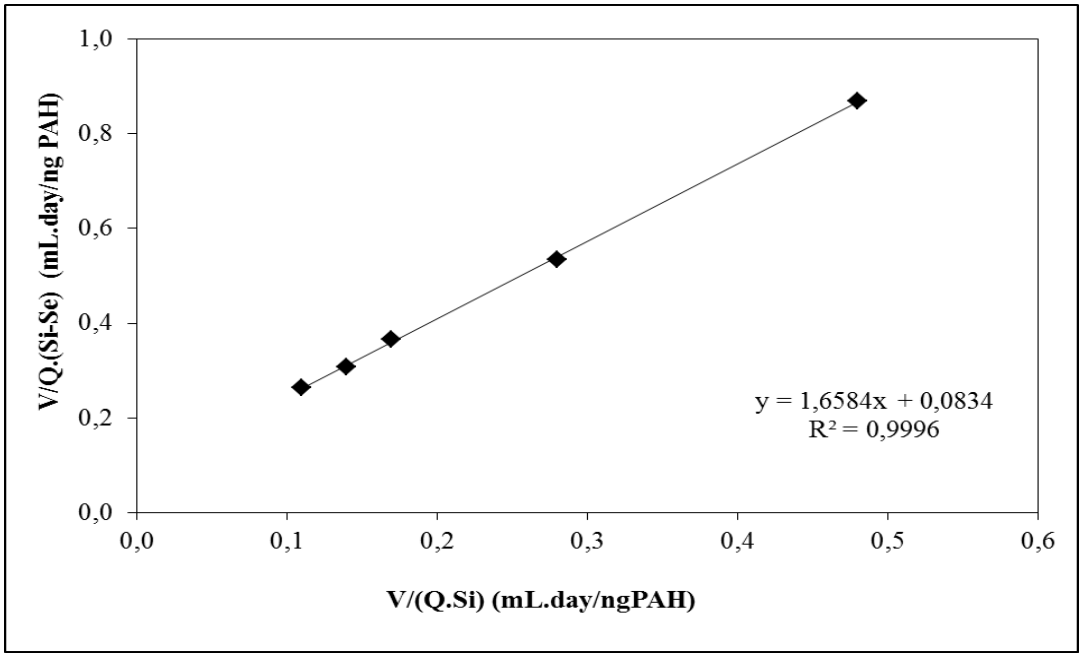

Figure 6.57 Determination of kinetic constants (R_{max} and K_B) in Modified Stover-Kincannon model for and PAHs

6.5.2.2 Contois Kinetic Model for COD_{dis} and PAHs Removal in the Anaerobic ITBR with 75 mg/L RD

6.5.2.2.1 Contois Kinetic Model for COD_{dis} Removal. Figure 6.58 was plotted using the Eq. (5.47) (See chapter 5.13.3.2) for determining the values of maximum specific growth rate (μ_{max}) (1/day) and Contois kinetic constant (β) ($gCOD_{dis}$/gVSS) in Contois kinetic model. μ_{max} and β values were calculated from the intercept and the slope of the straight line as illustrated in Figure 6.58 with regression coefficient of R^2=0.78, (y= 0.0652x+48.371) for COD_{dis}. μ_{max} and β values was calculated as 0.021 1/day and 0.001 $gCOD_{dis}$/gVSS, respectively.

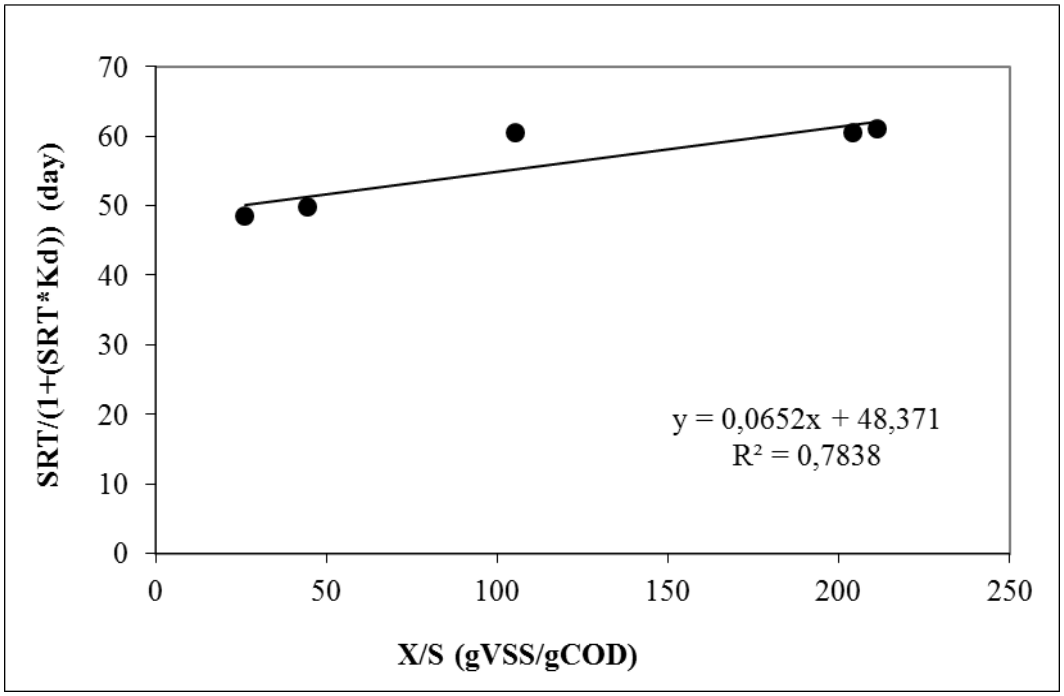

Figure 6.58 Determination of kinetic constants (μ_{max} and β) in Contois kinetic model for COD_{dis}

6.5.2.2.2 Contois Kinetic Model for PAHs Removal. Figure 6.59 was plotted using the Eq. (5.47) (See chapter 5.13.3.2) for determining the values of maximum specific grow rate (μ_{max}) (1/day) and Contois kinetic constant (β) (gPAHs/gVSS) in Contois kinetic model. μ_{max} and β values were calculated from the intercept and the slope of the straight line. The values of μ_{max} and β were found as 0.022 1/day and $3.73.10^{-7}$ ngPAH/ngVSS with regression coefficient R^2= 0.75, (y=0.00002x+46.084) for PAHs (Figure 6.59).

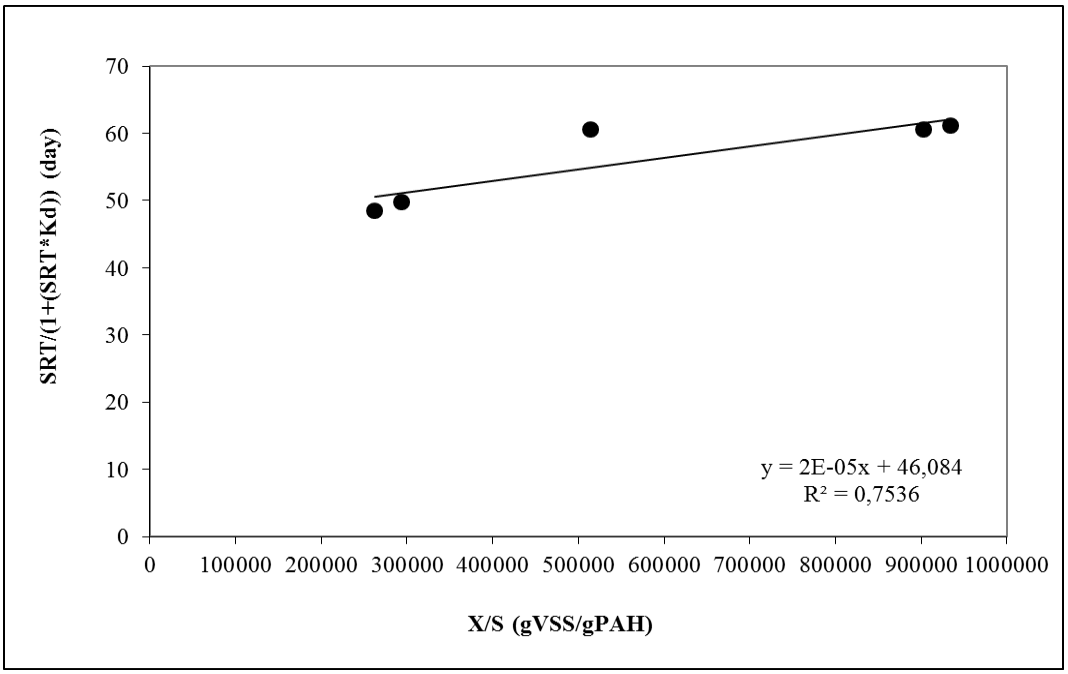

Figure 6.59 Determination of kinetic constants (μ_{max} and β) in Contois Kinetic model for PAHs

6.5.2.3 Monod Kinetic Model for COD_{dis} and PAHs Removal in the Anaerobic ITBR with 75 mg/L RD

6.5.2.3.1 Monod Kinetic Model for COD_{dis} Removal. Figure 6.60 was plotted using the Eq 5.61 (See chapter 5.13.3.3) for determining the values of Y and k_d in this model. Growth yield coefficient (Y) (mgVSS/mgCOD_{dis}) and endogenous decay coefficient (k_d) (1/day) values calculated from the intercept and the slope of the straight line as illustrated in Figure 6.60 as 0.50 mgVSS/mgCOD_{dis} and 0.006 1/day, respectively, with regression coefficient of R^2= 0.77, (y=1.9979x- 0.0136) for COD_{dis} at 75 mg/L RD in the ITBR system.

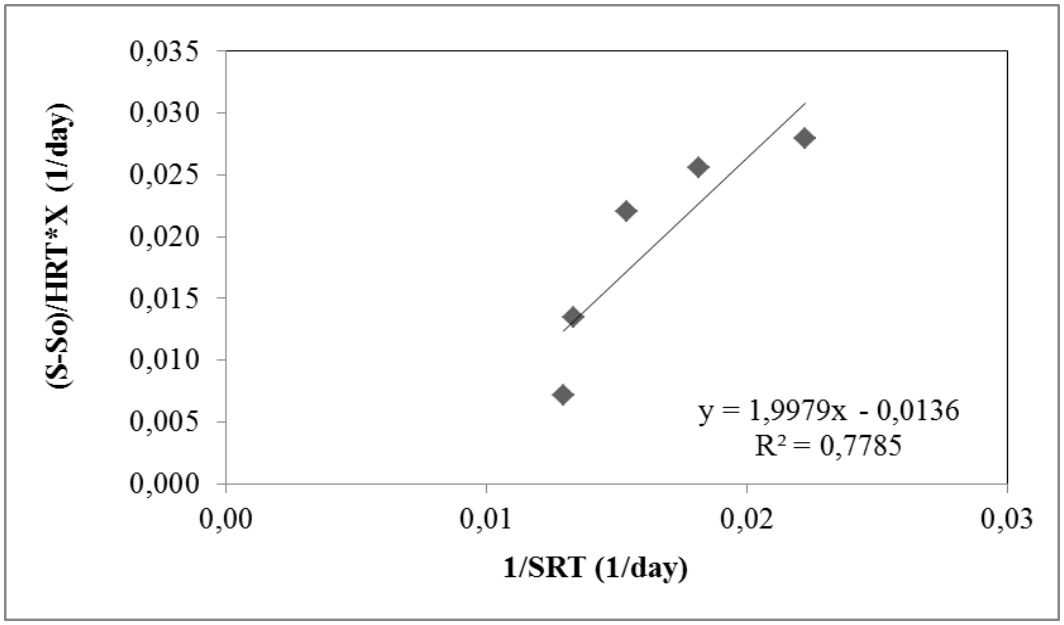

Figure 6.60 Determination of yield coefficient (Y) and death rate constant (k_d) values for COD_{dis}

The values of maximum specific substrate utilization rate **(**μ_{max}) (mgCOD/mgVSS.day) and half saturation concentration (K_s) (mg/L) for COD_{dis} were determined from Figure 6.61 using Eq (5.55) (see chapter 5.14.3.3). μ_{max} and K_s for COD_{dis} were calculated as 0.027 1/day and 0.13 mg/L, respectively with regression coefficient of R^2= 0.80, (y= 1626.1x+37.055),

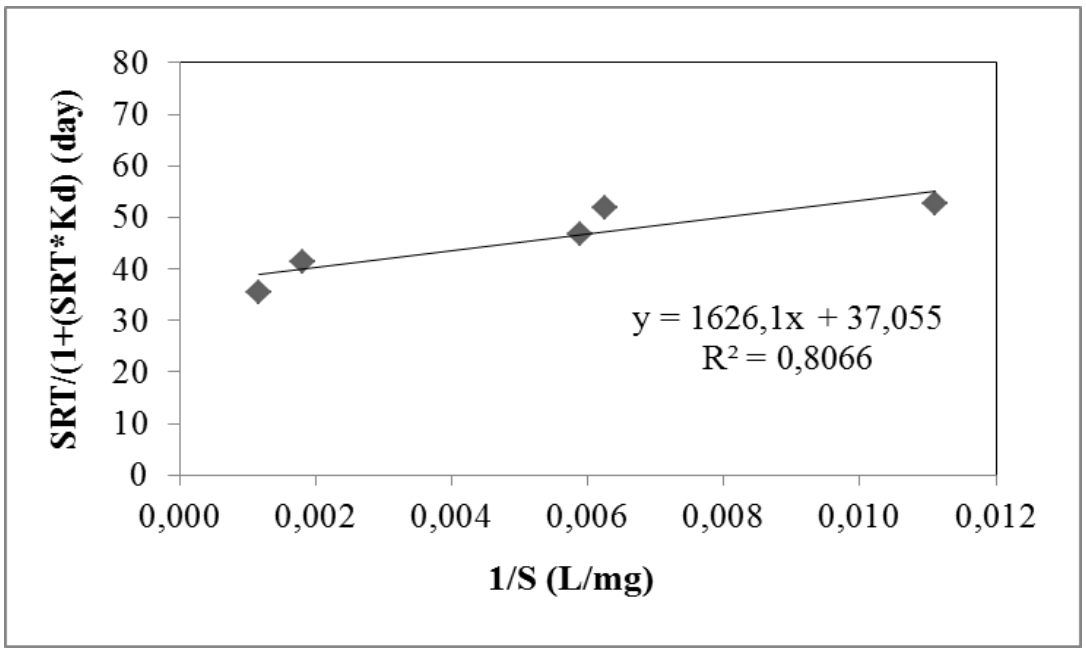

Figure 6.61 Determination of maximum specific substrate utilization rate (μ_{max}) and half saturation concentration (K_s) values for COD_{dis}

6.5.2.3.2 Monod Kinetic Model for PAHs Removal. Figure 6.62 was plotted using the Eq. 5.61 (See chapter 5.13.3.3) for determining the values of Y and k_d in this model. Growth yield coefficient (Y) (ngVSS/ngPAHs) and endogenous decay coefficient (k_d) (1/day) values calculated from the intercept and the slope of the straight line as illustrated in Figure 6.62 as 0.55 ngVSS/ngPAHs and 0.006 1/day, respectively, with regression coefficient of R^2= 0.86, (y=1.8155x- 0.0112) for PAHs at 75 mg/L RD in the ITBR system.

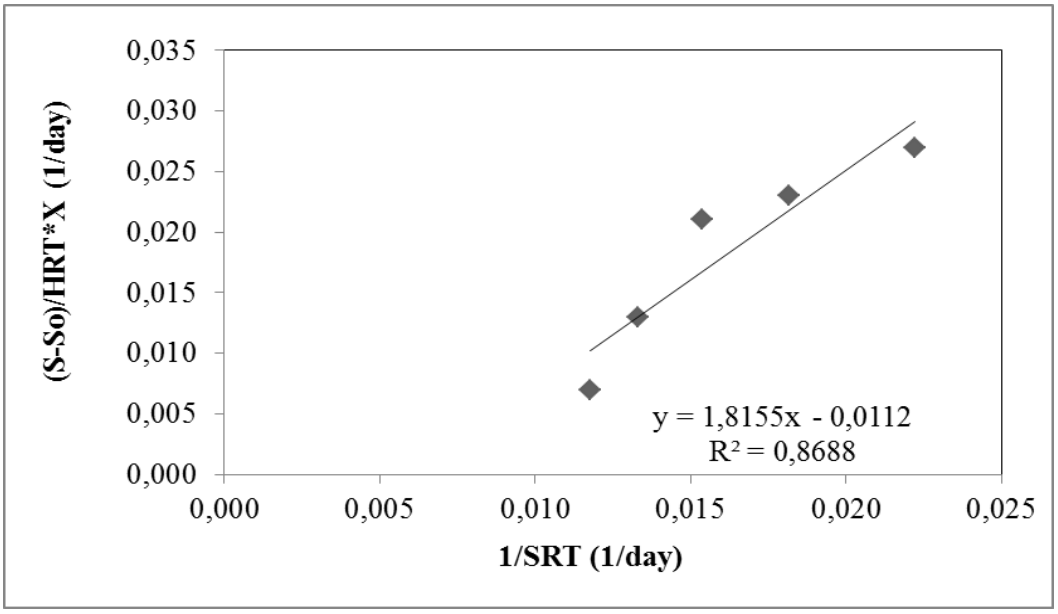

Figure 6.62 Determination of yield coefficient (Y) and death rate constant (k_d) values for PAHs

The values of maximum specific substrate utilization rate **(**μ_{max}) (mgPAH/mgVSS.day) and half saturation concentration (K_s) (ng/mL) for PAHs were determined from Figure 6.63 using Eq. (5.55) (See chapter 5.13.3.3). μ_{max} and K_s for PAHs were calculated as 0.03 1/day and 4 ng/mL, respectively with regression coefficient of R^2= 0.88, (y= 0.4899x+34.296),

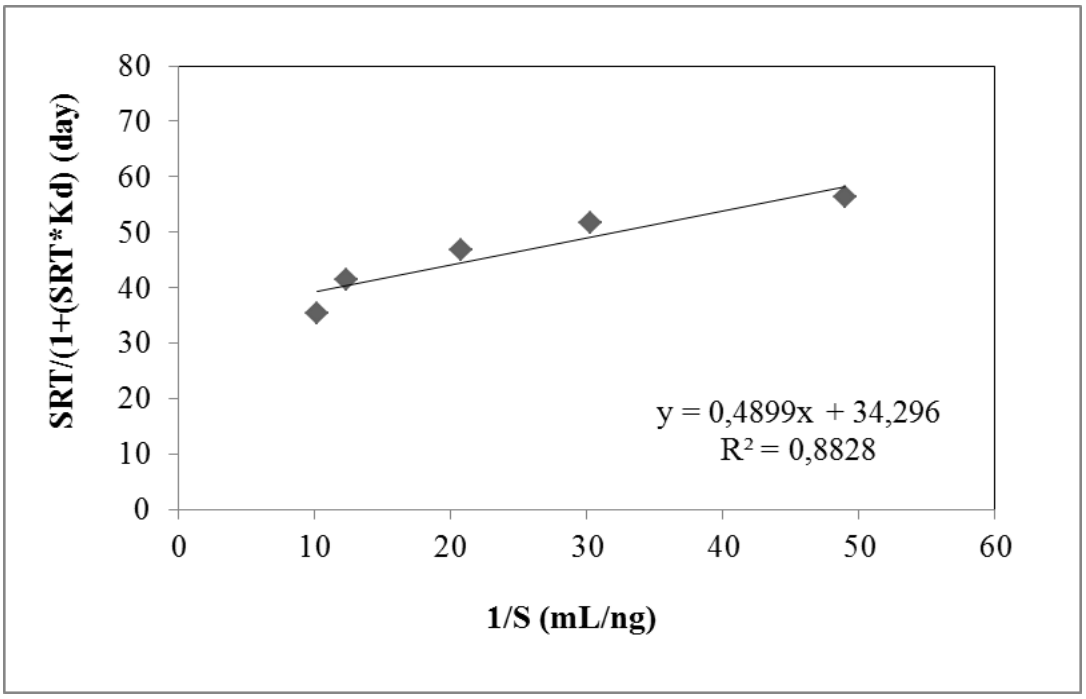

Figure 6.63 Determination of maximum specific substrate utilization rate (μ_{max}) and half saturation concentration (K_s) values for PAHs

6.5.2.4 Evaluation of the Kinetic Models in the Anaerobic ITBR System

The kinetic data showed that Modified Stover-Kincannon was more suitable kinetic model than the Contois and Monod kinetic models for the performance of the anaerobic ITBR system when the regression coefficients and the kinetic coefficients were compared with each other. The yield efficiency (Y) was extremely higher compared to the death rate coefficient (k_d) in Monod kinetic model. Half saturation constant (K_s) was lower compared to initial COD_{dis} and PAHs concentrations of 2850 mg/L and 292.00 ng/mL, respectively. Maximum specific growth rate (μ_{max}) (μ_{max}=0.03 1/day for COD_{dis}; μ_{max}=0.003 1/day for PAHs) was higher compared to the death rate constants (k_d) (k_d= 0.006 for COD_{dis} and PAHs) (See Table 6.42). High K_s value indicates a higher affinity of methanogens to COD_{dis} in anaerobic ITBR. K_s values estimated from the Monod model are very low (K_s= 0.13 mg/L for COD_{dis} (2850 mg/L±42) and K_s= 4 ng/mL for PAHs (292±1.85 ng/mL) than acceptable values for anaerobic reactors) treating toxic wastewaters (15-70 mg/L) (Metcalf and Eddy, 2005; Guha et al., 1999; Speece, 1996; Lee et al. 2003).

The maximum substrate utilization rate (R_{max}) (R_{max}=13.37mgCOD_{dis}/L.day; R_{max}=12.28 ngPAHs/mL.day) is lower and the saturation value constant (K_B) (K_B=44.91 gCOD_{dis}/L.day; K_B=20.12 ngPAHs/mL.day) is higher during COD_{dis} and PAHs degradation in Modified Stover Kincannon kinetic model (See Table 6.42). High COD_{dis} utilization rate (R_{max}) increase the reactor efficiency while high substrate saturation constant (K_B) indicates the utilization of COD_{dis} and PAHs by the methanogens in the ITBR. High saturation values (K_B) showed that there is no any accumulation of COD_{dis} and PAHs in the anaerobic reactor resulting in high affinity of substrate to the anaerobic methanogenic bacteria.

Table 6.42 Summary of kinetic constants of ITBR system treating PAHs.

	COD_{dis} removal			PAH removal		
Kinetic models	Kinetic parameters	Values	Regression coefficients (R^2)	Kinetic parameters	Values	Regression coefficients (R^2)
Modified Stover-Kincannon	K_B (mg COD_{dis}/L day)	44.91	0.99	K_B (ng PAH/mL day)	20.12	0.99
	R_{max} (mgCOD_{dis}/L day)	13.37		R_{max} (ngPAH/mL day)	12.28	
Contois	μ_{max} (1/day)	0,02	0.78	μ_{max} (1/day)	0,02	0.75
	β (g COD_{dis}/g VSS)	0,0014		β (ng PAH/ng VSS)	$3.73.10^{-7}$	
Monod	Y (mgVSS/mgCOD_{dis})	0.50	0.77	Y (ngVSS/ngPAH)	0.55	0.86
	k_d (1/day)	0.006		k_d (1/day)	0.006	
	μ_{max} (1/day)	0.03	0.80	μ_{max} (1/day)	0.003	0.88
	K_s (mg/L)	0.13		K_s (ng/mL)	4	

In Contois kinetic model the maximum specific growth rates (M_{max}) and the Contois kinetic constant (β) kinetic constants are very low with low regression coefficients (R^2=0.78 for COD_{dis} and R^2=0.75for PAHs). Therefore these kinetic constants cannot define the biodegradations of PAH and COD_{dis} in the anaerobic ITBR system. The regression coefficients for COD_{dis} and PAHs obtained at five

different HRTs were higher in Modified Stover Kincannon kinetic model (R^2=0.99) compared to Monod (R^2=0.88) and to Contois model (R^2=0.78). Furthermore the kinetic constants determined in Modified Stover Kincannon model (High R_{max} and K_B values) are more meaningful than that observed in Monod and Contois kinetic models.

In this study, the saturation constant (K_B) (44.91 mgCOD_{dis}/L.day) and maximum utilization rate (R_{max}) (13.37 mgCOD_{dis}/L day) values obtained from the Modified Stover-Kincannon model are lower than those obtained by Sahariah and Chakraborty (2011) (27.23 g/L.day and 15.08 g/L.day, respectively) in an moving bed biofilm reactor treating simulated petrochemical wastewater and lower than those obtained by Priya et al. (2009), (4.6 g/L.day and 3.4 g/L.day, respectively) in upflow anaerobic fixed film (UAFB) (HRT=6-24 hours) reactor treating petrochemical industry wastewater containing formaldehyde in Modified Stover-Kincannon model. The possible reasons for the differences may be variation in reactor configuration, wastewater characteristics and microorganisms used in the study. It is important to note that there is not much published information about the kinetics of anaerobic ITBR system treating PAHs in petrochemical industry wastewater using Monod and Contois kinetic models.

6.5.3 Biogas Production Kinetics in the Anaerobic ITBR System

Biogas production kinetic models namely, Modified Stover Kincannon and Van der Meer and Heertjes model were evaluated for biogas production in the anaerobic ITBR. The kinetic constants and regression coefficients of the Modified Stover Kincannon and Van der Meer and Heertjes biogas productions models were compared in anaerobic ITBR system.

6.5.3.1 Modified Stover-Kincannon Kinetic Model for Total Gas and Methane Gas Productions

6.5.3.1.1 Modified Stover-Kincannon Kinetic Model for Total Gas Productions. In order to determine the maximum specific gas production coefficient (G_{max})

(mL/L.day) and the gas kinetic constant (G_B) (mg/L.day), Eq. (5.66) (See Chapter 5.13.4.1) was used. The reciprocal of specific gas production (1/G) (L.day/mL) versus reciprocal of applied organic loading rates (1/OLR) (L.day/g) was plotted in order to determine the kinetic constants relevant to total gas productions (Figure 6.64).

G is the specific gas production rate (mL/L.day) and G_{max} is defined as the maximum specific gas production rate (mL/L.day). G_B is the gas kinetic constant (mg/L.day) and OLR is the organic loading rate (g/L.day). The intercept and slope of the line result in "$1/G_{max}$" and "G_B/G_{max}", respectively. "G_{max}", and "G_B" were found as 844 mL/L.day and 0.65 mg/L.day, respectively (R^2 = 0.99; y= 0.0008+0.0012).

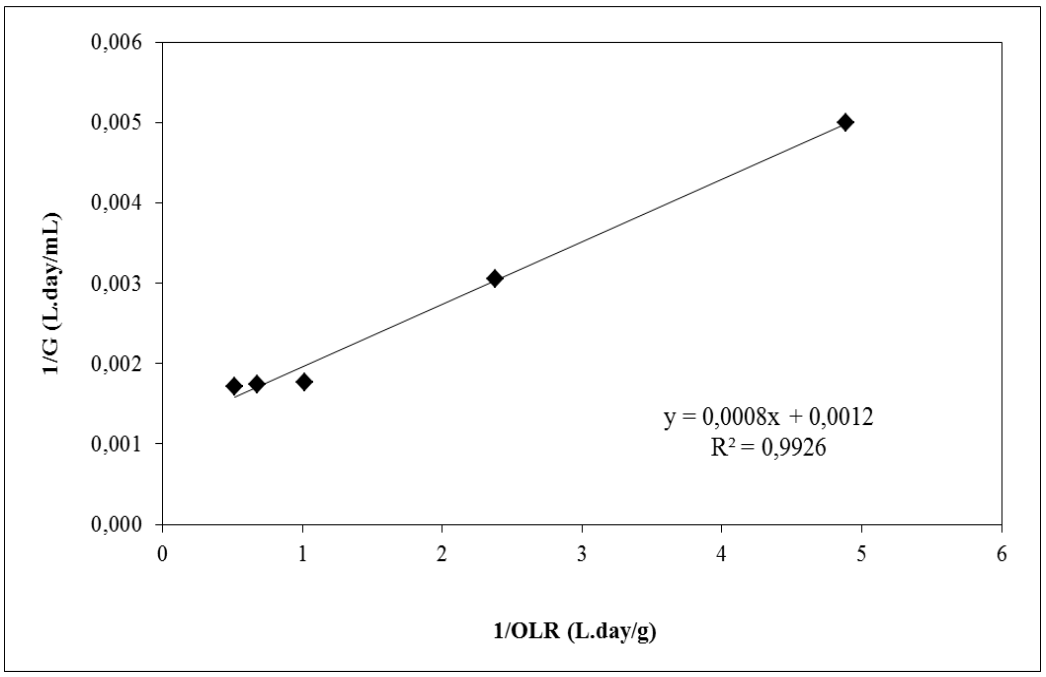

Figure 6.64 Determination of "G_{max}" and "G_B" in Modified Stover Kincannon model

In the Modified Stover Kincannon, G_{max} is related with the OLRs applied to the ITBR reactor and with proportionality constant as given in Eq. (5.66) (See chapter 5.13.4.1 in Materials and Methods). The total gas production can be calculated using the Eq. 5.66 (See chapter 5.13.4.1 in Material Methods).

$$\frac{1}{G} = \frac{G_B}{G_{max}} \frac{1}{OLR} + \frac{1}{G_{max}} \quad (5.66)$$

The total gas production can be estimated using the Eq. (6.1) by taken into consideration the kinetic constants determined previously (G_{max} and G_B)

$$\frac{1}{G} = \frac{0.65}{844} \frac{1}{OLR} + \frac{1}{844} \quad (6.1)$$

6.5.3.1.2 Modified Stover-Kincannon Model for Methane Gas. M, M_{max} and M_B can be explained as the specific methane gas production rate (mL/L.day), the maximum specific methane gas production rate (mL/L.day) and the methane gas kinetic constant (mg/L.day) for methane production, respectively. In order to determine the M_{max} Eq. (5.67) (See chapter 5.13.4.1) was used to calculate the methane gas productions in the ITBR. The reciprocal of specific gas production (1/M) (L.day/mL) versus reciprocal of applied organic loading rates (1/OLR) (L.day/g) were plotted in order to determine the kinetic constants relevant to methane gas productions (Figure 6.65).

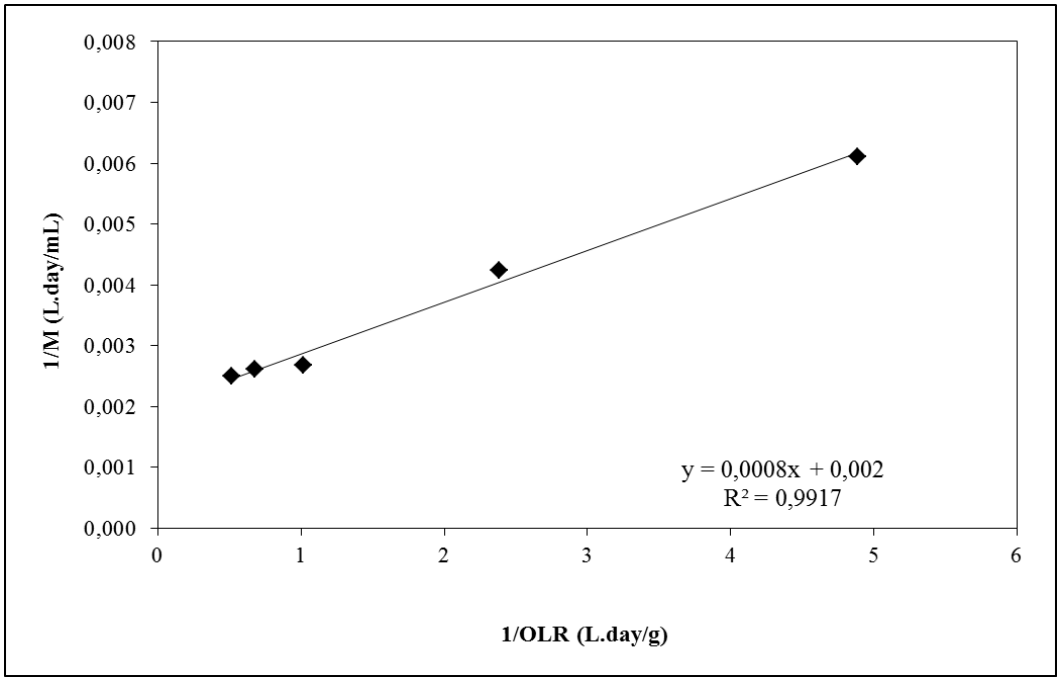

Figure 6.65 Determination of "M_{max}" and "M_B" in Modified Stover Kincannon model

The intercept and slope of the line result in "$1/M_{max}$" and "M_B/M_{max}", respectively. "M_{max}", and "M_B" were found as 495 mL/L.day and 0.42 mg/L.day, respectively (R^2 = 0.99; y= 0.0008+0.002) (See Figure6.65). The methane gas production can be calculated using the Eq. 5.67 (See chapter 5.13.4.1 in Material and Methods).

$$\frac{1}{M} = \frac{M_B}{M_{max}}\frac{1}{OLR} + \frac{1}{M_{max}} \quad (5.67)$$

The methane gas production can be estimated using the Eq. (6.2) by taken into consideration the kinetic constants determined previously (M_{max} and M_B)

$$\frac{1}{M} = \frac{0.42}{495}\frac{1}{OLR} + \frac{1}{495} \quad (6.2)$$

6.5.3.2 Van der Meer & Heertjes Kinetic Model

Van der Meer & Heertjes kinetic constant (k_{sg}) was determined empirically from the slope of the line plotted between "Q.(Si-Se)" and "G_{CH4}" using Eq. (5.68) (See chapter 5.13.4.2 in Material and Methods) (R^2=0.98,y= 0.0845x+839.88) (See Figure 6.66). G_{CH4} is the methane gas production (L/day), S_i is the influent COD_{dis} concentration (mg/L), S_e is the effluent COD_{dis} concentration (mg/L) and Q is the flow rate (L/day).

The k_{sg} was found as 0.0845 mL CH_4/mg$COD_{dis.rem}$ removal with high R^2 (0.98) value. In this model the methane gas production is related with gas kinetic constant applied to the anaerobic ITBR system (Wang et al., 2009). The methane gas production can be calculated by taken into consideration the Van der Meer & Heertjes kinetic constant, the flow rate and the influent and effluent substrate concentrations using Eq. (5.68) (chapter 5.13.4.2 in Material and Methods).

$$G_{CH4} = k_{sg} Q\ (S_i - S_e) \quad (5.68)$$

The methane gas production can be estimated using the Eq.(5.3) by taken into consideration the Van der Meer & Heertjes kinetic constant determined previously (k_{sg}) in the anaerobic ITBR reactor treating the PAHs.

$$G_{CH4} = 0.0845\ Q\ (S_i - S_e) \qquad (6.3)$$

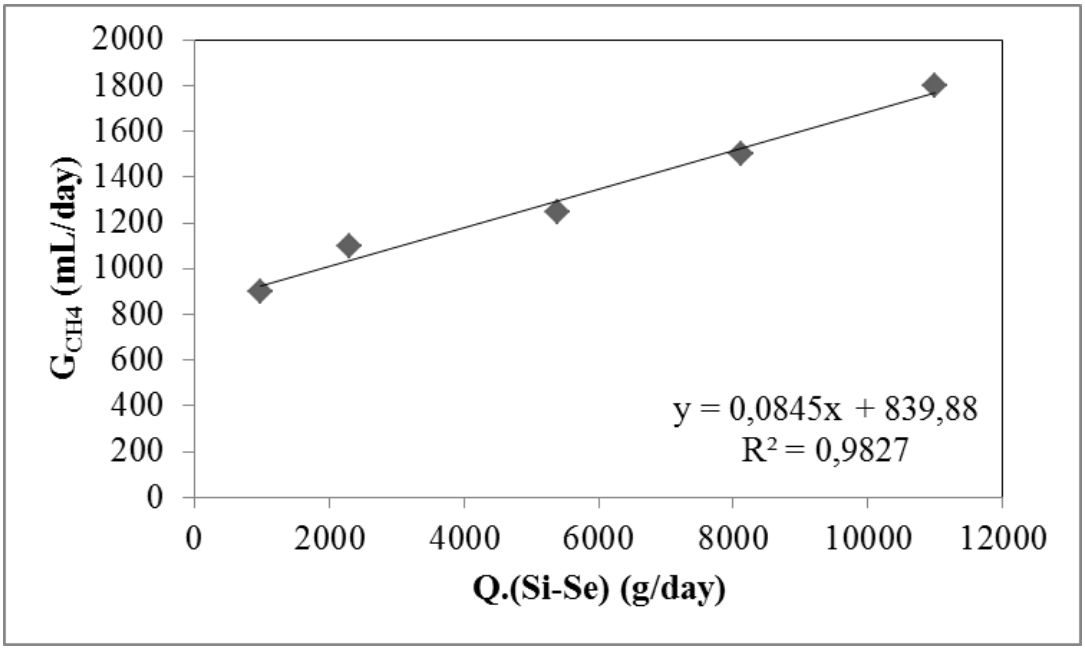

Figure 6.66 Determination of k_{sg} in Van der Meer and Heertjes model

6.5.3.3 Evaluation of the Biogas Kinetic Models in the Anaerobic ITBR System

The methane and total gas productions were calculated from the experimental studies using two different kinetic models and they were correlated. The kinetic constants calculated from the models are summarized in Table 6.43. G_{max} and G_B were found as 844 mL/L.day and 0.65 mg/L.day, respectively, in the Modified Stover Kincannon biogas kinetic model. M_{max}, and M_B were found to be 495 mL/L.day and 0.42 mg/L.day, respectively, in the Modified Stover Kincannon biogas kinetic model.

Table 6.43 Comparison of kinetic constants for Modified Stover-Kincannon, and Van der Meer & Heertjes models for total and methane gas productions (HRTs=1.38-1.83-2.75-5.5-11 days, SRT=63 days, influent COD_{dis}=2850 mg/L, influent PAHs=292 ng/mL, OLRs=2.05-1.56-1.04-0.45-0.19 mg/L.day).

Model	Kinetic constants	Methane gas production	Total gas production
Modified Stover-Kincannon Model	G_{max} (mL/L.day)	-	844
	G_B (mg/L.day)	-	0.65
	M_{max} (mL/L.day)	495	
	M_B (mg/L.day)	0.42	
Van derMeer and HeertjesModel	k_{sg} (mL CH_4/g COD removed)	0.0845	-

The results show that higher total and methane gas productions occurred in the removed substrates (COD_{dis} and PAHs) in modified Modified Stover-Kincannon model. Furthermore, the G_B and the M_B values are high according to the anaerobic treatment although it was studied with petrochemical wastewater containing PAH concentration as high as 292 ng/mL.

k_{sg} was found as 0.0845 mL CH_4/mgCOD_{dis} in Van der Meer & Heertjes methane gas kinetic model throughout metabolization of COD_{dis} to the CH_4. The k_{sg} value is lower for anaerobic gas production treating PAHs in Van der Meer & Heertjes model (Ni et al., 2010).

As a conclusion it was found that the total and methane gas productions can be defined with the kinetic constants in the Modified Stover-Kincannon model.

In this study, the kinetic constants values obtained from Modified Stover-Kincannon and Van der Meer and Heertjes kinetic models cannot be compared with the literature since it was not found enough kinetic data about the methane production rate in the anaerobic ITBR treating petrochemical industry wastewater containing PAHs.

6.5.3.4 Comparison of the Experimental and Theoretical Total and Methane Gas Productions in the Modified Stover Kincannon and Van der Meer & Heertjes Kinetic Models for the Anaerobic ITBR System

The experimental total and methane gas productions were compared with theoretical gas productions in two different kinetic models. In Modified Stover Kincannon kinetic model, the theoretical total and methane gas productions were calculated using Eq. (6.1) and Eq. (6.2), respectively. In Van der Meer & Heertjes kinetic model, the theoretical methane gas production was calculated using Eq. (6.3). The results for experimental and theoretical total and methane gas productions for both models are given in Table 6.44.

The experimental and theoretical methane gas productions in HRTs were compared for two kinetic models (See Table 6.44). In experimental studies the total gas amounts for different HRTs, which vary from 1.38 and 11 days were found between 2400 and 3200 L/day, where the Modified Stover-Kincannon model theoretical total gas results varied between 2333 and 3185 mL/day of the total gas with a regression coefficient of R^2 =0.99. This similarity is repeated for the theoretical methane gas productions. Experimental methane results change between 900 and 2100 mL/day and the Modified Stover-Kincannon model theoretical methane gas results range is calculated as between 1141 and 2022 mL/day. But the Van der Meer & Heertjes Model model theoretical methane gas results are not compatible with experimental results, since the range of theoretical methane production in this model was found to be as low as 95-906 mL/day with a regression coefficient of R^2 =0.98. Hence these methane productions from the Van der Meer & Heertjes Model model is also much lower than the methane production of the Modified Stover-Kincannon model.

Table 6.44 Comparison of experimental and theoretical results for total and methane gas productions in two different models versus HRTs (SRT=63 days, influent COD_{dis}=2850 mg/L, influent PAHs=292 ng/mL).

		Experimental		Theoretical					
				Modified Stover-Kincannon Model				Van derMeer & Heertjes Model	
OLR (mg/L.day)	HRT (day)	Total gas (mL/day)	Methane gas (mL/day)	Total gas (mL/day)	R^2	Methane gas (mL/day)	R^2	Methane gas (mL/day)	R^2
2.07	1.38	3100	2000	3145		2018		906	
1.56	1.83	3200	2100	3185		2022		686	
1.04	2.75	3100	2050	3073	0.99	1954	0.99	458	0.98
0.52	5.50	2500	1200	2779		1579		195	
0.25	11.00	2400	900	2333		1141		95	

Furthermore the kinetic constants relevant to gas production rates and the proportionality constants found from this model are meaningful for maximum anaerobic total and methane gas production rates.

The total and methane gas productions values calculated with the Modified Stover Kincannon gas model were almost the same with the total and methane gas productions found in the experimental studies (See Table 6.44). However the theoretical methane gas production values calculated according to the Van der Meer & Heertjes model were not closer to the experimental ones (See Table 6.44). The methane gas produced from the removed COD_{dis} in Van der Meer & Heertjes kinetic are low (between 95 and 906 $mLCH_4$/day) in the anaerobic ITBR system (See Table 6.44) since the Van der Meer & Heertjes kinetic constant for methane gas production (k_{sg}) is low (Table 6.43).

The theoretical methane gas productions from the Modified Stover-Kincannon model were very close to the experimental data compared to the Van derMeer & Heertjes Model model at six different HRTs. A linear relationship between the experimental and theoretical methane gas productions was observed in the Modified Stover Kincannon model with a regression coefficient of R^2 =0.99 (y= 0.0008x+0.002).

These results showed that although both kinetic models can be successfully used for modeling the gas productions in the ITBR system, the Modified Stover Kincannon kinetic approach is more suitable than the Van der Meer & Heertjes model since the total and methane gas productions in the Modified Stover Kincannon gas kinetic model, are related to maximum specific total and methane gas production rates, gas kinetic constants and OLR applied to the anaerobic ITBR system.

The OLR versus experimental and theoretical total and methane gas productions showed that the theoretical total and methane gases are closer to the experimental values when the calculated kinetic constants were placed into Modified Stover-Kincannon kinetic model.

6.5.4 Inhibition Kinetics of PAHs in the Presence of Biosurfactants

6.5.4.1 Biodegradation Kinetics of PAHs in the Absence of Biosurfactants

Among the kinetic models used it was found that the Monod kinetic is appropriate for the utilization of PAHs as substrate (S) and PAH degrading biomass as given in section 5.14.5 (See Table 5.28). When the inverse of substrate (PAH) utilization rate 1/R is plotted against the reciprocal of substrate (PAH) "1/S", a straight line is obtained (Lineweaver–Burk plot) as showed in section 5.14.5 (See Table 5.28). As summarized in Table 6.45, the parameters estimated using integrated Monod kinetic are 0.02 1/day for the maximum specific growth rate (μ_{max}), 42 ng/mL for the K_s and 9.25 ng/mL day for the R_{max} through aerobic degradation of a real petrochemical wastewater without biosurfactant at a SRT of 25 days and at an initial total PAH concentration of 65.32 ng/mL (See Figures 6.36 and 6.37 in section 6.5). Among the SRTs applied it was found that the maximum μ_{max} and R_{max} values were obtained at a SRT of 25 days in biosurfactant free samples.

Table 6.45 Biodegradation and inhibition kinetic constants calculated for total PAHs at increasing RD, SR and EM concentrations at a SRT of 25 days (initial total PAH conc.: 65.32 ng/mL)

Biosurfactant (mg/L)	Type of inhibition	μ_{max} (1/day)	R_{max} (ng/mL.day) [k]	K_s (ng/mL) [l]	K_{ID} (ng/mL) [m]	R^2 [n]
0	No- inhibition (Monod Kinetic)	0.02	9.25	42	-	0.99
RD 15 [a]	No- inhibition (Monod Kinetic)	0.14	21	13	-	0.99
SR 15 [b]	No- inhibition (Monod Kinetic)	0.10	12.56	16	-	0.99
EM 15 [c]	No- inhibition (Monod Kinetic)	0.12	11.96	14	-	0.99
RD 25 [d]	Competitive	0.016	21	48	1.78	0.99
RD 25 [d]	Non-competitive	15	49	300	78	0.80
RD 25 [d]	Un-competitive	98	89	267	98	0.81
RD 25 [d]	Haldane	1.34	0.45	0.3	86	0.85
SR 25 [e]	Competitive	0.012	12.56	52	1.23	0.98
SR 25 [e]	Non-competitive	67	76	99	120	0.65
SR 25 [e]	Un-competitive	120	345	138	340	0.60
SR 25 [e]	Haldane	0.34	0.99	12	78	0.63
EM 25 [f]	Competitive	0.010	11.96	54	1.32	0.99
EM 25 [f]	Non-competitive	87	89	121	129	0.58
EM 25 [f]	Un-competitive	134	487	187	378	0.59
EM 25 [f]	Haldane	0.21	0.56	11	98	0.61

a: 15 mg/L rhamnolipid; b: 15 mg/L surfactin; c: 15 mg/L emulsan; d: 25 mg/L rhamnolipid ; e: 25 mg/L surfactin concentration; f: 25 mg/L emulsan concentration g: μ_{max} : maximum specific growth rate;k: R_{max} : maximum substrate utilization rate: ; l: K_s: half saturation constant; m: K_{ID} : inhibition constant; n: regression coefficient

6.5.4.2 Inhibition Kinetics of PAHs in the Presence of Biosurfactants

The double reciprocal plot in Monod kinetic can also give valuable information on inhibition of PAH biodegradation at high biosurfactant concentrations. The possible effects of increasing RD, EM and SR concentrations on Lineweaver–Burk plot can be seen by the linearization of Eqs. 5.76 and 5.79 in Table 5.28 (see section 5.13.5) in competitive, non-competitive, uncompetitive and Haldane. Inhibition models are classified according to the effect of toxic compounds (in this study excess biosurfactant) on the R_{max} and K_s. In the presence of increasing concentrations of biosurfactants, the impacts of excess biosurfactant were explained by the modified Monod equations namely competitive, non-competitive, uncompetitive and Haldane. Generally, the effect of biosurfactants is related to μ_{max} and R_{max} values. As aforementioned, depending on the type of biosurfactant and its excess concentration to the variations in μ_{max} and R_{max} values and the inhibitions were expressed by the Eqs. 5.76 and 5.79 in Table 5.28 (see section 5.14.5) in competitive, non-competitive, uncompetitive and Haldane. Subsequently the R_{max}, K_s and μ_{max} values obtained in the Monod kinetic were substituted into the integrated competitive, non-competitive, uncompetitive and Haldane equations to obtain the relationships between K_s, K_{ID} and R_{max} at increasing RD, EM and SR concentrations (25 mg/L). μ_{max} and K_s values calculated at SRT of 25 days for 15 and 25 mg/L RD, EM and SR concentrations were placed in integrated Monod equations to determine $\mu_{max,}$ K_s and K_{ID} values in all inhibition kinetics. (See section 5.14.5, Table 5.28).

When the inverse of substrate (PAH) utilization rate 1/R is plotted against the reciprocal of substrate (PAH) "1/S", a straight line is obtained (Lineweaver–Burk plot) as showed in See section 5.14.5, Table 5.28. This line will have a slope of "$[(K_s/R_{max})\ (1+I_D/K_{ID})]$", an intercept of "$1/R_{max}$" on the "1/R" axis, and an intercept of "$[-1/(K_s(1+I_D/K_{ID}))]$" on the "1/S" axis in competitive inhibition model. The slope is increased by a factor of $(1+I_D/K_{ID})$. From this relationship K_s, R_{max} and K_{ID} can be calculated.

Since the plot of "1/R" versus "1/S" is linear, this line will have a slope of "[(K_s/Rmax) (1+I_D/K_{ID})]", an intercept of "[(1/R_{max}) (1+I_D/K_{ID})]" on the "1/R" axis, and an intercept of "−1/K_s" on the "1/S" axis in the non-competitive inhibition kinetic. The slope is increased by a factor of (1+I_D/K_{ID}). From this relationship K_s, R_{max} and K_{ID} can be calculated.

Since the plot of "1/R" versus "1/S" linear, this line will have a slope of "K_s/R_{max}", an intercept of "[(1/R_{max}) (1+I_D/K_{ID})]" on the "1/R" axis, and an intercept of "[−(1+I_D/K_{ID})/K_s]" on the "1/S" axis for un-competitive inhibition model. The intercept on the "1/R" is increased by a factor of (1+I_D/K_{ID}). From this relationship K_s, R_{max} and K_{ID} can be calculated.

The addition of RD, EM and SR biosurfactants up to 15 mg/L increased the μ_{max} values (Table 6.45). The μ_{max} values increased from 0.02 1/day to 0.10 1/day, 0.12 1/day and to 0.14 1/day at 15 mg/L SR, EM and RD, respectively. The R_{max} values increased from 9.25 ng/mL.day to 11.96, 12.56 and to 21 ng/mL.day at a SRT of 25 days in CSTR reactors containing 15 mg/L EM, SR and RD, respectively (Table 6.45). The K_s values decreased from 42 ng/mL to approximately 13 and to 16 ng/mL indicating the utilization of PAHs by the PAH degrading biomass in CSTR reactors containing 15 mg/L RD, and SR, respectively. At 15 mg/L EM, the K_s values decreased from 42 to 14 ng/mL. The K_s values increased from 42 ng/mL to 48 and to 52 ng/mL, respectively, at 25 mg/L RD and SR, respectively, in competitive inhibition kinetics at a SRT of 25 days.

In non-competitive inhibition, the addition of RD, EM and SR biosurfactants up to 25 mg/L were increased extremely the μ_{max} values which are not between the limits values known for specific growth rate. As a result this kinetic constant is not realistic, therefore this inhibition kinetic can be rejected (Table 6.45). Similarly, extremely high K_s values were obtained in this type of inhibition. The K_s values increased from 42 ng/mL to approximately 99 and to 120 ng/mL at 25 mg/L SR, and EM, respectively. The K_s values increased from 42 to 300 ng/mL at 25 mg/L RD in non-competitive inhibition kinetic at a SRT of 25 days.

In uncompetitive inhibition, the μ_{max} values increased from 0.02 1/day to 98 1/day, 120 1/day and to 134 1/day at 25 mg/L RD, SR and EM, respectively. The R_{max} values increased from 9.25 ng/mL.day to 89, 345 and to 487 ng/mL.day at a SRT of 25 days in CSTR reactors containing 25 mg/L RD, SR and EM, respectively. Similarly, it can be said that the kinetic constants calculated from the uncompetitive inhibition are not suitable to describe the inhibition of PAHs in the case of excess biosurfactant concentration (Table 6.45). The K_s values increased from 42 ng/mL to approximately 138, 187 and to 267 ng/mL at 25 mg/L SR, EM and RD, respectively in un-competitive inhibition kinetic at a SRT of 25 days.

In Haldane inhibition, the μ_{max} values increased enormous while the R_{max} values decreased minorly at a SRT of 25 days in CSTR reactors containing 25 mg/L RD, EM and SR, respectively (Table 6.45). Similarly, the K_s values also deceased to very low levels. These results showed that the Haldane inhibition kinetic is not appropriate to mention the PAH inhibition at high biosurfactant concentrations at a SRT of 25 days.

The results of this step showed that the competitive inhibition equation gave the correct fit for high biosurfactant concentrations since the model fitted the experimental data was very well with an R^2 of 0.99. The excess concentrations of the biosurfactants (25 mg/L) mentioned above decreased the μ_{max} levels from 0.02 to 0.016, 0.012 and to 0.010 1/day, for 25 mg/L RD, SR and EM, respectively while no differentiation was observed on R_{max} values at a SRT of 25 days. The K_s values also increased from 42 to 48, 52 and to 48 ng/mL at RD, SR and EM concentrations of 25 mg/L, respectively, at a SRT of 25 days in competitive inhibition kinetic model. The K_s reflects the fact that the high K_s values (48 ng/mL and 52 ng/mL) caused accumulation of the PAHs. This means low affinity of biomass to the PAH-substrate in the presence of 25 mg/L RD and SR biosurfactants. The slopes and the intercepts, obtained from the Lineweaver–Burk plots in the competitive inhibition kinetic model at increasing RD concentrations are illustrated in Figure 6.67. The slope of the RD plot for 15 mg/L is "K_s/R_{max}" whereas the slope of plots containing high RD concentration is "$(K_s/R_{max})\ (1+I_D/K_{ID})$" (Figure 6.67).

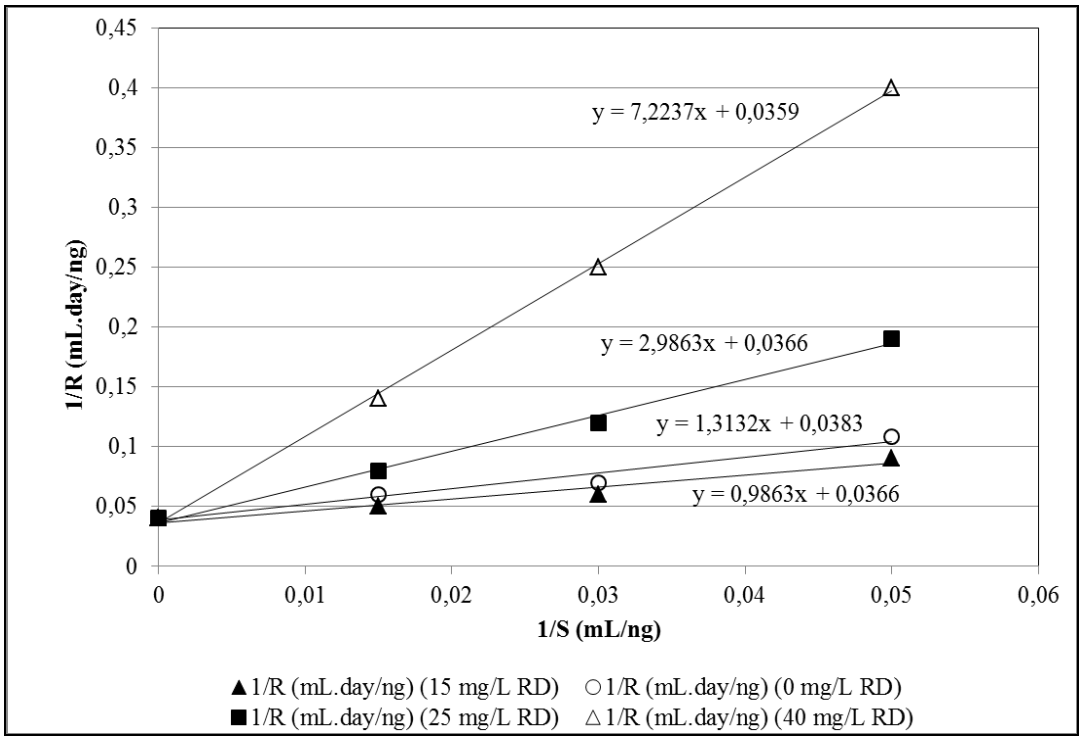

Figure 6.67 Reciprocal of Monod without RD (0 mg/L), 15 mg/L RD, and competitive inhibitions of excess RD concentrations (25 mg/L and 40 mg/L),

The inhibition coefficients (K_{ID}) were found as 1.23 ng/mL, 1.32 ng/mL and 1.78 ng/mL for 25 mg/L SR, EM and RD biosurfactants, respectively in competitive inhibition kinetic. (Table 6.67). The K_{ID} decreased as the biosurfactant concentration increased from 15 to 25 mg/L (Table 6.45). From this data, it can be concluded that the PAH inhibition occurs for 25 mg/L, RD, SR and EM concentrations.

Figure 6.67 shows that high biosurfactant concentrations cause competitive inhibition on PAH degradation resulting in increases in K_s. In the competitive inhibition, R_{max} did not change but the K_s values increased ($1/K_s$ decreased) while the K_{ID} values decreased ($1/K_{ID}$ increased) as the RD concentrations increased. The apparent K_s for PAH degrading samples at RD= 25 mg/L is greater than the K_s measured in samples treating petrochemical wastewater with 15 mg/L RD. Increases in the intercept on the "1/S" axis and the slopes of plots containing RD concentrations > 15 mg/L indicate that the slopes in inhibited fits increased by a factor "$1+(I_D/K_{ID})$" (See section 4.14.5, Table 5.28), (Figure 6.67). However, as aforementioned, high RD concentrations causing competitive inhibition do not affect

the intercept on the 1/R axis (R_{max} values) indicating that they do not interfere with the substrate (PAH) rate through the formation of enzyme-substrate complex. In this type of inhibition the inhibitor (RD >15 mg/L) combined with the free enzyme in such a way that it competes with the normal substrate for binding at the active site. Larger K_s values seem to indicate a low affinity between the primary substrate/PAH and the aerobic microorganisms resulting in accumulation of PAHs. This accumulation shows that the PAH was not utilized and the benzene bonds in the PAHs were not cleaved.

The calculated R_{max}, μ_{max} and K_s values are unrealistically high in non-competitive, uncompetitive and Haldane inhibition kinetics as seen in Table 6.67. For this reason non-competitive, un-competitive and Haldane inhibition equations have to be rejected. The threshold limitations for competitive inhibition are: $K_s \geq K_{ID}$; $K_s \leq 65.32$ ng/mL; $\mu \leq \mu_{max} \leq 3\mu$ for 65.32 ng/mL total PAH both for 15 mg/L RD and SR concentrations at a SRT of 25 days.

Substantial inhibitory effect on K_s value was observed for PAHs, as evidenced by a decrease in K_{ID} values for RD higher than 15 mg/L in competitive inhibition. Higher Ks and lower K_{ID} values, in the competitive inhibition, can be characterized as a system with low affinity to substrate (PAHs).

No data is reported for PAH inhibition in the specialized literature and a direct comparison is difficult in the presence of excess biosurfactant concentrations. Stringfellow and Aitken (1995) found competitive inhibition of PHE degradation by naphthalene, methylnaphthalene, and FLN in binary mixtures using pure cultures. The occurrence of competitive inhibition was observed with two different *Pseudomonas* species throughout PAH degradation.

6.6 Acute Toxicity Evaluations in the Petrochemical Industry Wastewater

6.6.1 Acute Toxicity Evaluations in the Petrochemical Industry Wastewater in the CSTR System

6.6.1.1. Effect of Increasing SRTs on the Acute Toxicity Removal without Biosurfactant in the CSTR System

6.6.1.1.1 Effect of Increasing SRTs on the Daphnia magna Acute Toxicity without Biosurfactant in the CSTR System. The COD_{dis} and total PAHs concentrations were 1200 mg/L and 65.32 ng/mL in the influent of the raw petrochemical industry wastewater. The operational conditions for this study are summarized in Table 5.4 in the section Material and Methods. The *Daphnia magna* acute toxicity test is accepted as an acute toxicity test to determine the toxicity of refractory organics (Zheng et al., 2007; Gomez et al., 2001; Eom et al., 2007). In order to determine the acute toxicity of samples, dilutions varying between 1/1, 1/2, 1/4, 1/8 and 1/16 were performed in the influent and effluents of the CSTR system. Table 6.46 shows the alive numbers and the percentage inhibition of *Daphnia magna* in the influent wastewater. The inhibition percentage was calculated by the ratio of the number of dead *Daphnia magna* to the total number of living *Daphnia magna*. It was found that the percentage of dead *Daphnia magna* was higher at high wastewater ratios. In other words, the inhibition percentage increased at low dilutions in the influent samples. For example, the inhibitions (the percentage of dead *Daphnia magna*) decreased from 100% to 90% and 50% as the dilution ratio increased from 1/1 to 1/2 and 1/8, respectively, in the influent wastewater (Table 6.46).

Table 6.46 The number of *Daphnia magna* and inhibition percentages in the influent of CSTR

	Alive *Daphnia magna* number		Inhibition percentage (%)
Dilution ratio	First start	24 hours	
1/1 (0 mL DW+100 mL WW)	10	0	100
1/2 (50 mL DW+50 mL WW)	10	1	90
1/4 (75 mL DW+ 25 mL WW)	10	3	70
1/8 (87.5 mL DW+ 12.5 mL WW)	10	5	50
1/16 (93.75 mL DW+ 6.25 mL WW)	10	7	30

DW: distilled water (mL); WW: wastewater (mL)

Figures 6.68-6.72 and Table 6.47 show the EC_{50} values (the concentration affected 50% of *Daphnia magna* number*)* in the influent and effluent of the CSTR at increasing SRTs based on COD_{dis}. EC_{50} value was obtained as 143 mg/L in the influent wastewater. As the SRTs were increased from 5 to 10 and 25 days, the EC_{50} values increased from 467 to 471 and to 678 mg/L, respectively, at a HRT of 5 days in the effluent of the CSTR (Figures 6.68-6.71). The EC_{50} values decreased from 678 to 535 mg/L when the SRT was increased from 25 to 40 days (Figure 6.72). The acute toxicity reductions in the CSTR were 69%, 70%, 79% and 73% at SRTs 5, 10, 25 and 40 days, respectively. The *Daphnia magna* acute toxicity test results in the CSTR system showed that the EC_{50} values increased from 467 to 678 mg/L as the SRTs were increased from 5 to 25 days and the maximum acute toxicity removal efficiency was 79% at a SRT of 25 days. This corresponds to a COD_{dis} yield of 79% in the CSTR at a SRT of 25 days (See section 6.3 in Table 6.3).

In this study it was found that the EC_{50} value increased from 143 to 467 mg/L from the influent of the petrochemical industry wastewater to the effluent of the CSTR system at a SRT of 5 days. As the SRTs were increased from 5 to 25 days, the EC_{50} values increased in the effluent of the CSTR system. At high EC_{50} value (678 mg/L), the maximum acute toxicity removal (79%) was observed at a SRT of 25 days.

The acute toxicity of aromatic hydrocarbon compounds (naphthalene, benzene, toluene and xylene) and the toxic effects after an activated sludge continuous-flow completely mixed reactor were studied with the *Daphnia magna* by Gomez et al. (2001). Although the removals of the organics in question varied between 77% and 93% the acute toxicity removals were low (34-56%) in untreated and treated effluents. The EC_{50} values were observed at between 18.44 and 30.81 at 24 h (v/v). Eom et al. (2007) evaluated the toxicity of 16 PAHs (total concentration 17.7 ng/mL) in contaminated soil samples using *Daphnia magna* bioassay. The ecotoxicity results of this study showed that the EC_{50} values were very toxic to *Daphnia magna*. In our study the EC_{50} values in the effluent of CSTR system exhibited higher acute toxicity removals than those of Gomez et al. (2001) and Eom et al. (2007). Sepic et al. (2003) investigated the toxicity of 20 mg/L FLN and its biodegradation metabolites to pure bacterial strain *Pasteurella sp*. and *Daphnia magna*. 34% and 56% acute toxicity removals were obtained for the organisms in question, respectively. These results are comparably lower than the toxicity yields obtained in our study.

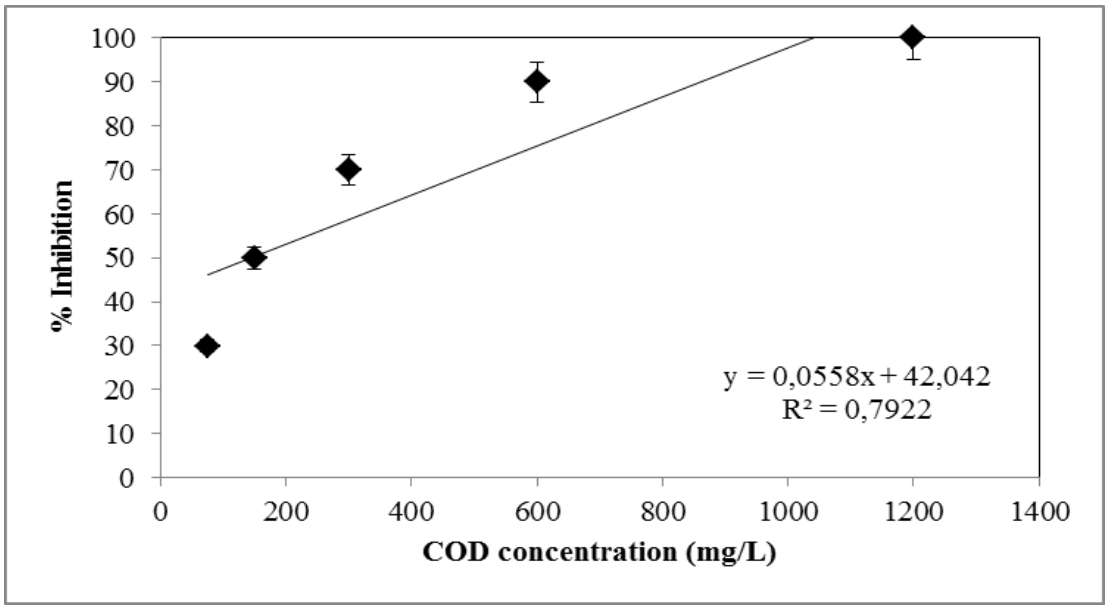

Figure 6.68 Variations of % inhibition versus COD concentration in the influent of the aerobic CSTR (EC_{50}=143 mg/L, HRT= 5 days)

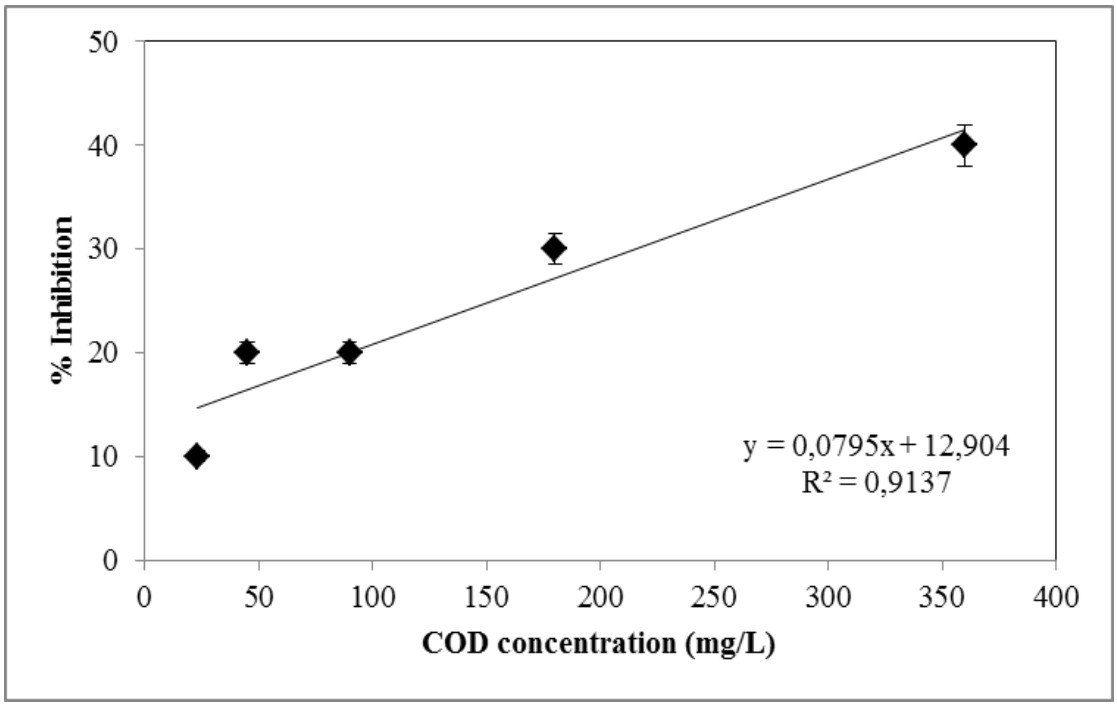

Figure 6.69 Variations of % inhibition versus COD concentration in the effluent of the aerobic CSTR for SRT 5 days without RD at a HRT of 5 days (EC_{50}= 467 mg/L)

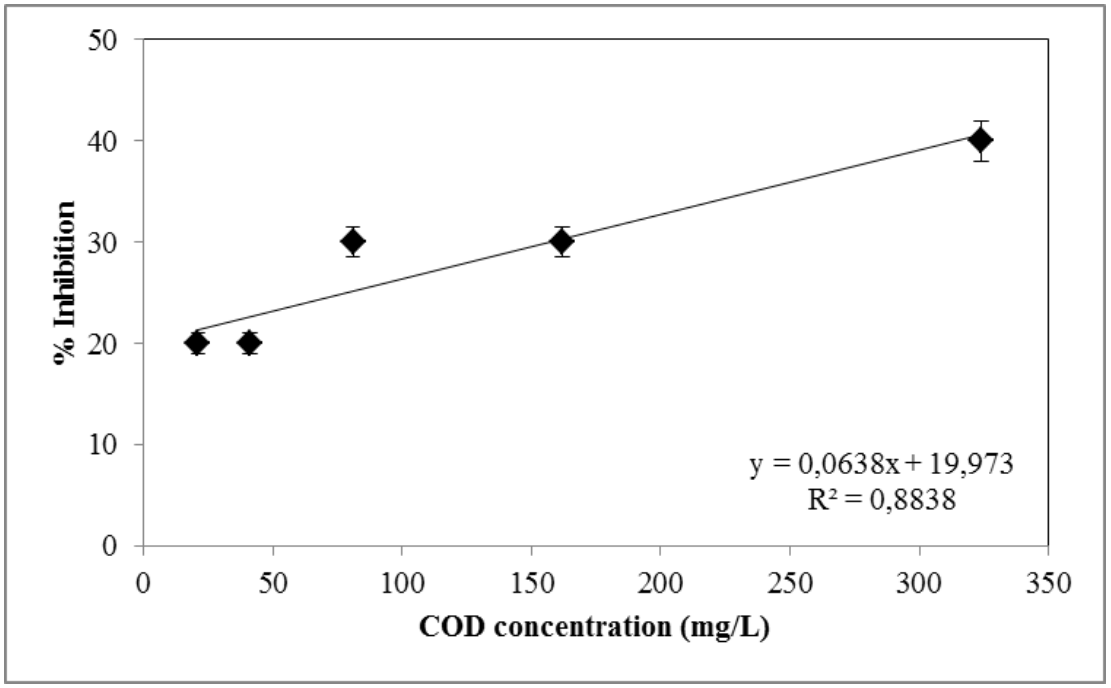

Figure 6.70 Variations of % inhibition versus COD concentration in the effluent of the aerobic CSTR for SRT 10 days without RD at a HRT of 5 days (EC_{50}= 471 mg/L)

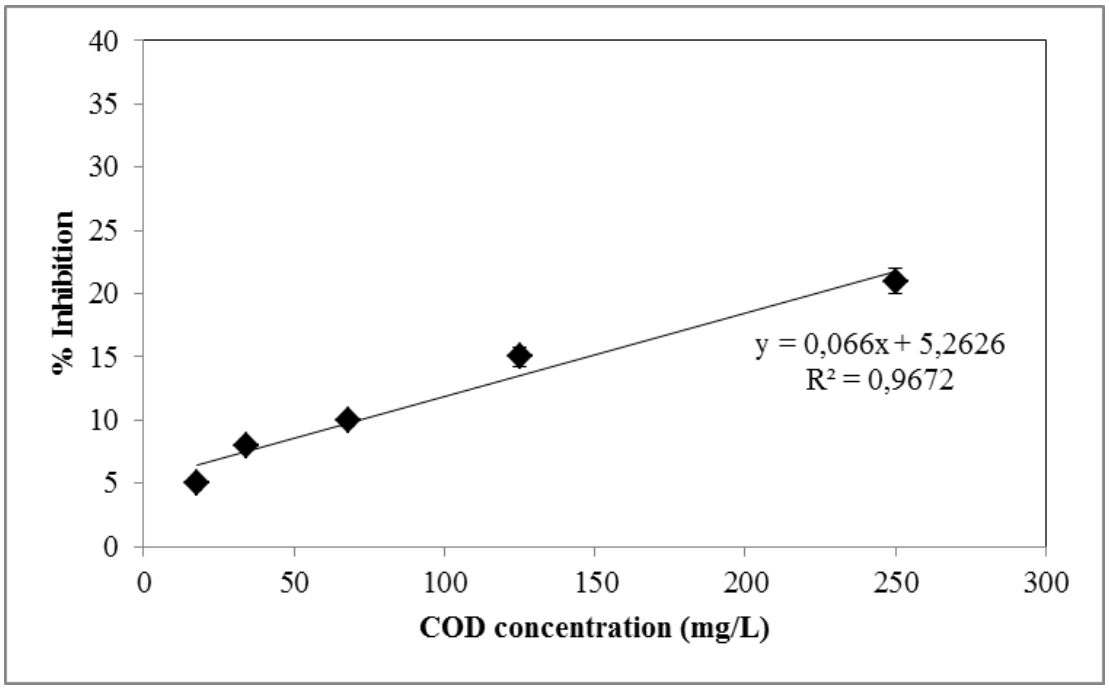

Figure 6.71 Variations of % inhibition versus COD concentration in the effluent of the aerobic CSTR for SRT 25 days EC_{50}= 678 mg/L without RD at a HRT of 5 days

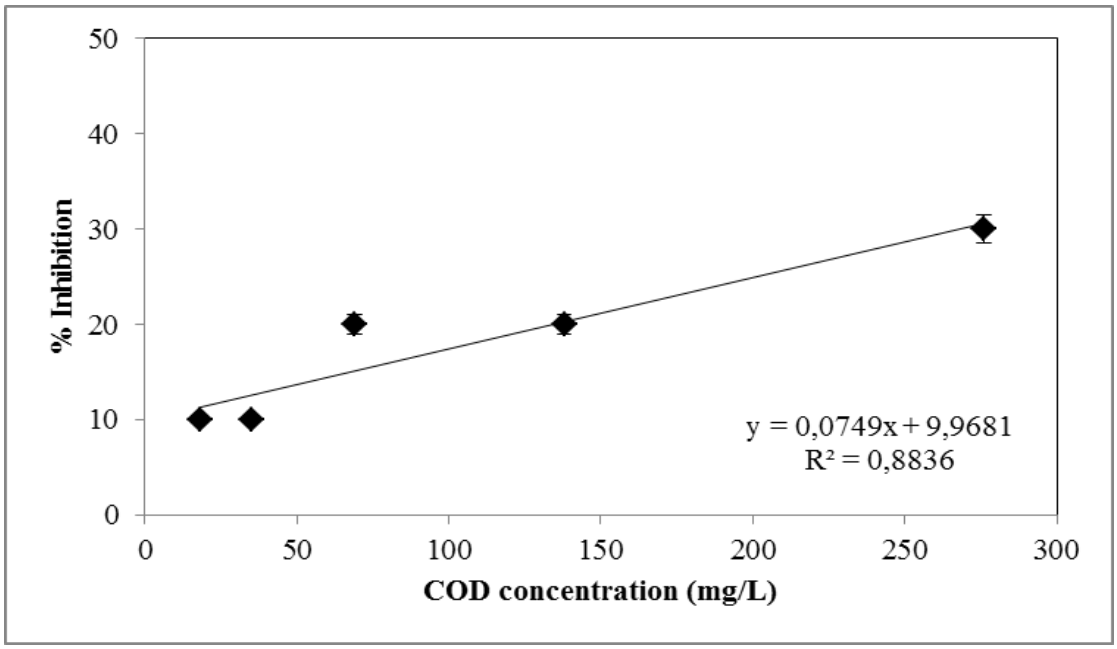

Figure 6.72 Variations of % inhibition versus COD concentration in the effluent of the aerobic CSTR for SRT 40 days EC_{50}= 535 mg/L without RD at a HRT of 5 days

Table 6.47 The EC_{50} values in the influent and effluent of the CSTR without RD (COD_{dis}=1200 mg/L PAHs=65.32 ng/mL, HRT= 5 days)

SRT (days)	Influent EC_{50} value (mg/L)	Effluent EC_{50} value (mg/L)	*Daphnia magna* acute toxicity removal (%)
5	143	467	69
10	143	471	70
25	143	678	79
40	143	535	73

6.6.1.1.2 Effect of Increasing SRTs on the Vibrio fischeri Acute Toxicity without Biosurfactant in the CSTR. Microtox test is an acute toxicity test. Toxicity was estimated in terms of EC_{50}, defined as the concentration of the toxicant causing 50% reduction in the activity of the *Vibrio fischeri*. A specific strain of the luminescent bacteria, *Vibrio fischeri* (LCK 491, NRRL-B-11177), was used in this test to determine the acute toxicity of PAHs. Reductions in light intensity at 30^{th} minute were chosen to measure the acute toxicity (Lange, 1994). The *Vibrio fischeri* acute toxicity tests were performed in the influent and effluent samples of the aerobic CSTR at different dilution ratios (1/1-1/2-1/4-1/8-1/16). The average results of triplicate acute toxicity test results performed in the influent and effluent wastewaters without RD were shown in Figures 6.73-6.77 and Table 6.48. The COD_{dis} and total PAHs concentrations were 1200 mg/L and 65.32 ng/mL in the influent of the wastewater. The operational conditions for this study are summarized in Table 5.4 in the section Material and Methods.

The 50% inhibition of the *Vibrio fischeri* ($EC_{\mathbf{50}}$ value) was obtained as 100 mg/L in the influent wastewater, when COD_{dis} were taken into consideration (Figure 6.73). As the SRTs were increased from 5 to 10 and to 25 days, the EC_{50} values increased from 270 to 307 and to 398 mg/L, respectively, in the effluent of the CSTR (Figures 6.74-6.76). The EC_{50} values decreased from 398 to 330 mg/L when the SRT was increased from 25 to 40 days, respectively (Figure 6.77). The *Vibrio fischeri* acute

toxicity reductions in the CSTR were 63%, 67%, 75% and 70% at SRTs 5, 10, 25 and 40 days, respectively. The *Vibrio fischeri* acute toxicity test results in the CSTR system showed that the EC_{50} values increased from 100 to 398 mg/L at a SRT of 25 days and the maximum acute toxicity removal efficiency was 75% in this SRT (Figure 6.76).

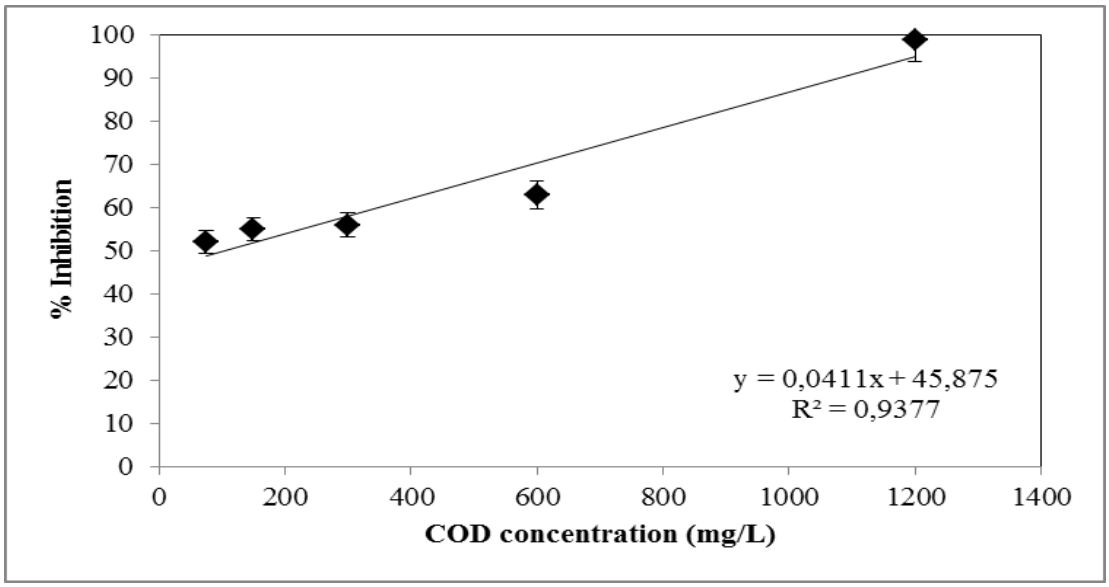

Figure 6.73 Variations of inhibition percentages versus COD_{dis} concentrations in the influent raw petrochemical wastewater (EC_{50}= 100 mg/L, HRT=5 days)

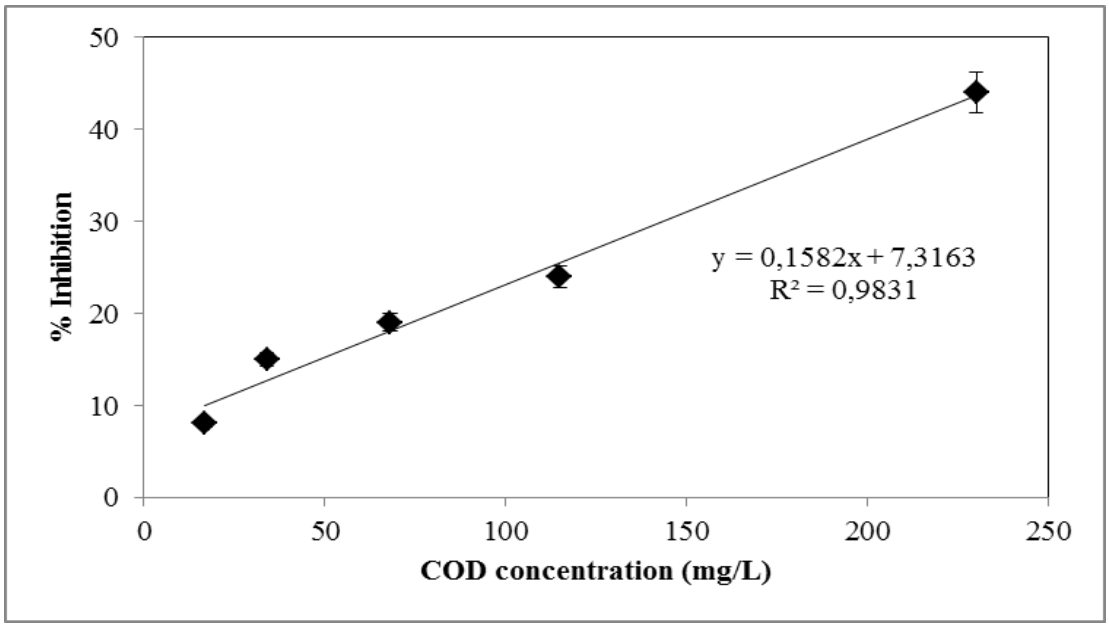

Figure 6.74 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a SRT of 5 days without RD (EC_{50}= 270 mg/L, HRT=5 days)

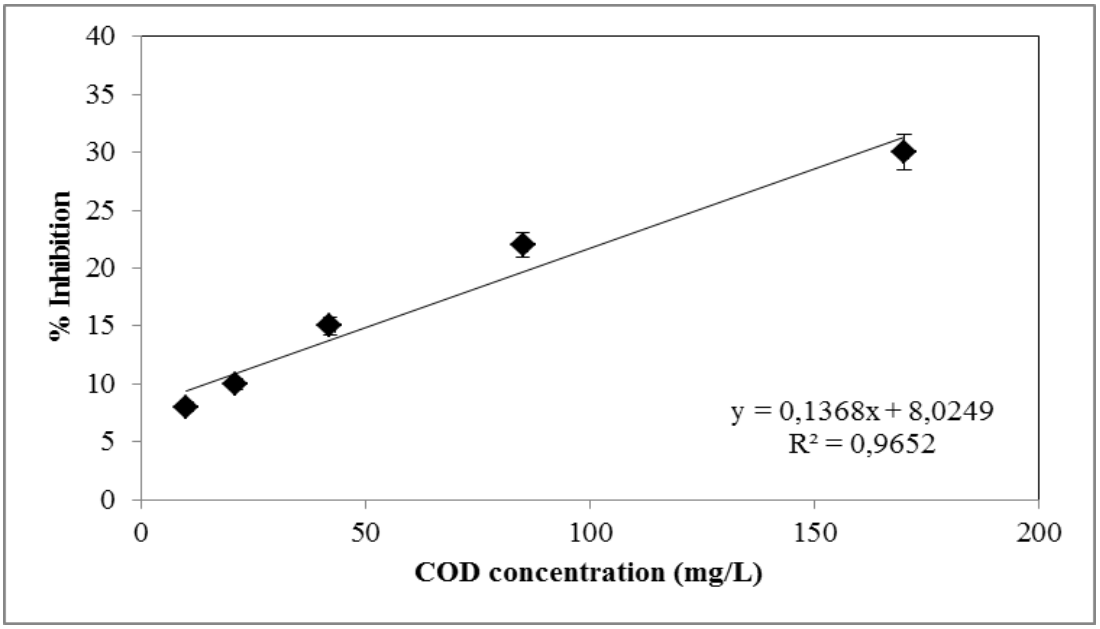

Figure 6.75 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a SRT of 10 days without RD (EC_{50}= 307 mg/L, HRT=5 days)

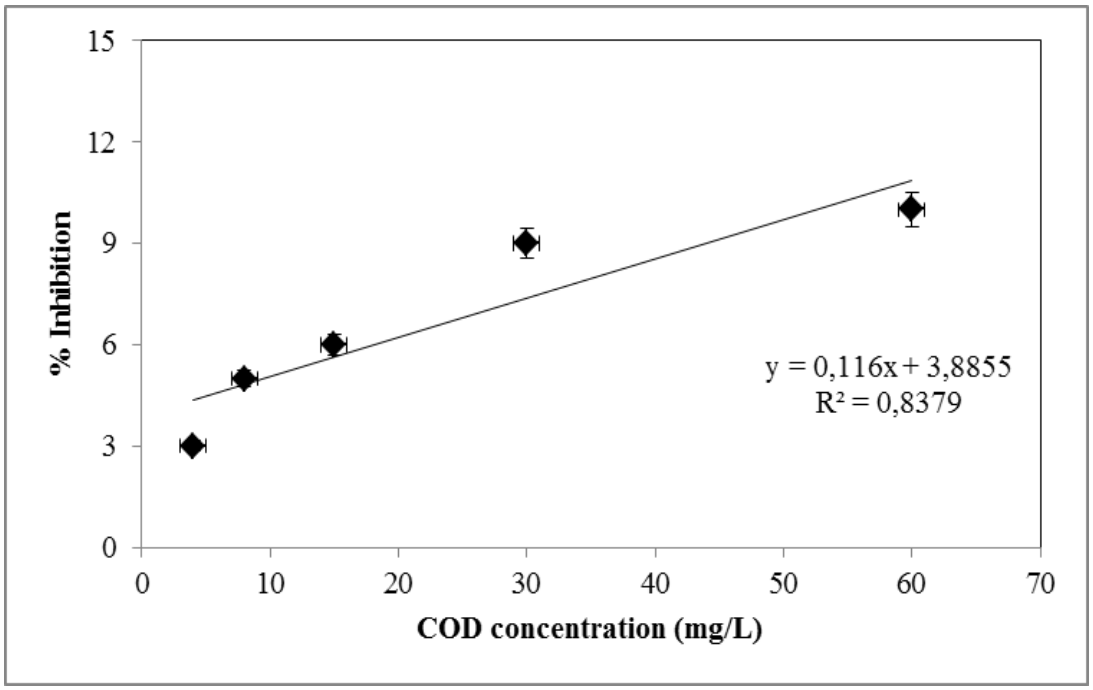

Figure 6.76 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a SRT of 25 days without RD (EC_{50}= 398 mg/L, HRT=5 days)

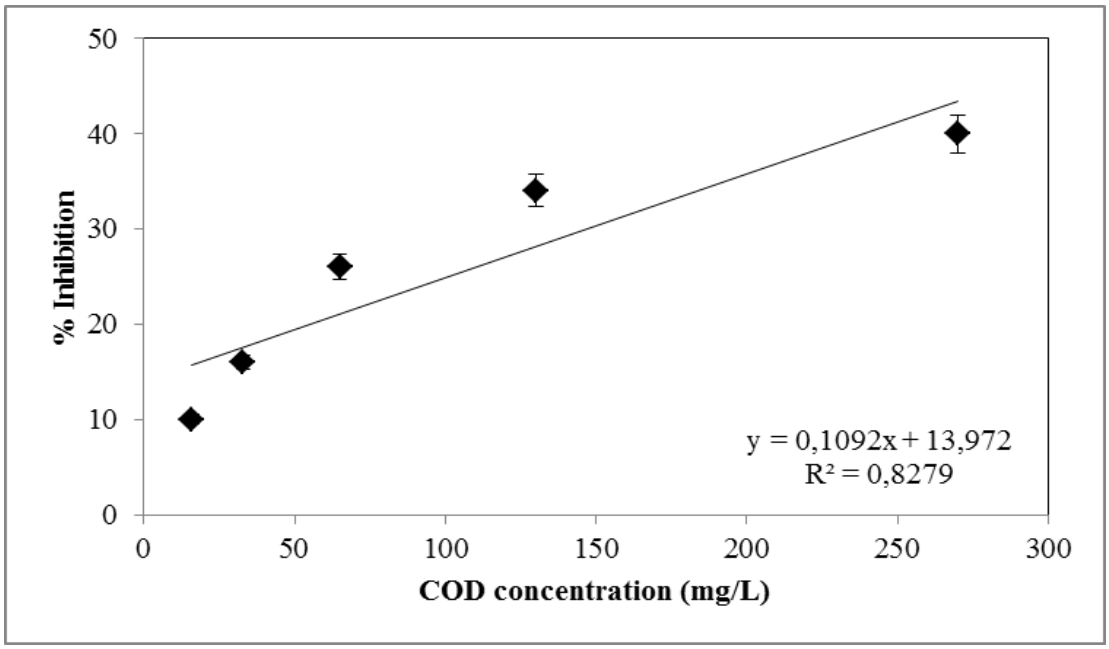

Figure 6.77 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a SRT of 40 days without RD (EC_{50}= 330 mg/L, HRT=5 days)

Table 6.48 The EC_{50} values in the influent and effluent of the CSTR without RD (COD_{dis}=1200 mg/L PAHs=65.32 ng/mL, HRT=5 days)

SRT (days)	Influent EC_{50} value (mg/L)	Effluent EC_{50} value (mg/L)	*Vibrio fischeri* acute toxicity removal (%)
5	100	270	63
10	100	307	67
25	100	398	75
40	100	330	70

6.6.1.2 Effect of Increasing SRTs on the Acute Toxicity Removal with 15 mg/L RD in the CSTR System

6.6.1.2.1 Effect of Increasing SRTs on the Daphnia magna Acute Toxicity at 15 mg/L RD in the CSTR System. Figures 6.78-6.81 and Table 6.49 show the EC_{50} values in the influent and effluent of the CSTR containing 15 mg/L RD at increasing SRTs from 5 days up to 40 days. The operational conditions for this study are summarized in Table 5.6 in the section Material and Methods. The EC_{50} value was

found as 143 mg/L in the influent wastewater. The EC_{50} values increased from 143 to 678, 876 and to 1450 mg/L, at SRTs 5, 10 and 25 days, respectively, in the effluent of the CSTR. When the SRT was increased from 25 to 40 days, the EC_{50} values decreased from 1447 to 773 mg/L at 15 mg/L RD (Figures 6.78-6.81). The acute toxicity reductions in the CSTR were 79%, 84%, 90% and 82% at SRTs 5, 10, 25 and 40 days, respectively. The EC_{50} was 143 mg/L in the influent of CSTR while this value increased to 1450 mg/L at a SRT of 25 days in the effluent of this reactor. The maximum acute toxicity removal was 90% at a SRT of 25 days with 15 mg/L RD in the CSTR (Figure 6.80).

The petrochemical wastewater was not treated effectively at short SRTs since short SRTs did not provide enough contact time for bacteria and PAHs to metabolize the total PAHs in the petrochemical industry wastewater in the CSTR. Therefore, high acute toxicity was observed at low SRTs such as 5 and 10 days. Similarly, the acute toxicity was high at SRTs > 25 days since the PAHs in the petrochemical industry wastewater caused toxicity to the activated sludge bacteria at a SRT of 40 days. The low acute toxicity and the maximum acute toxicity removal were obtained at a SRT of 25 days with 15 mg/L RD. This could be explained by the uptake of the PAHs by the activated bacteria cells through fast PAH diffusion with 15 mg/L RD at a SRT of 25 days. The COD_{dis} is taken up by the activated sludge bacteria degrading PAHs in a matter of minutes and metabolized, giving rise to a high unit rate of oxygen demand for synthesis resulting in low inhibitions to *Daphnia magna* high acute toxicity removals.

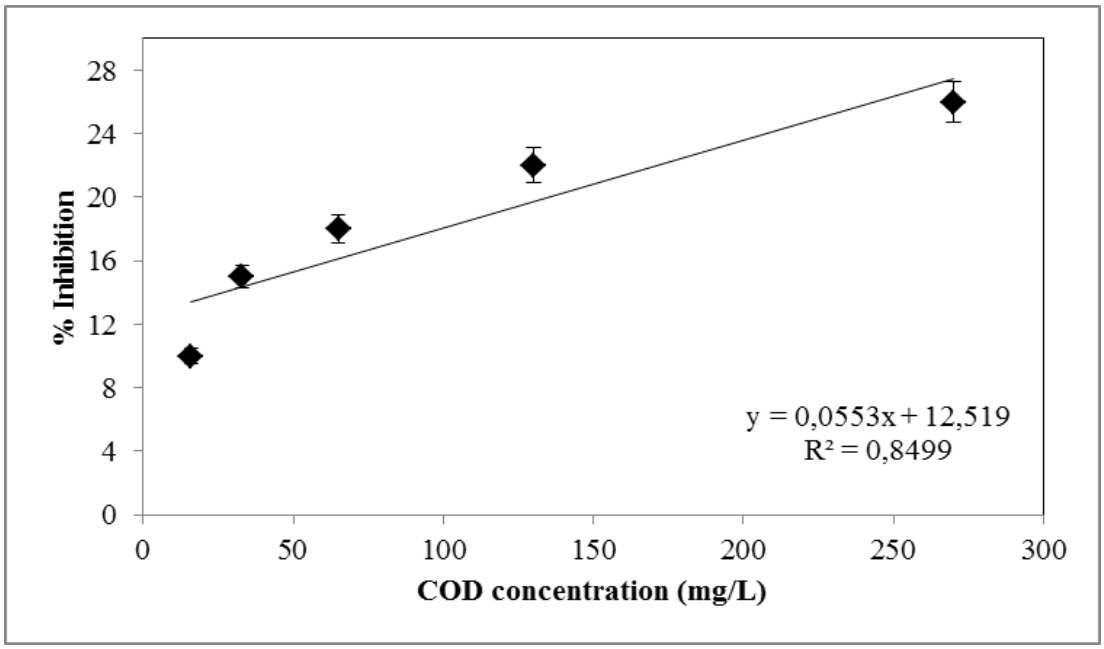

Figure 6.78 Variations of % inhibition versus COD concentration in the effluent of the aerobic CSTR for SRT 5 days with 15 mg/L RD at a HRT of 5 days (EC_{50}= 678 mg/L)

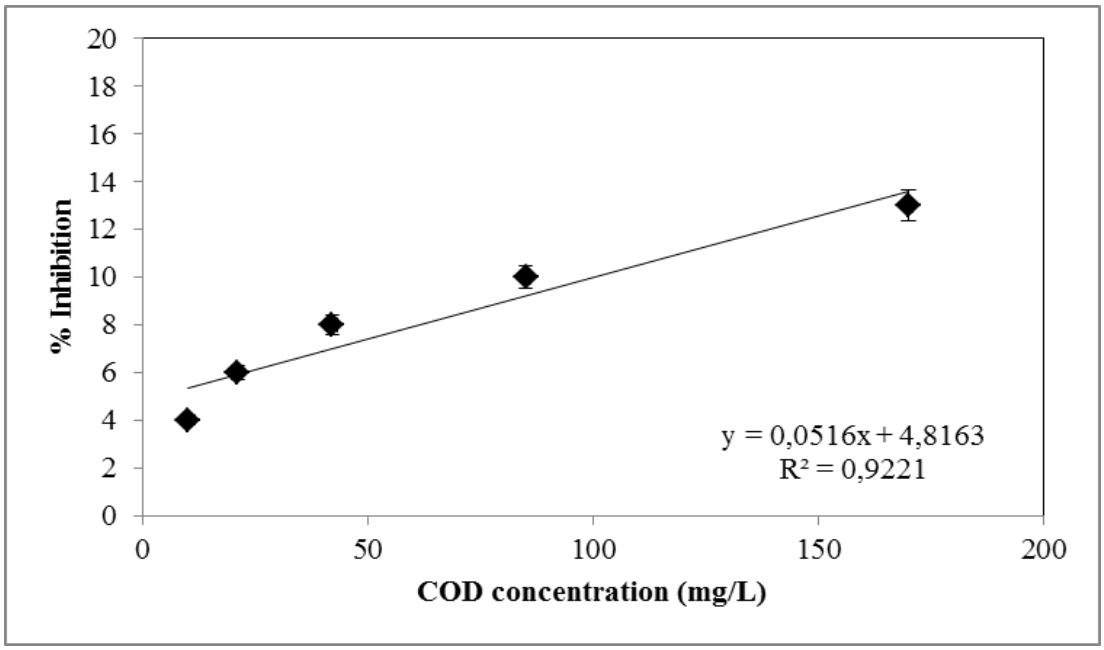

Figure 6.79 Variations of % inhibition versus COD concentration in the effluent of the aerobic CSTR for SRT 10 days with 15 mg/L RD at a HRT of 5 days (EC_{50}= 876 mg/L)

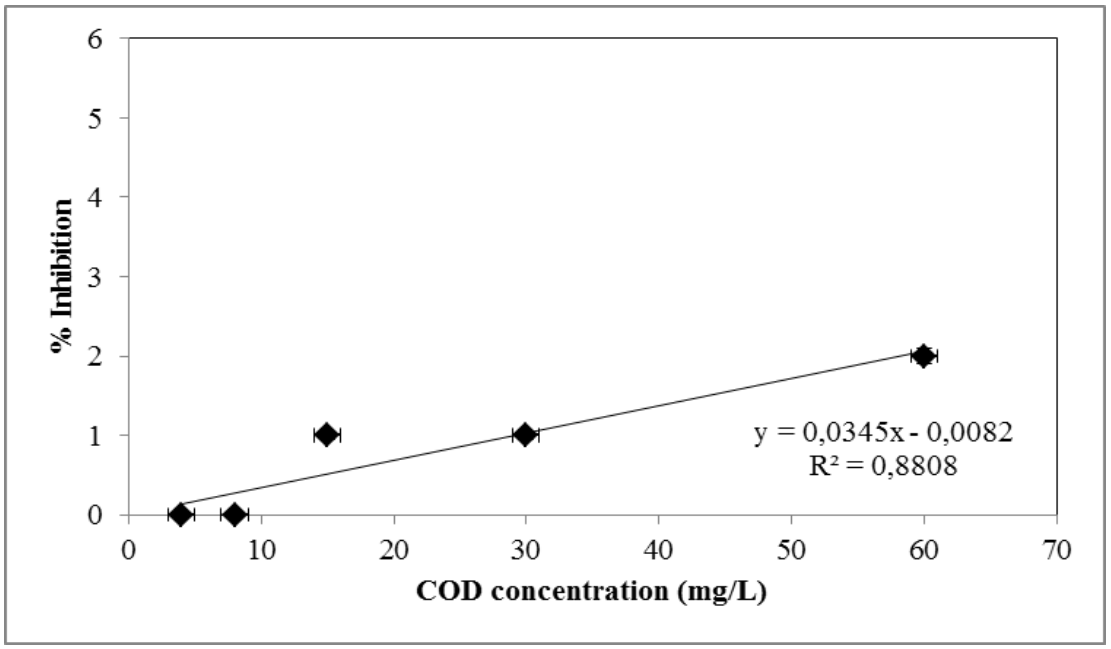

Figure 6.80 Variations of % inhibition versus COD concentration in the effluent of the aerobic CSTR for SRT 25 days with 15 mg/L RD at a HRT of 5 days (EC_{50}=1450 mg/L)

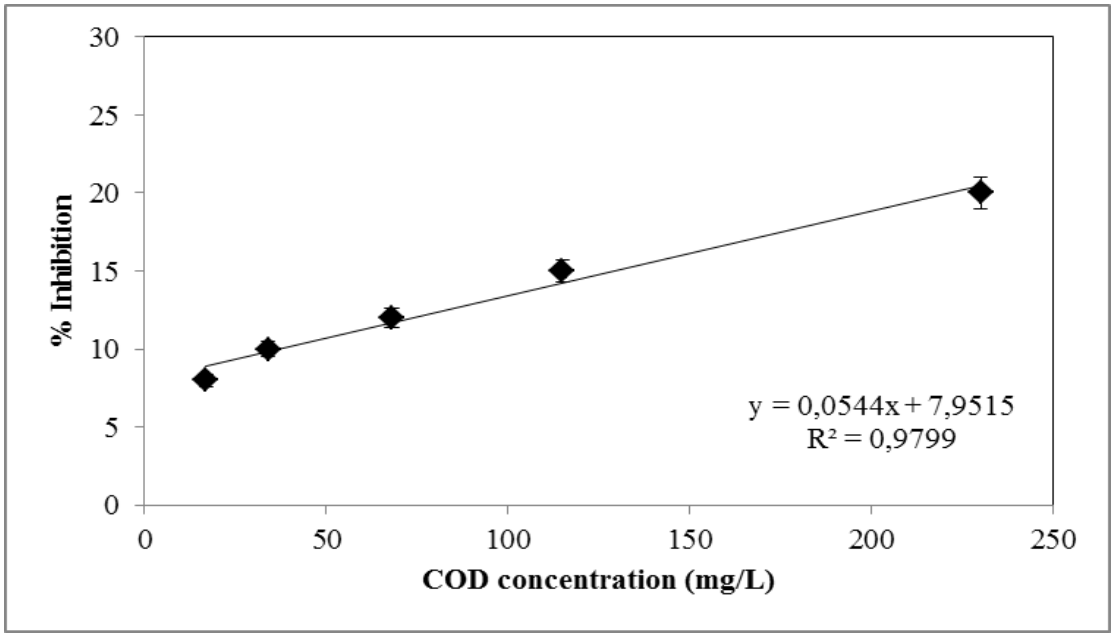

Figure 6.81 Variations of % inhibition versus COD concentration in the effluent of the aerobic CSTR for SRT 40 days with 15 mg/L RD at a HRT of 5 days (EC_{50}=773 mg/L)

Table 6.49 The EC_{50} values in the influent and effluent of the CSTR at 15 mg/L RD (COD_{dis}=1200 mg/L PAHs=65.32 ng/mL, HRT=5 days)

SRT (days)	Influent EC_{50} value (mg/L)	Effluent EC_{50} value (mg/L)	*Daphnia magna* acute toxicity removal (%)
5	143	678	79
10	143	876	84
25	143	1450	90
40	143	773	82

The administration of 15 mg/L RD increased the acute toxicity removals from the samples containing no RD to the samples containing RD (Tables 6.47 and 6.49). The acute toxicity removal increased from 69% to 79% at a SRT of 5 days (See Table 6.49). The acute toxicity removals increased from 79% to 84% as the SRT was increased from 5 to 10 days at 15 mg/L RD. Similarly, the acute toxicity removal increased to 90% for SRT of 25 days in the CSTR system containing 15 mg/L RD. At a SRT of 40 days, the acute toxicity removal decreased from %90 to 82% in the CSTR system containing 15 mg/L RD. In this study, it was found that 15 mg/L RD was beneficial to remove the acute toxicity with a maximum yield of 90% in comparison to 79% in the RD-free case at a SRT of 25 days (See Table 6.47).

6.6.1.2.2 Effect of Increasing SRTs on the Vibrio fischeri Acute Toxicity at 15 mg/L RD in the CSTR System. Figures 6.82-6.85 and Table 6.50 show the *Vibrio fischeri* acute toxicity results in the aerobic CSTR effluent samples containing 15 mg/L RD at increasing SRTs from 5 days up to 40 days at a HRT of 5 days. The operational conditions for this study are summarized in Table 5.6 in the section Material and Methods**.** The EC_{50} value was found as 100 mg/L in the influent wastewater of the CSTR system. The EC_{50} values increased from 100 to 354, 460 and 596 mg/L, respectively, at SRTs 5, 10 and 25 days in the effluent of the CSTR (Figures 6.82-6.84). When the SRT increased from 25 to 40 days, the EC_{50} values decreased from 596 to 433 mg/L at 15 mg/L RD (Figure 6.85). The acute toxicity reductions in the CSTR were 72%, 78%, 83% and 77% at SRTs 5, 10, 25 and 40

days, respectively. The EC_{50} value was 100 mg/L in the influent of CSTR while this value increased to 596 mg/mL at a SRT of 25 days in the effluent of this reactor. The maximum acute toxicity removal was 83% at a SRT of 25 days with 15 mg/L RD in the CSTR.

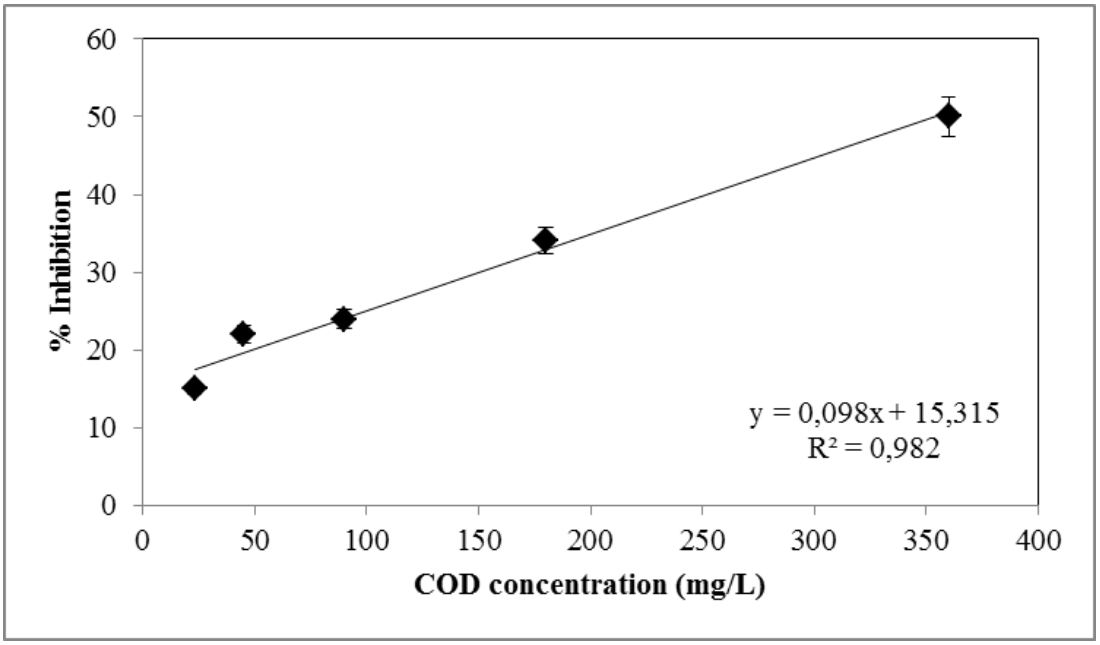

Figure 6.82 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a SRT of 5 days with 15 mg/L RD (EC_{50}= 354 mg/L, HRT=5 days)

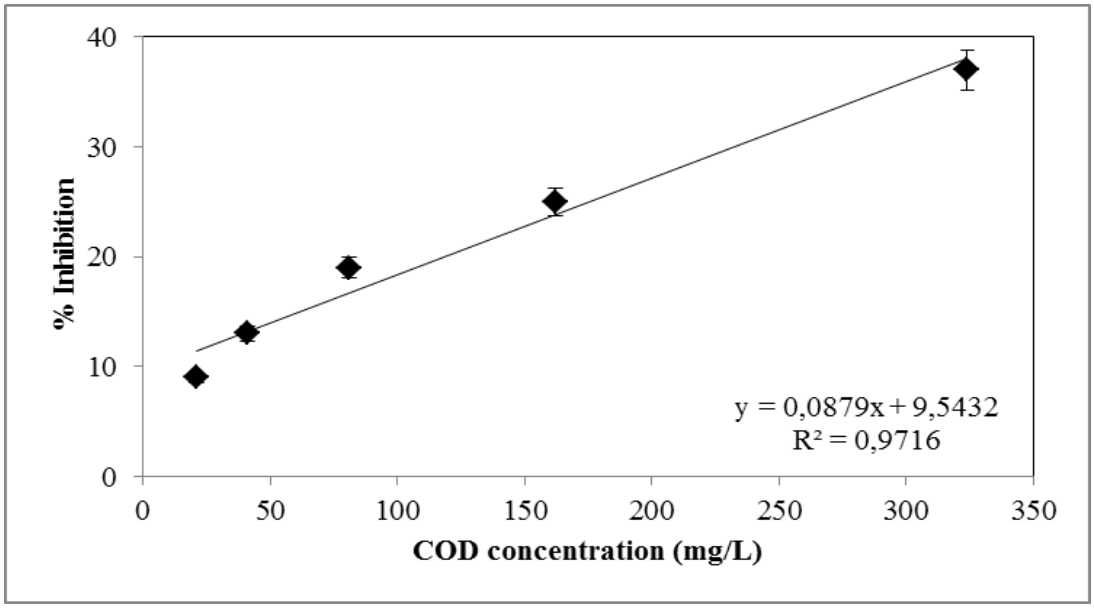

Figure 6.83 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a SRT of 10 days with 15 mg/L RD (EC_{50}= 460 mg/L, HRT=5 days)

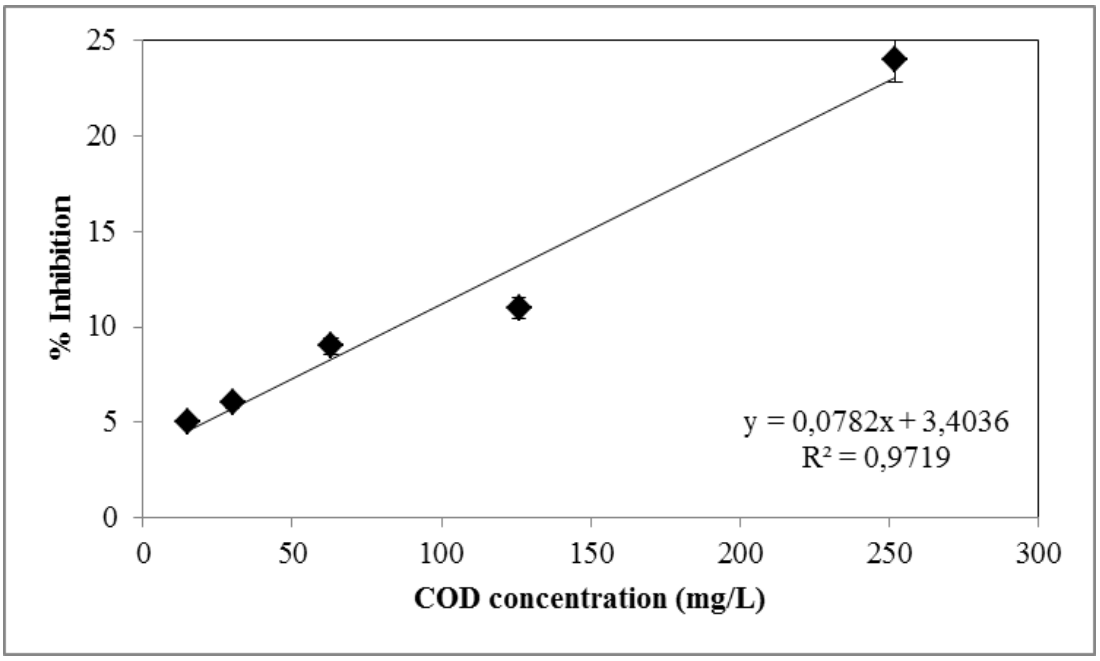

Figure 6.84 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a SRT of 25 days with 15 mg/L RD (EC_{50}= 596 mg/L, HRT=5 days)

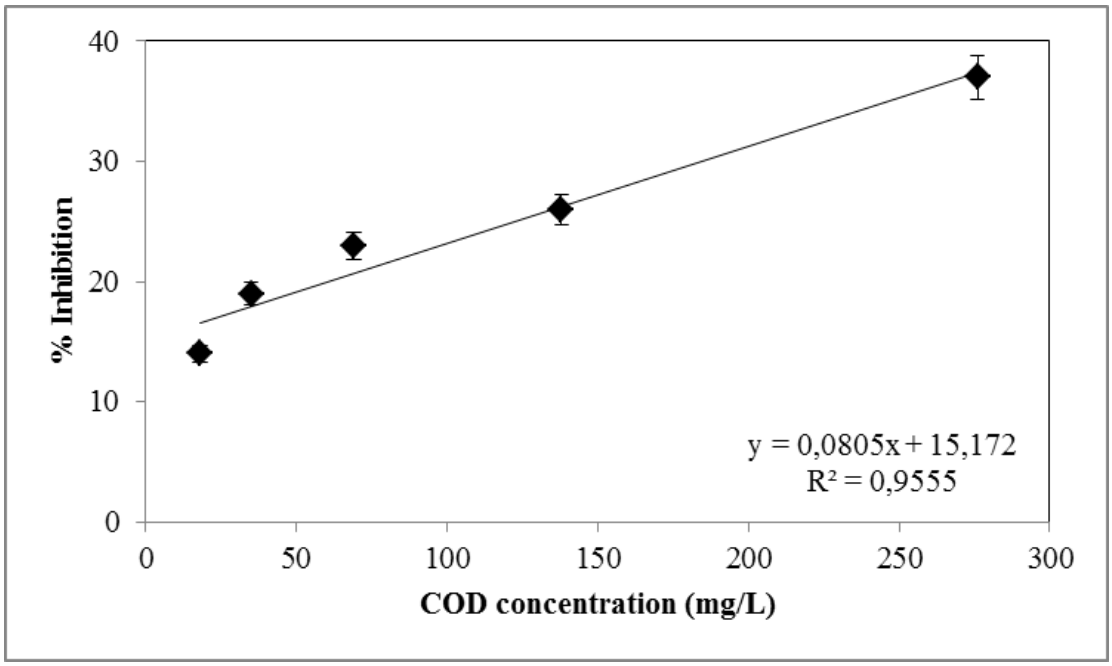

Figure 6.85 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a SRT of 40 days with 15 mg/L RD (EC_{50}= 433 mg/L, HRT=5 days)

Table 6.50 The EC_{50} values in the influent and effluent of the CSTR at 15 mg/L RD (COD_{dis}=1200 mg/L PAHs=65.32 ng/mL, HRT=5 days)

SRT (days)	Influent EC_{50} value (mg/L)	Effluent EC_{50} value (mg/L)	*Vibrio fischeri* acute toxicity removal (%)
5	100	354	72
10	100	460	78
25	100	596	83
40	100	433	77

6.6.1.3 Effect of Increasing HRTs on the Acute Toxicity Removal without Biosurfactant in the CSTR System

6.6.1.3.1 Effect of Increasing HRTs on the Daphnia magna Acute Toxicity without Biosurfactant in the CSTR System. The COD_{dis} and total PAHs concentrations were 2850 mg/L and 119.76 ng/mL in the influent of the raw petrochemical industry wastewater. The operational conditions for this study are summarized in Table 5.8 in the section Material and Methods. Figures 6.86-6.90 and Table 6.51 show the EC_{50} values in the influent and effluent of the CSTR at increasing HRTs. The EC_{50} value was obtained as 174 mg/L in the influent wastewater (Figure 6.86). As the HRTs were increased from 2.5 to 3.3 and to 5 days, the EC_{50} values increased from 675 to 770 and to 809 mg/L, respectively, in the effluent of the CSTR at a SRT of 25 days (Figures 6.87-6.89). The EC_{50} values decreased from 809 to 596 mg/L when the HRT was increased from 5 to 10 days (Figure 6.90). The acute toxicity reductions in the CSTR were 74%, 77%, 78% and 71% at HRTs 2.5, 3.3, 5 and 10 days, respectively. In this study it was found that the EC_{50} value increased from 174 to 675 mg/L from the influent of the petrochemical wastewater to the effluent of the CSTR system at a HRT of 2.5 days. As the HRTs were increased from 2.5 to 5 days, the EC_{50} values increased in effluent of the CSTR system. At this EC_{50} value (809 mg/L) the maximum acute toxicity removal (78%) was observed.

The petrochemical industry wastewater was not treated effectively at short HRTs since short HRTs did not provide enough contact time for bacteria and PAHs to metabolize the total PAHs in the CSTR system. Therefore, high acute toxicity was observed at high HRT such as 10 days. The acute toxicity was high at HRT > 5 days since the PAHs in the petrochemical industry wastewater accumulated in the CSTR and caused acute toxicity to the activated sludge bacteria at a HRT of 10 days. The continuous accumulation of PAHs could not be metabolized by the bacteria in the activated sludge resulting in high acute toxicity at a HRT of 10 days.

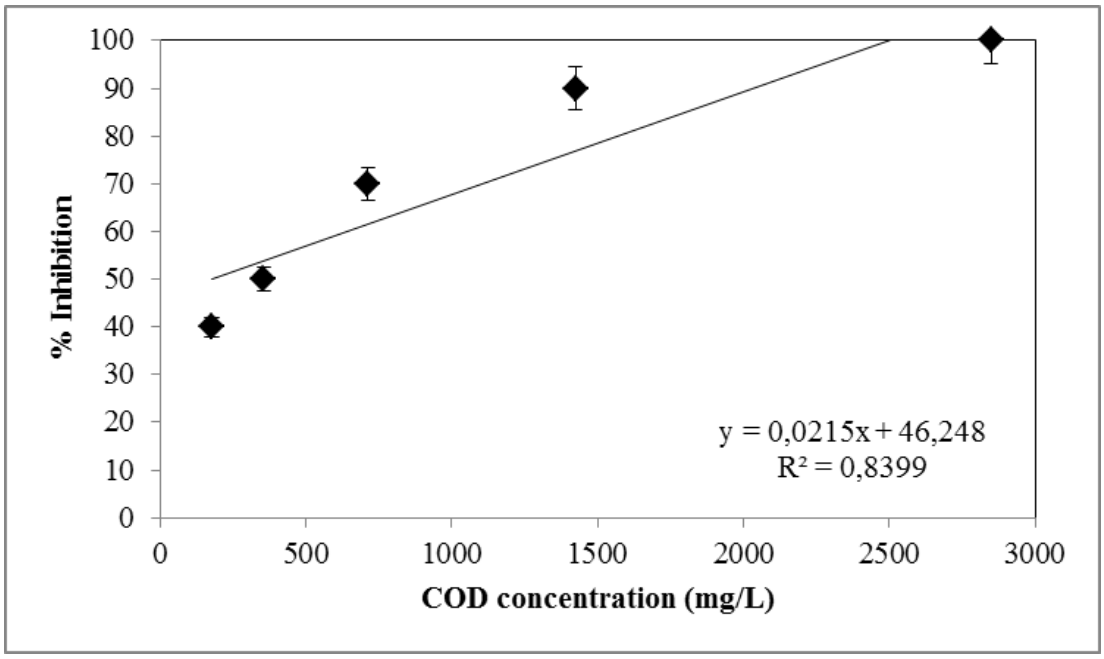

Figure 6.86 Variations of inhibition percentages versus COD_{dis} concentrations in the influent raw petrochemical wastewater (EC_{50}= 174 mg/L, SRT=25 days)

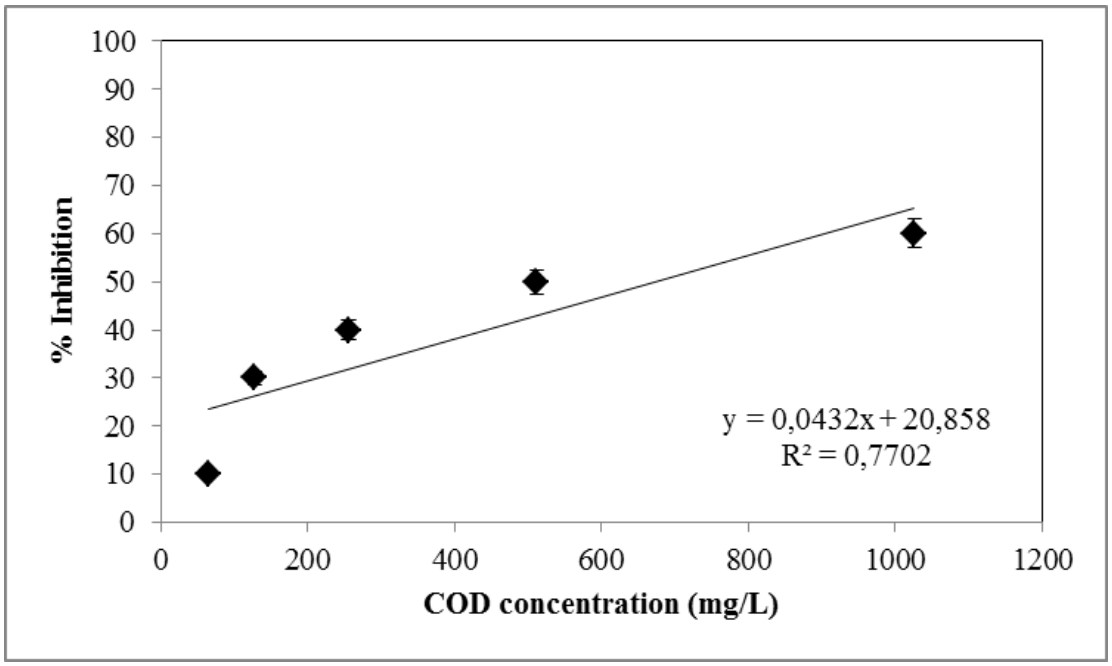

Figure 6.87 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a HRT of 2.5 days without RD (EC_{50}= 675 mg/L, SRT=25 days)

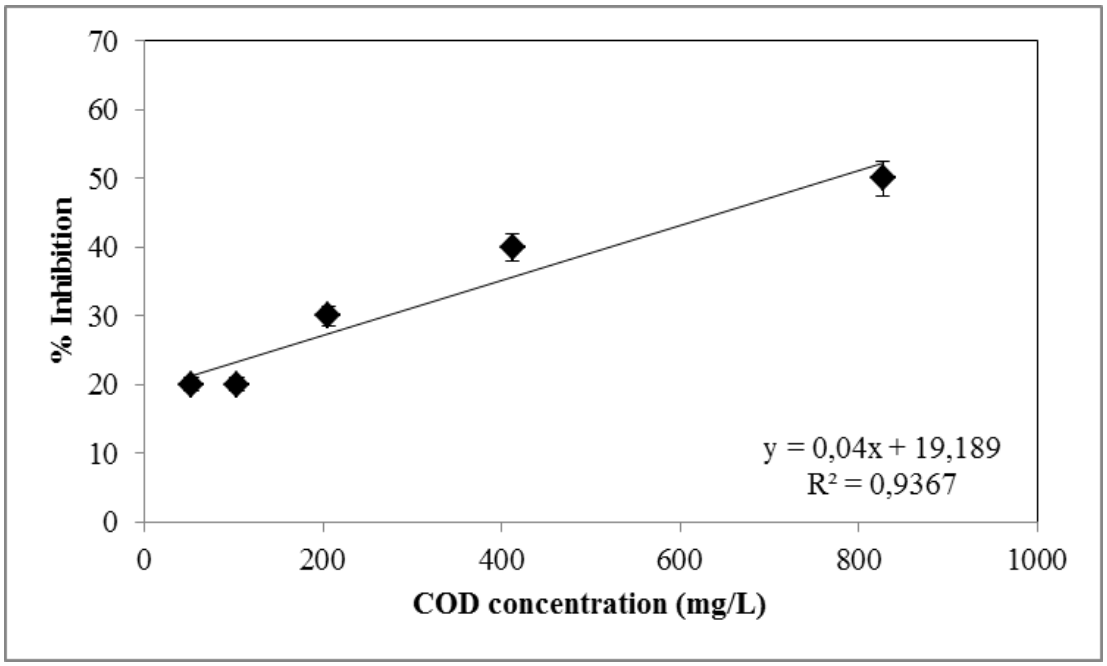

Figure 6.88 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a HRT of 3.3 days without RD (EC_{50}= 770 mg/L, SRT=25 days)

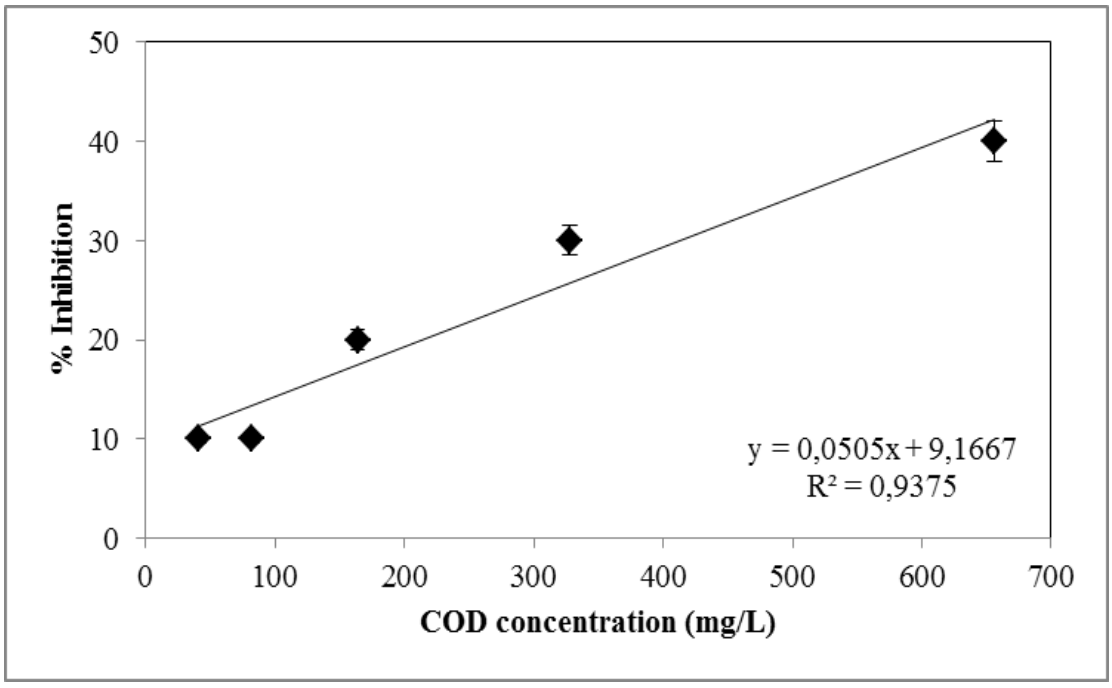

Figure 6.89 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a HRT of 5 days without RD (EC_{50}= 809 mg/L, SRT=25 days)

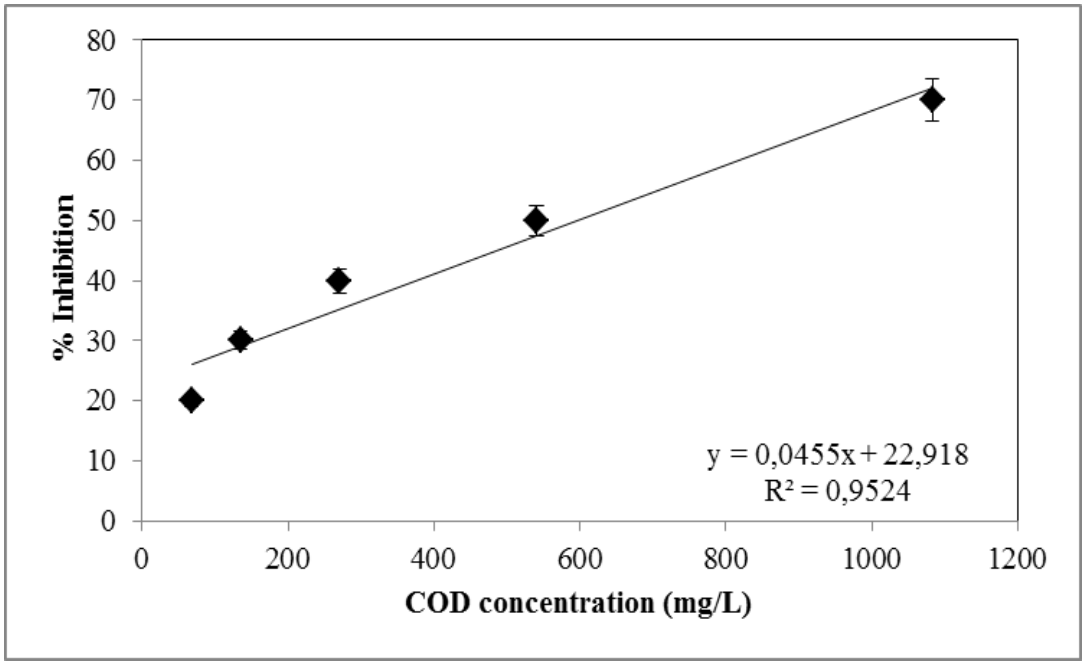

Figure 6.90 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a HRT of 10 days without RD (EC_{50}= 596 mg/L, SRT=25 days)

Table 6.51 The EC_{50} values in the influent and effluent of the CSTR without RD (COD_{dis}=2850 mg/L PAHs=119.76 ng/mL, HRT=5 days, SRT=25 days)

HRT (days)	Influent EC_{50} value (mg/L)	Effluent EC_{50} value (mg/L)	*Daphnia magna* acute toxicity removal (%)
2.5	174	675	74
3.3	174	770	77
5	174	809	78
10	174	596	71

6.6.1.3.2 Effect of Increasing HRTs on the Vibrio fischeri Acute Toxicity without Biosurfactant in the CSTR. The COD_{dis} and total PAHs concentrations were 2850 mg/L and 119.76 ng/mL in the influent wastewater. The operational conditions for this study are summarized in Table 5.8 in the section Material and Methods. The EC_{50} value was obtained as 140 mg/L in the influent wastewater (Figure 6.91). As the HRTs were increased from 2.5 to 3.3 and 5 days, the EC_{50} values increased from 428 to 464 and to 507 mg/L, respectively, in the effluent of CSTR (Figures 6.92-6.94). The EC_{50} values decreased from 507 to 393 mg/L when the HRT increased from 5 to 10 days (Figure 6.95). The *Vibrio fischeri* acute toxicity reductions in the CSTR were 67%, 70%, 72% and 64% at HRTs 2.5, 3.3, 5 and 10 days, respectively. In this study it was found that the EC_{50} value increased from 140 to 428 mg/L from the influent of the petrochemical wastewater to the effluent of the CSTR system at a HRT of 2.5 days. In this study it was found that as the HRTs were increased from 2.5 to 5 days, the EC_{50} values increased in the effluent of the CSTR system (Table 6.52). The maximum acute toxicity removal (72%) was observed at a HRT of 5 days.

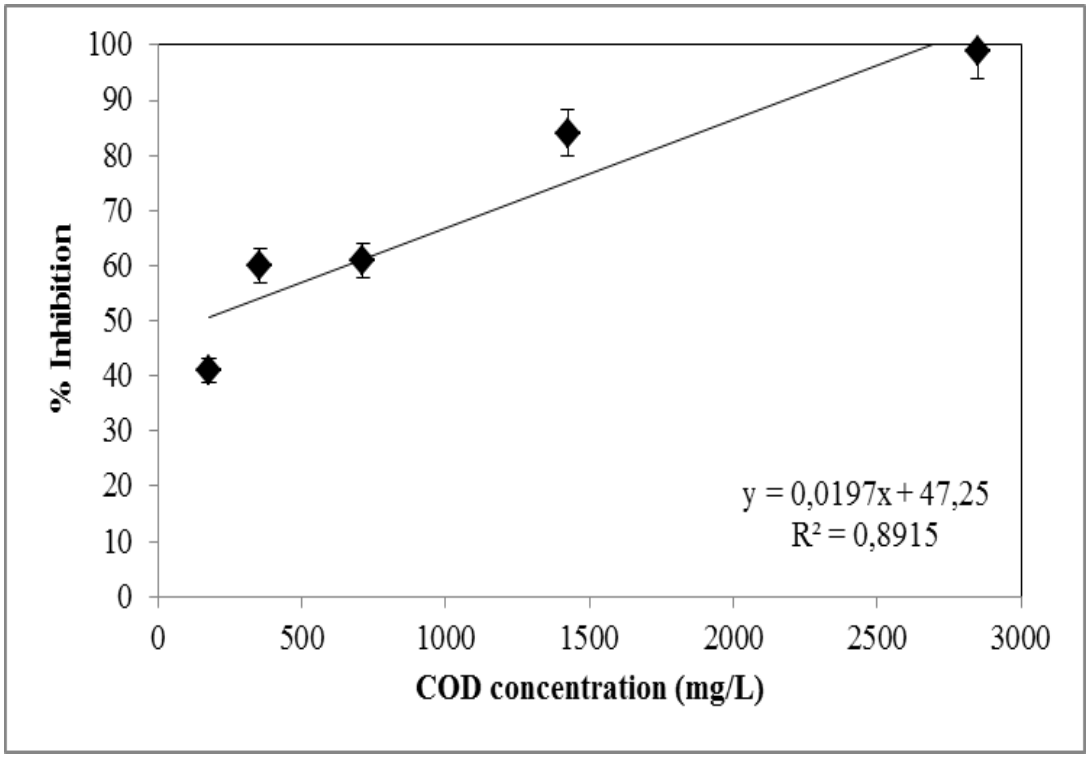

Figure 6.91 Variations of inhibition percentages versus COD_{dis} concentrations in the influent raw petrochemical wastewater (EC_{50}= 140 mg/L)

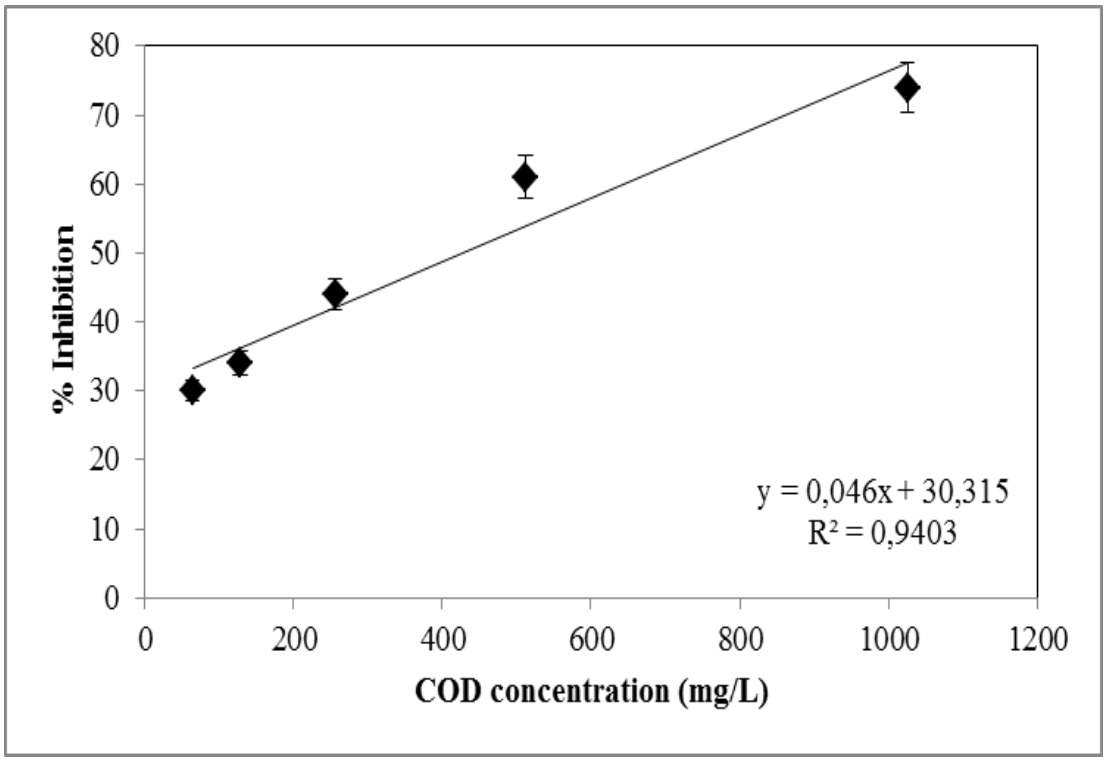

Figure 6.92 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a HRT of 2.5 days without RD (EC_{50}= 428 mg/L, SRT=25 days)

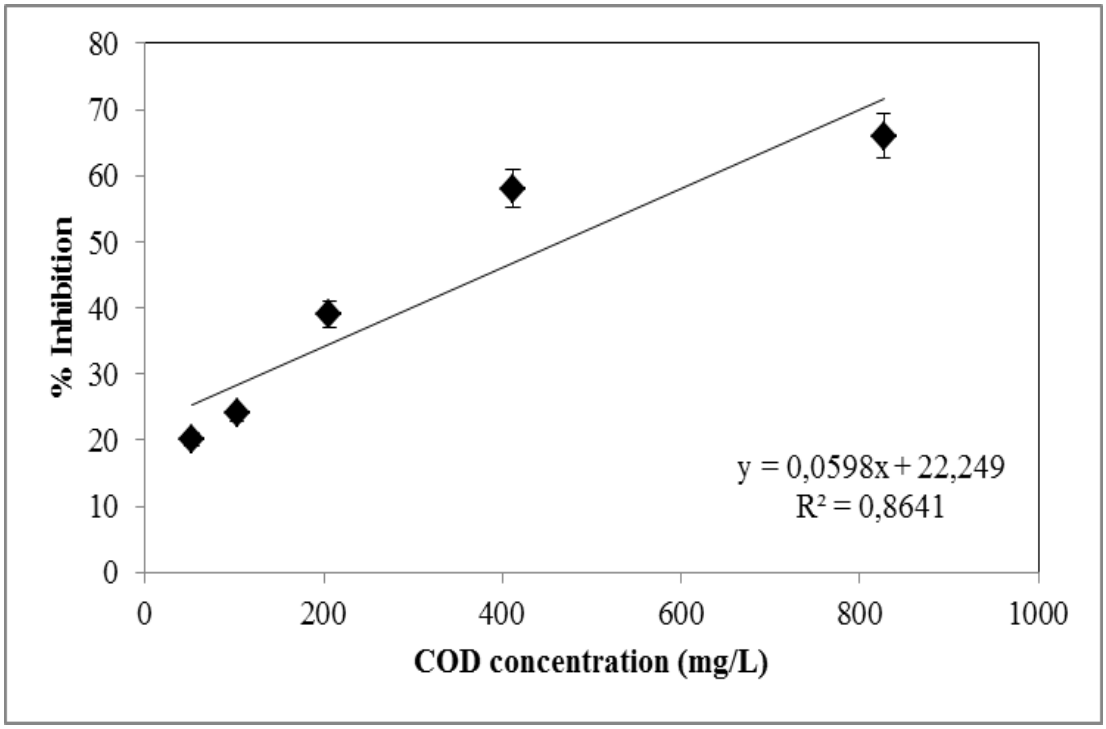

Figure 6.93 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a HRT of 3.3 days without RD (EC_{50}= 464 mg/L, SRT=25 days)

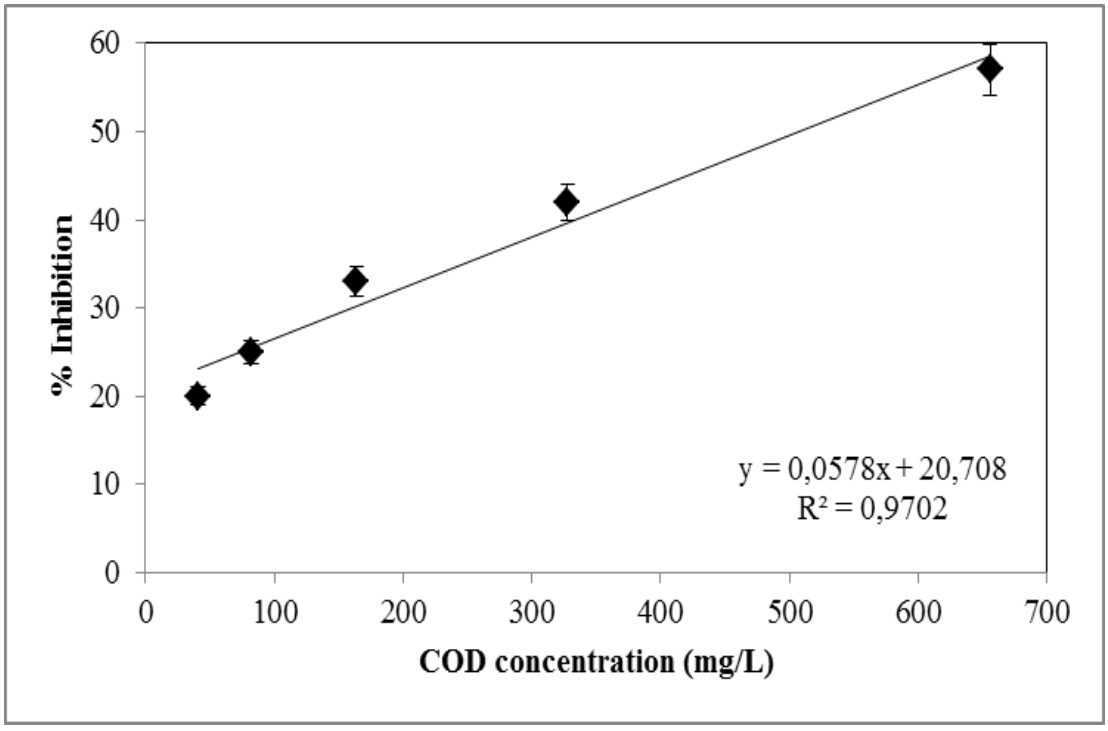

Figure 6.94 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a HRT of 5 days without RD (EC_{50}= 507 mg/L, SRT=25 days)

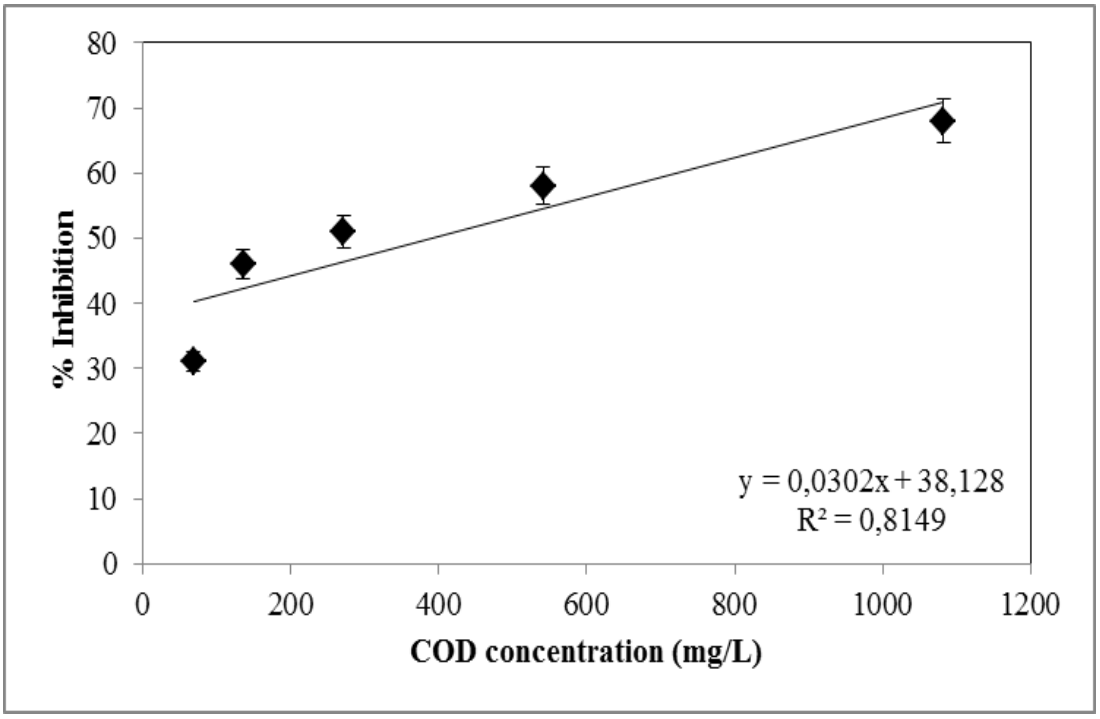

Figure 6.95 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a HRT of 10 days without RD (EC_{50}= 393 mg/L, SRT=25 days)

Table 6.52 EC_{50} values in the influent and effluent of the CSTR without RD (COD_{dis}=2850 mg/L PAHs=119.76 ng/mL, SRT=25 days)

HRT (days)	Influent EC_{50} value (mg/L)	Effluent EC_{50} value (mg/L)	*Vibrio fischeri* acute toxicity removal (%)
2.5	140	428	67
3.3	140	464	70
5	140	507	72
10	140	393	64

6.6.1.4 Effect of Increasing HRTs on the Acute Toxicity Removal with 15 mg/L RD in the CSTR

6.6.1.4.1 Effect of Increasing HRTs on the Daphnia magna Acute Toxicity at 15 mg/L RD in the CSTR System. Figures 6.96-6.99 and Table 6.53 show the *Daphnia magna* acute toxicity results in the aerobic CSTR effluent samples containing 15 mg/L RD at increasing HRTs from 2.5 days up to 10 days at a SRT of 25 days. The

operational conditions for this study are summarized in Table 5.8 in the section Material and Methods. The EC_{50} value was found as 174 mg/L in the influent wastewater. The EC_{50} values increased from 174 to 897, 1064 and to 1725 mg/L, at HRTs 2.5, 3.3 and 5 days, respectively, in the effluent of the CSTR (Figures 6.96-6.99). When the HRT was increased from 5 to 10 days, the EC_{50} values decreased from 1725 to 675 mg/L at 15 mg/L RD (Figure 6.99). The acute toxicity reductions in the CSTR were 81%, 84%, 90% and 74% at HRTs 2.5, 3.3, 5 and 10 days, respectively. As the HRTs were increased from 2.5 to 5 days, the EC_{50} values increased in the effluent of the CSTR system. The high EC_{50} value indicated the reducing acute toxicity. The maximum acute toxicity removal was 90% at a HRT of 5 days with 15 mg/L RD. This result show that the aerobic CSTR system decreased the acute toxicity of the influent wastewater and produced less toxic intermediate products under these conditions. The presence of 15 mg/L RD at optimum HRT (5 days) may stimulate the biodegradation of total PAHs to be taken up by the aerobic bacteria.

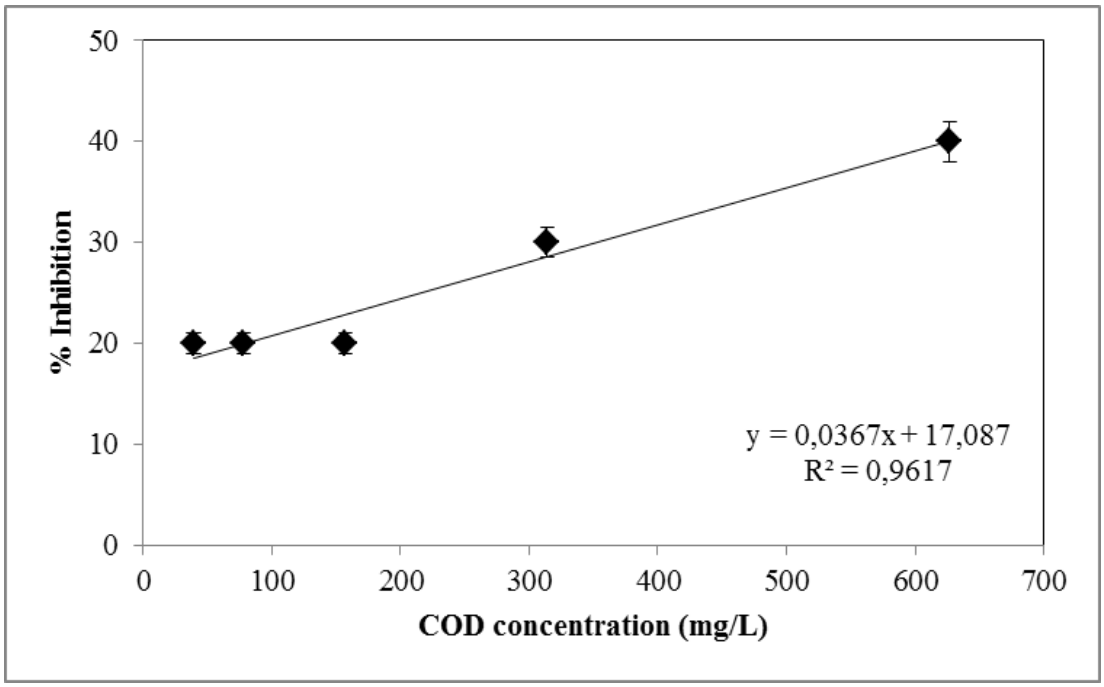

Figure 6.96 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a HRT of 2.5 days with 15 mg/L RD (EC_{50}= 897 mg/L, SRT=25 days)

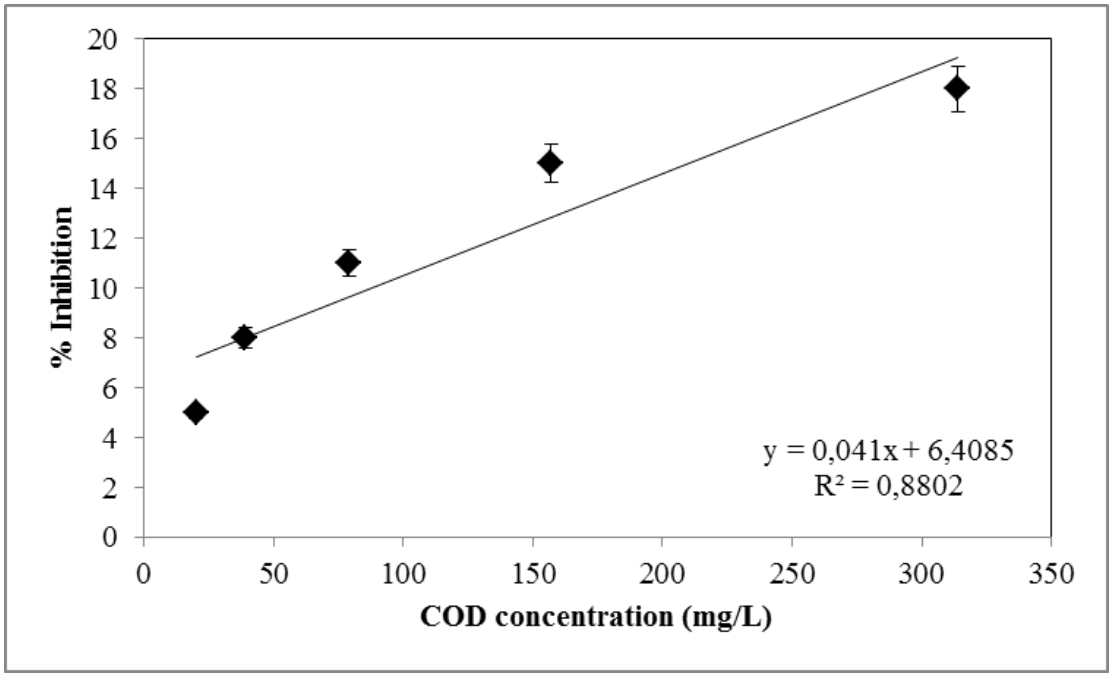

Figure 6.97 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a HRT of 3.3 days with 15 mg/L RD (EC_{50}= 1064 mg/L, SRT=25 days)

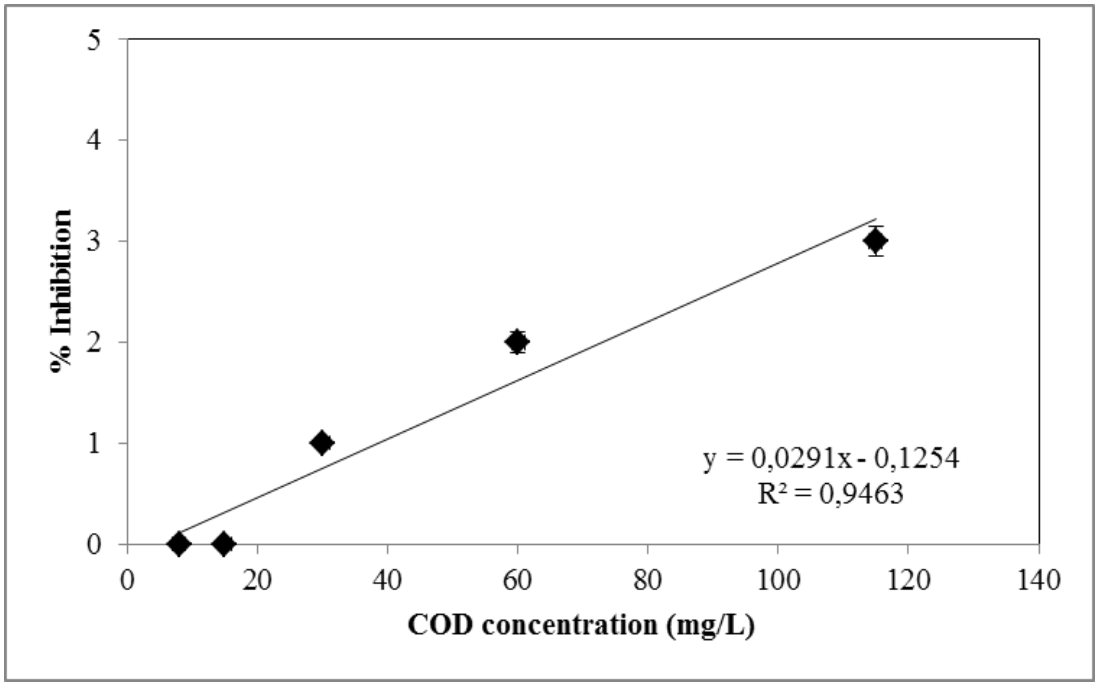

Figure 6.98 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a HRT of 5 days with 15 mg/L RD (EC_{50}= 1725 mg/L, SRT=25 days)

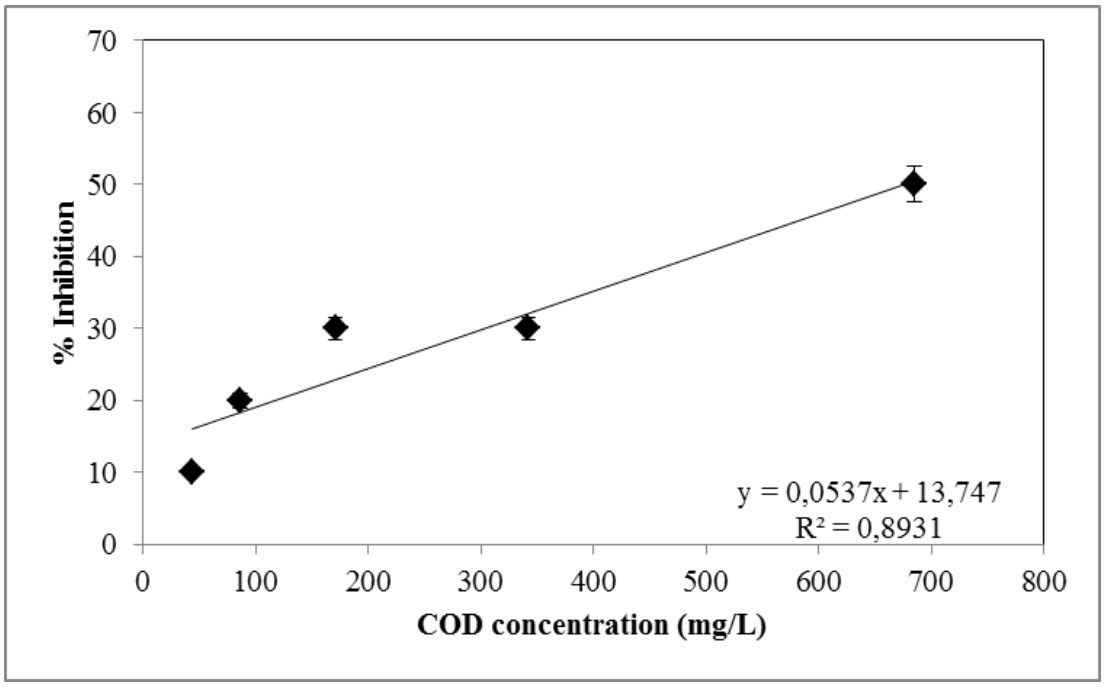

Figure 6.99 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a HRT of 10 days with 15 mg/L RD (EC_{50}= 675 mg/L, SRT=25 days)

Table 6.53 The EC_{50} values in the influent and effluent of the CSTR with 15 mg/L RD (COD_{dis}= 2850 mg/L PAHs =119.76 ng/mL, SRT=25 days)

HRT (days)	Influent EC_{50} value (mg/L)	Effluent EC_{50} value (mg/L)	*Daphnia magna* acute toxicity removal (%)
2.5	174	897	81
3.3	174	1064	84
5	174	1725	90
10	174	675	74

6.6.1.4.2 Effect of Increasing HRTs on the Vibrio fischeri Acute Toxicity at 15 mg/L RD in the CSTR System. Figures 6.100-6.103 and Table 6.54 show the *Vibrio fischeri* acute toxicity results in the aerobic CSTR effluent samples containing 15 mg/L RD at increasing HRTs from 2.5 days up to 10 days at a SRT of 25 days. The operational conditions for this study are summarized in Table 5.8 in the section Material and Methods. The EC_{50} value was found as 140 mg/L in the influent wastewater. The EC_{50} values increased from 542 to 595 and to 663 mg/L, as the

HRTs were increased from 2.5 to 3.3 and 5 days, respectively in the effluent of the CSTR (Figures 6.100-6.102). When the HRT was increased from 5 to 10 days, the EC_{50} values decreased from 663 to 442 mg/L at 15 mg/L RD (Figure 6.103). The acute toxicity reductions in the CSTR were 74%, 76%, 79% and 68% at HRTs 2.5, 3.3, 5 and 10 days, respectively.

The EC_{50} value increased from 140 to 542 mg/L from the influent of the petrochemical industry wastewater to the effluent of CSTR system. As the HRTs were increased from 2.5 to 5 days, the EC_{50} values increased in the effluent of the CSTR system. The maximum acute toxicity removal was 79% at a HRT of 5 days in the CSTR. In this study it was found that the optimum HRT was 5 days for the highest EC_{50} value at 15 mg/L RD.

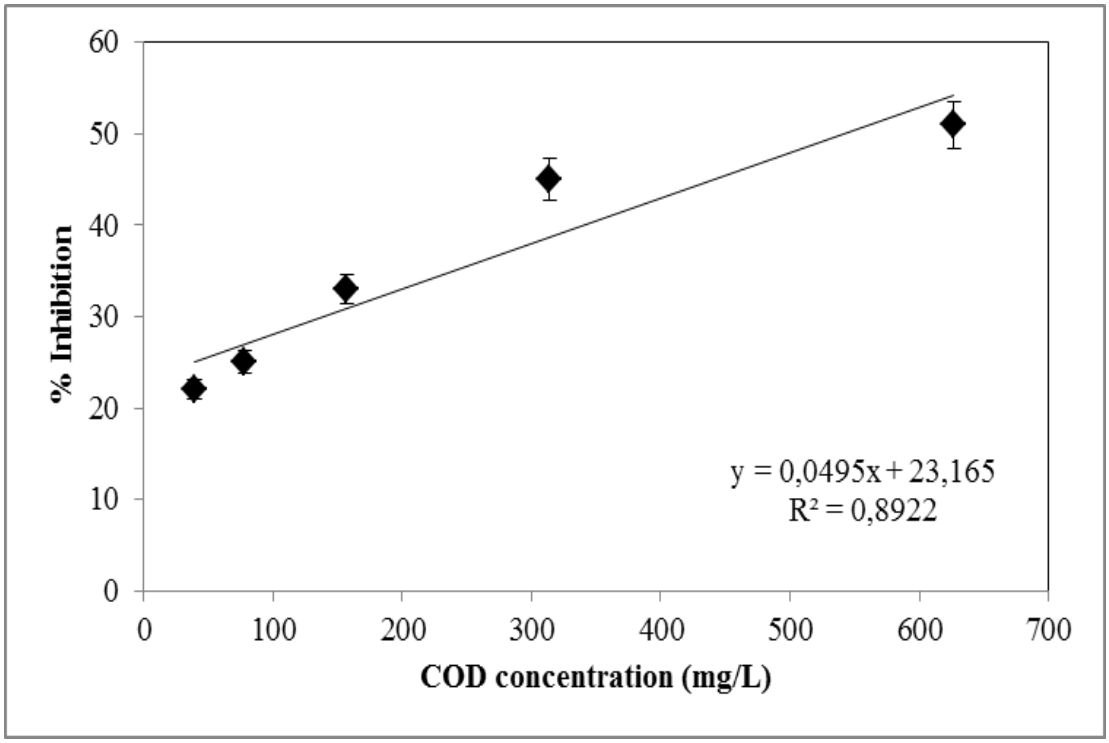

Figure 6.100 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a HRT of 2.5 days with 15 mg/L RD (EC_{50}= 542 mg/L, SRT=25 days)

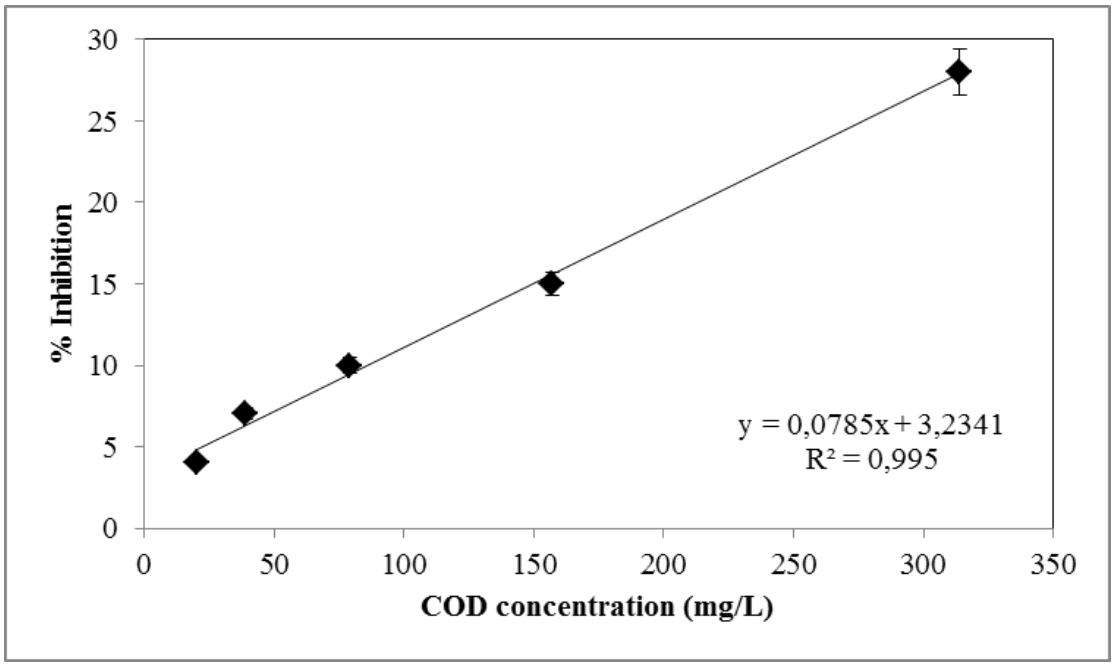

Figure 6.101 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a HRT of 3.3 days with 15 mg/L RD (EC_{50}= 595 mg/L, SRT=25 days)

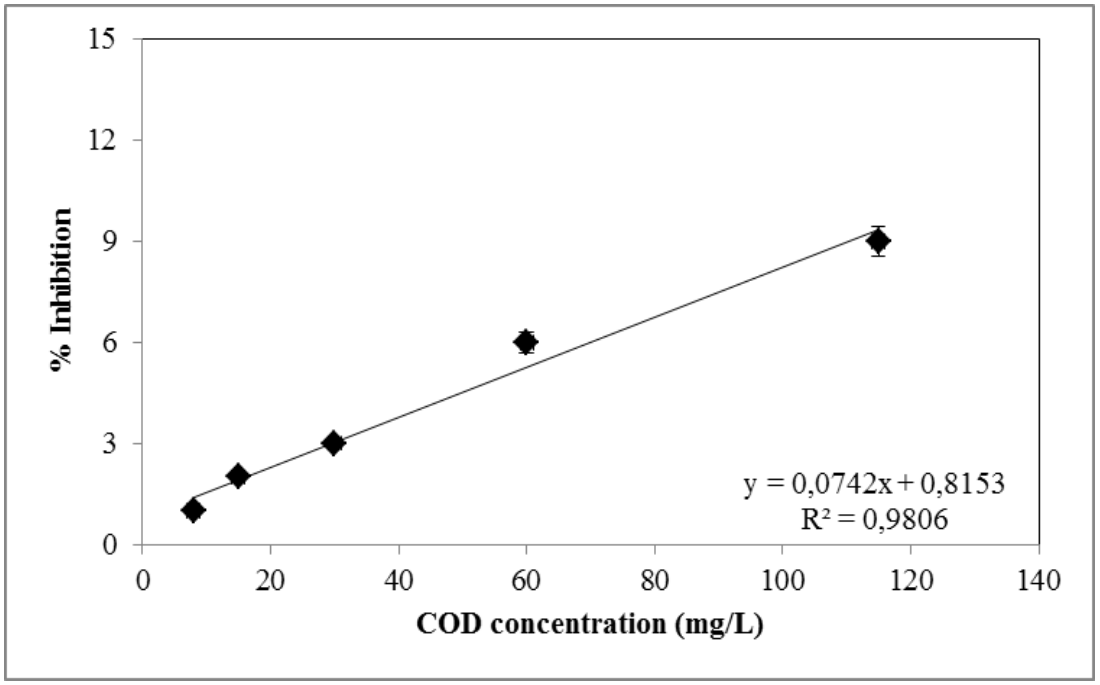

Figure 6.102 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a HRT of 25 days with 15 mg/L RD (EC_{50}= 663 mg/L, SRT=25 days)

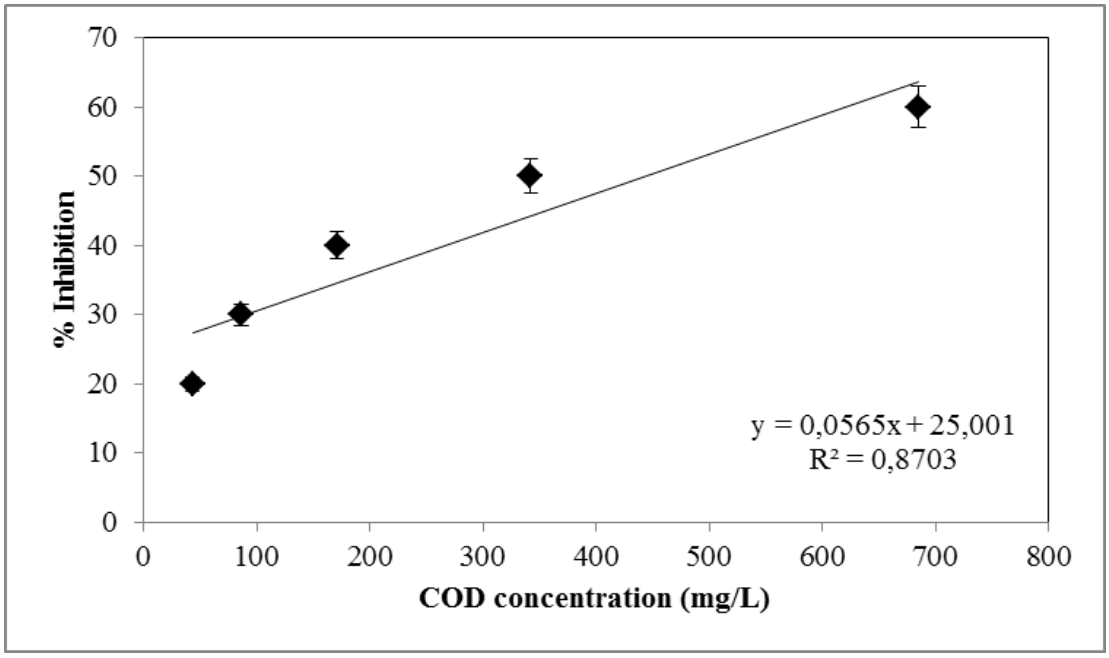

Figure 6.103 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of aerobic CSTR system at a HRT of 40 days with 15 mg/L RD (EC_{50}= 442 mg/L, SRT=25 days)

Table 6.54 The EC_{50} values in the influent and effluent of the CSTR at 15 mg/L RD (COD_{dis}=2850 mg/L PAHs=119.76 ng/mL, SRT=25 days)

HRT (days)	Influent EC_{50} value (mg/L)	Effluent EC_{50} value (mg/L)	*Vibrio fischeri* acute toxicity removal (%)
2.5	140	542	74
3.3	140	595	76
5	140	663	79
10	140	442	68

6.6.2 Acute Toxicity Evaluations in the Petrochemical Wastewater in the Anaerobic ITBR System

6.6.2.1 Effect of Increasing RD on the Acute Toxicity in the Anaerobic ITBR System

6.6.2.1.1 Effect of Increasing RD on the Daphnia magna Acute Toxicity in the Anaerobic ITBR System. In this step, the effects of increasing RD concentrations (0-15-50-75-100 and 150 mg/L) on the removals of acute toxicity in the petrochemical industry wastewater were investigated in the anaerobic ITBR system. In order to determine the optimum RD dose for the maximum removals of acute toxicity increasing RD concentrations were administered to the feed of the ITBR system.

The EC_{50} value was obtained as 174 mg/L in the influent wastewater (Figure 6.104). As the RD concentration were increased from 0 to 15, 50 and to 75 mg/L, the EC_{50} values increased from 768 to 973, 1038 and to 3181 mg/L, respectively, in the effluent of the anaerobic ITBR at a HRT of 2.75 days and SRT of 63 days (Figures 6.105-6.108). The EC_{50} values decreased from 3181 to 1182 and to 890 mg/L when the RD concentrations were increased from 75 to 100 and 150 mg/L, respectively (Figures 6.109-6.110). The *Daphnia magna* acute toxicity removals in the anaerobic ITBR were 77%, 82%, 83%, 95%, 85% and 80% at non-added, 15, 50, 75, 100 and 150 mg/L RD, respectively. In this study it was found that the *Daphnia magna* acute toxicity test results in the anaerobic ITBR system showed that EC_{50} values increased from 174 to 3181 at 75 mg/L RD and acute toxicity removal efficiency was 95%. As the RD concentrations were increased from 15 to 75 mg/L, the EC_{50} values increased in effluent of the ITBR system (Table 6.55). The high EC_{50} values indicated the reducing acute toxicity. The high EC_{50} values indicate the resistance of *Daphnia magna* to the real petrochemical industry wastewater at 75 mg/L RD. At this EC_{50} value (3181 mg/L), the maximum acute toxicity removal (95%) was observed. Among the rhamnolipid concentrations used it was found that 75 mg/L was the optimum RD concentration for acute toxicity removal in an anaerobic ITBR system. As the RD concentrations were increased from 75 to 100 and to 150 mg/L days, the

acute toxicity removal decreased from 95% to 85% and to 80% in the anaerobic system. The further addition of the RD concentration (100 and 150 mg/L) could have detrimental effects on anaerobic bacteria in the anaerobic ITBR system. This may be due to the toxic effect of the high RD concentrations on the biomass and due to the low rate of mass transport between biosurfactant micelles and bacteria in the aqueous phase of the anaerobic ITBR.

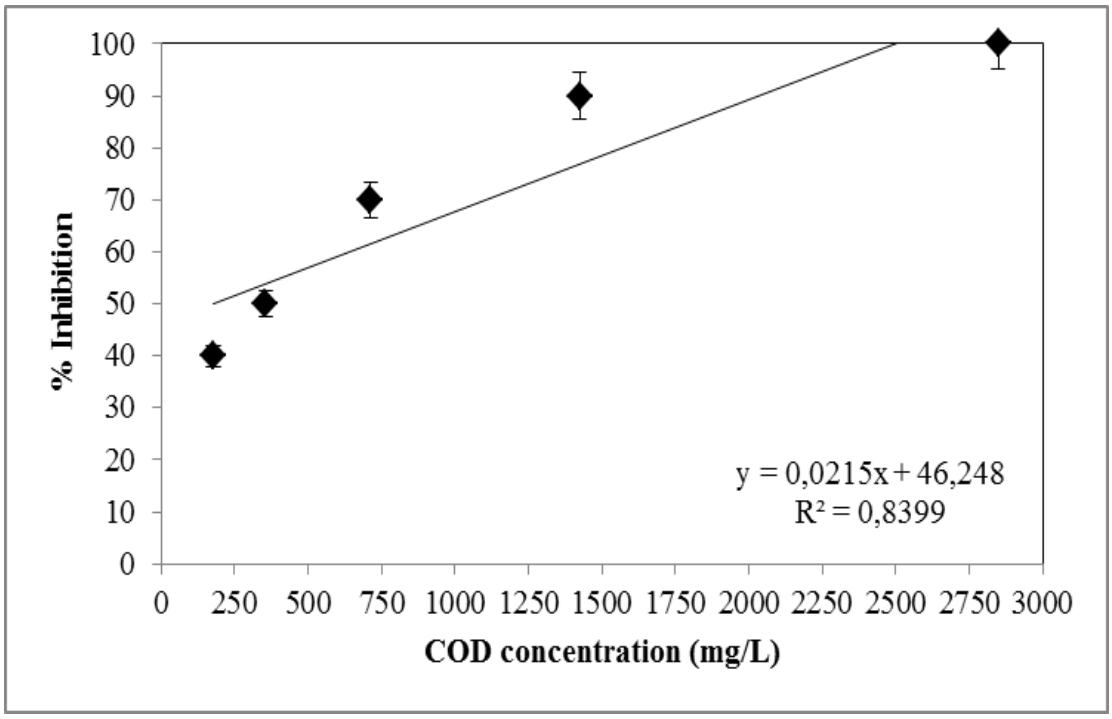

Figure 6.104 Variations of inhibition percentages versus COD_{dis} concentrations in the influent raw petrochemical wastewater (EC_{50}= 174 mg/L, SRT=63 days, HRT= 2.75 days)

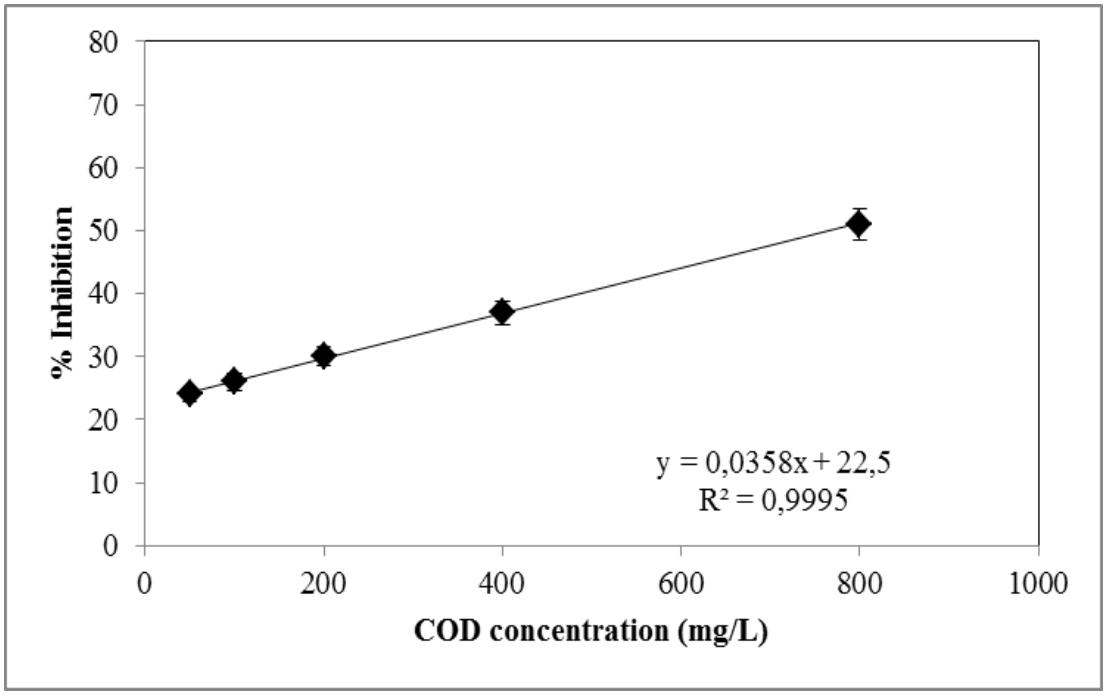

Figure 6.105 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system without RD (EC_{50}= 768 mg/L, SRT=63 days, HRT= 2.75 days)

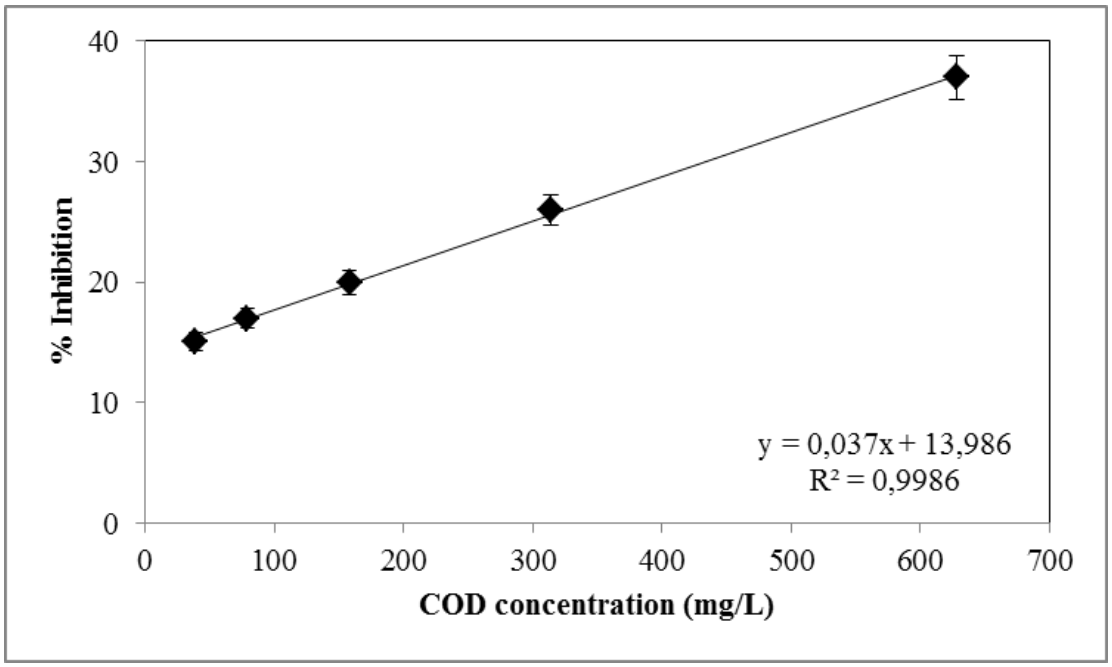

Figure 6.106 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system with containing 15 mg/L RD (EC_{50}= 973 mg/L, SRT=63 days, HRT= 2.75 days)

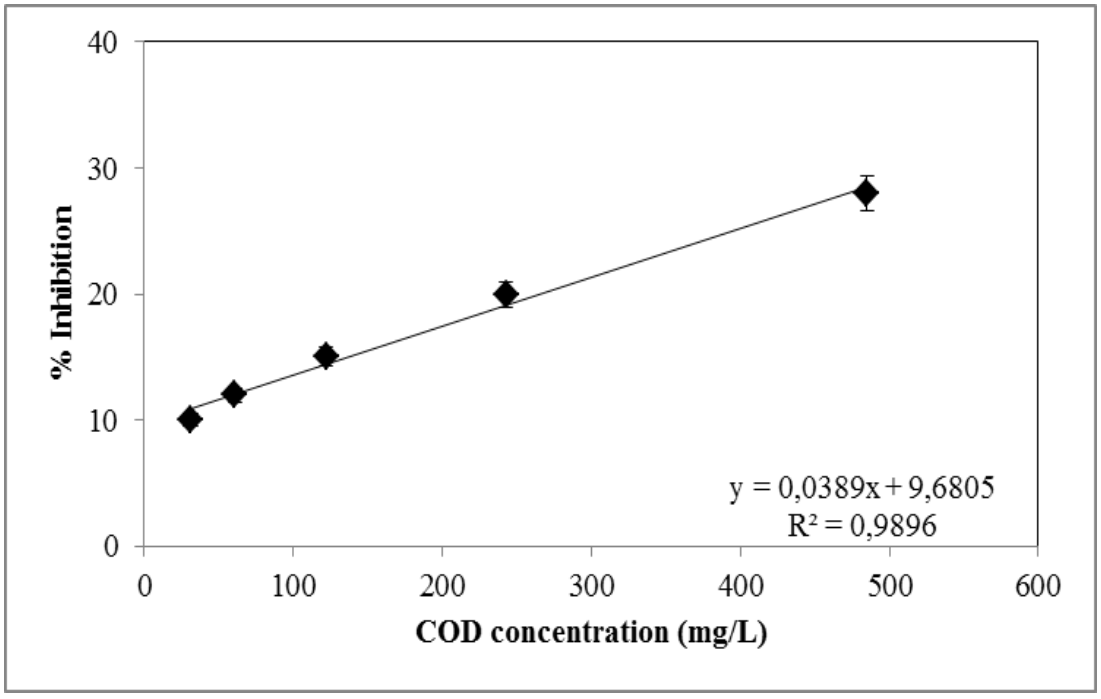

Figure 6.107 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system with containing 50 mg/L RD (EC_{50}= 1038 mg/L, SRT=63 days, HRT= 2.75 days)

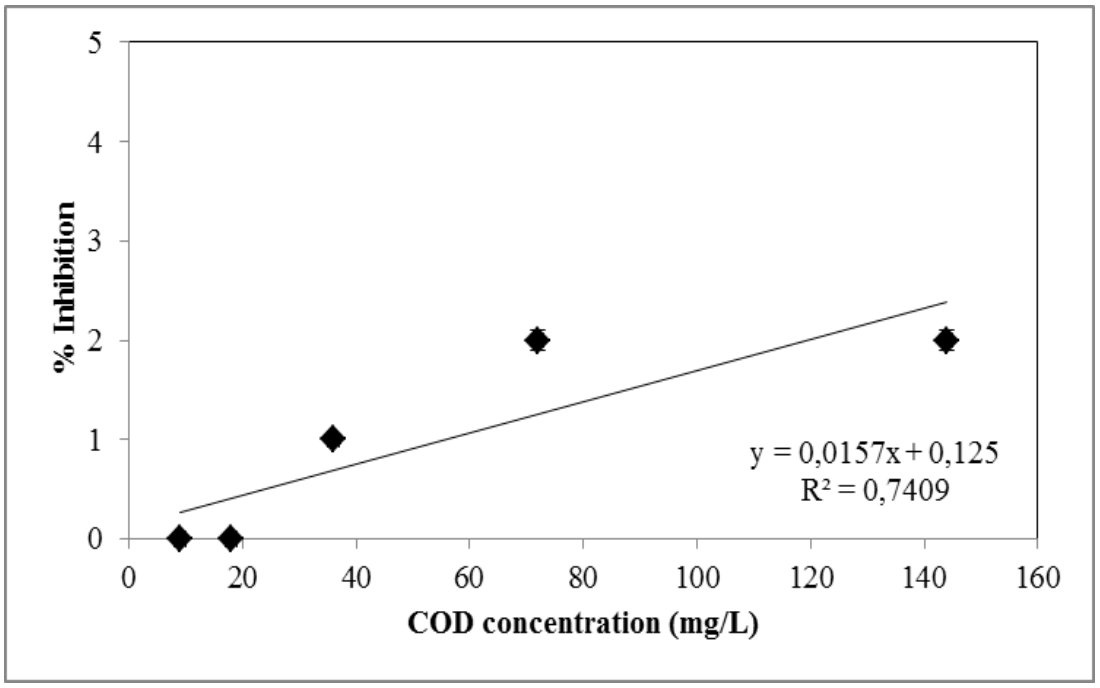

Figure 6.108 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system with containing 75 mg/L RD (EC_{50}= 3181 mg/L, SRT=63 days, HRT= 2.75 days)

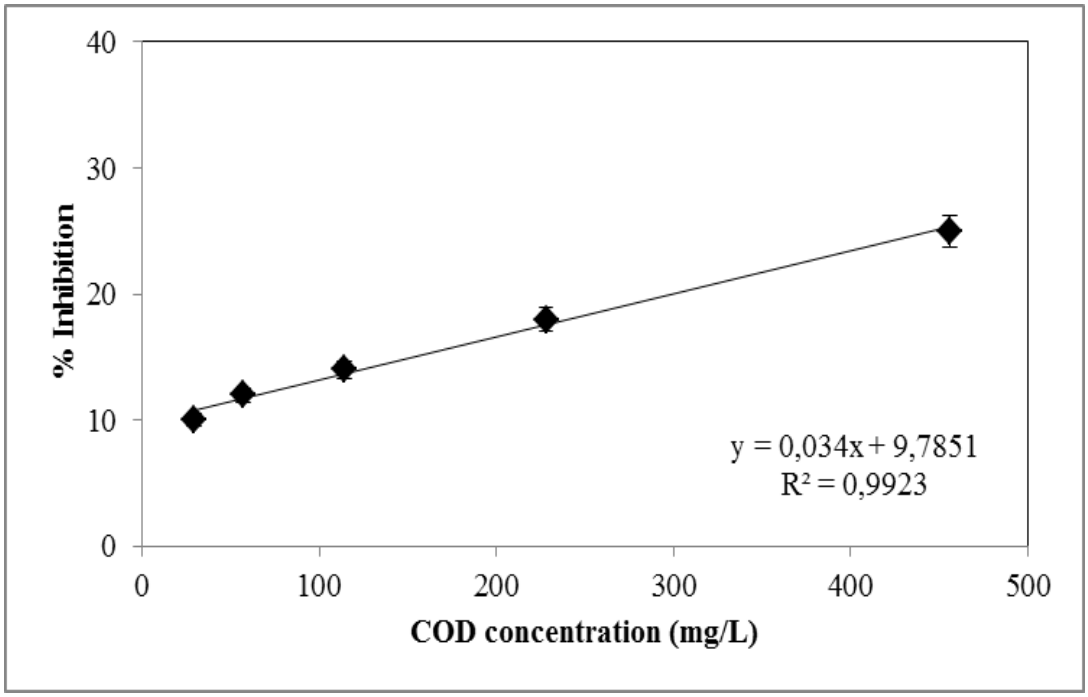

Figure 6.109 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system with containing 100 mg/L RD (EC_{50}= 1182 mg/L, SRT=63 days, HRT= 2.75 days)

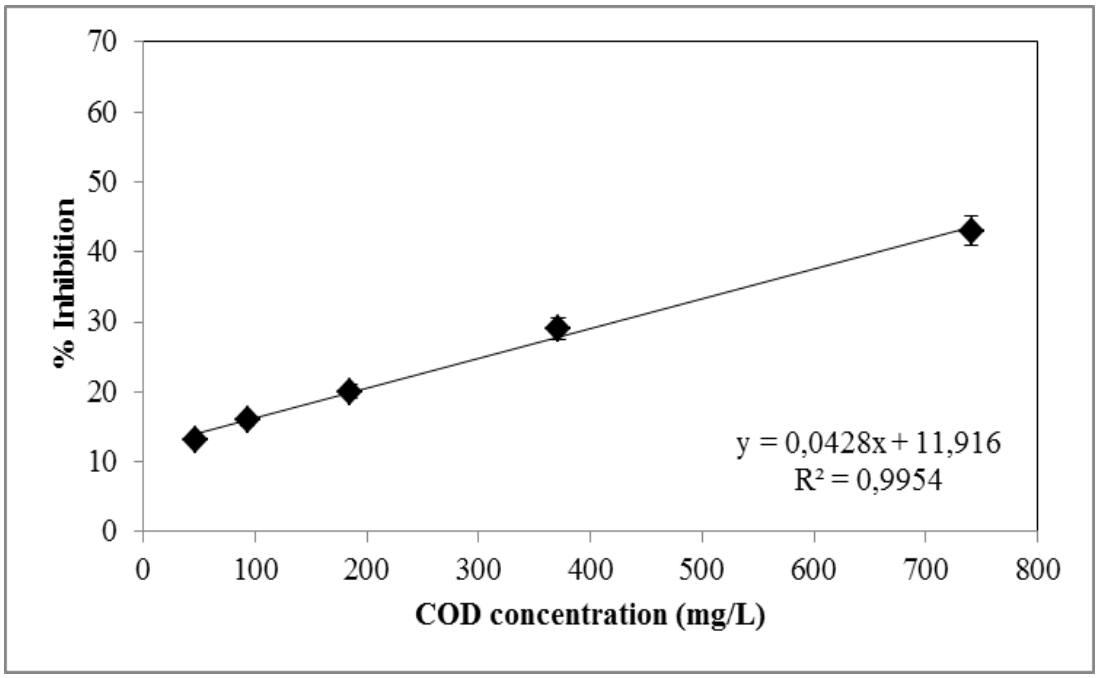

Figure 6.110 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system with containing 150 mg/L RD (EC_{50}= 890 mg/L, SRT=63 days, HRT= 2.75 days)

Table 6.55 The EC_{50} values in the influent and effluent of the anaerobic ITBR at increasing RD (COD_{dis}=2850 mg/L PAHs=292 ng/mL, SRT=63 days, HRT= 2.75 days)

RD concentration (mg/L)	Influent EC_{50} value (mg/L)	Effluent EC_{50} value (mg/L)	*Daphnia magna* acute toxicity removal (%)
0	174	768	77
15	174	973	82
50	174	1038	83
75	174	3181	95
100	174	1182	85
150	174	890	80

6.6.2.1.2 Effect of Increasing RD on the Vibrio fischeri Acute Toxicity in the Anaerobic ITBR System. Figures 6.111-6.117 and Table 6.56 show the EC_{50} values in the influent and effluent of the anaerobic ITBR at increasing RD. The COD_{dis} and total PAHs concentrations were 2850 mg/L and 292 ng/mL in the influent of the raw petrochemical industry wastewater. The anaerobic ITBR system was operated at a HRT of 2.75 days and SRT of 63 days. The operational conditions for this study are summarized in Table 5.10 in the section Material and Methods. The EC_{50} value was obtained as 140 mg/L in the influent wastewater (Figure 6.111). As the RD concentrations were increased from 15 to 50 and 75 mg/L, the EC_{50} values increased from 504 to 615 and to 918 mg/L, respectively, in the effluent of the anaerobic ITBR (Figures 6.112-6.115). The EC_{50} values decreased from 918 to 631 and to 448 mg/L when the RD concentrations were increased from 75 to 100 and to 150 mg/L (Figures 6.116-6.117). The acute toxicity removals in the anaerobic ITBR were 65%, 72%, 77%, 85%, 78% and 69% at RD of without RD, 15, 50, 75, 100 and 150 mg/L, respectively. In this study it was found that the EC_{50} value increased from 140 to 400 mg/L from the influent of the petrochemical wastewater to the effluent of the ITBR system at 15 mg/L RD. As the RD concentrations were increased from 15 to 75 days,

the EC_{50} values increased in the effluent of the anaerobic ITBR system. The maximum acute toxicity removal (85%) was observed at this RD concentration.

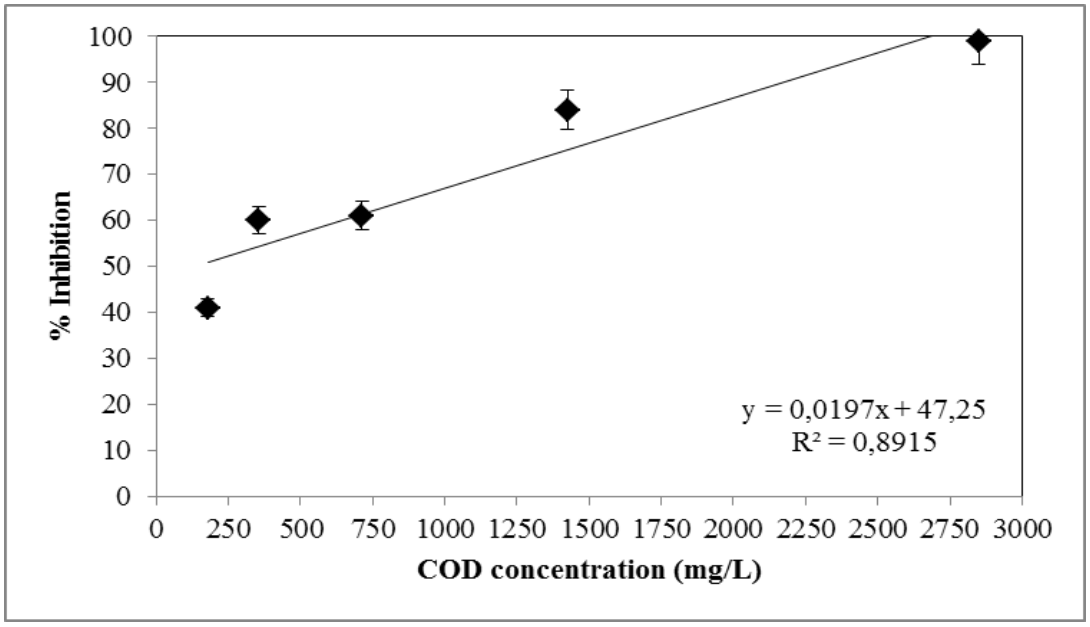

Figure 6.111 Variations of inhibition percentages versus COD_{dis} concentrations in the influent of the raw petrochemical wastewater (EC_{50}= 140 mg/L SRT=63 days, HRT= 2.75 days)

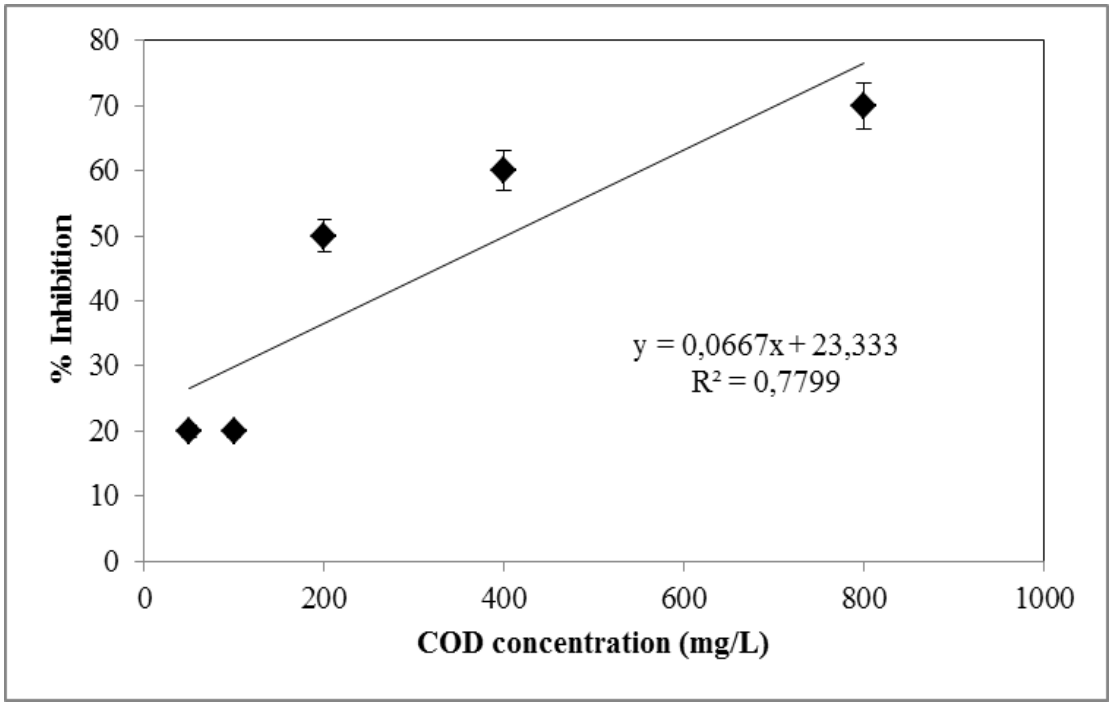

Figure 6.112 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of the anaerobic ITBR system without RD (EC_{50}= 400 mg/L SRT=63 days, HRT= 2.75 days)

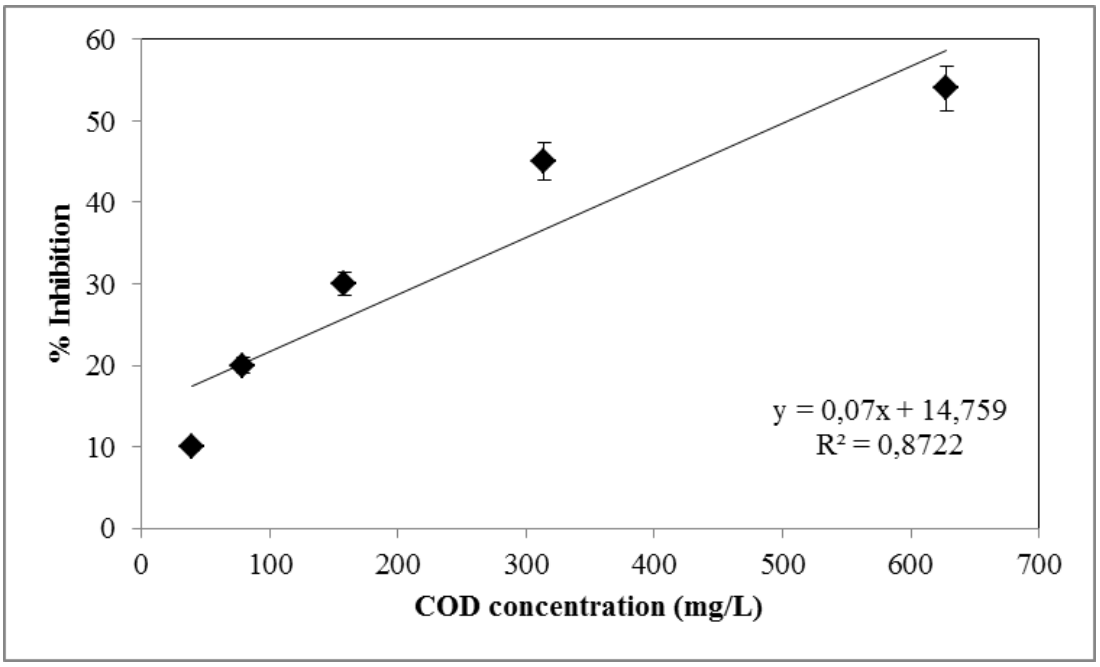

Figure 6.113 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of the anaerobic ITBR system with containing 15 mg/L RD (EC_{50}= 504 mg/L SRT=63 days, HRT= 2.75 days)

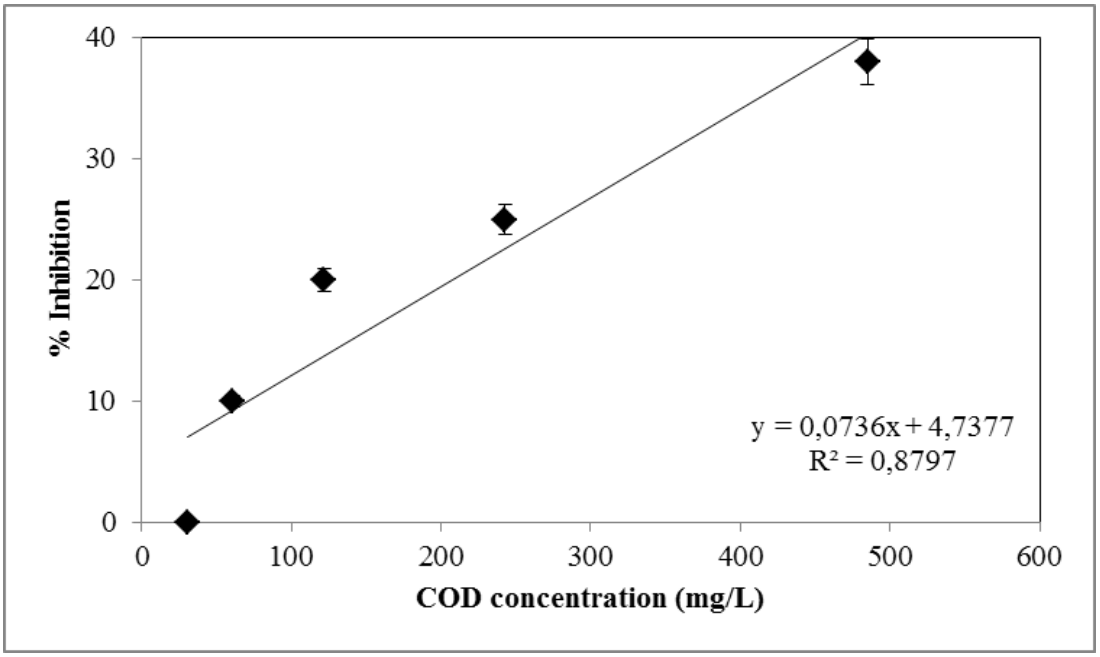

Figure 6.114 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of the anaerobic ITBR system with containing 50 mg/L RD (EC_{50}= 615 mg/L SRT=63 days, HRT= 2.75 days)

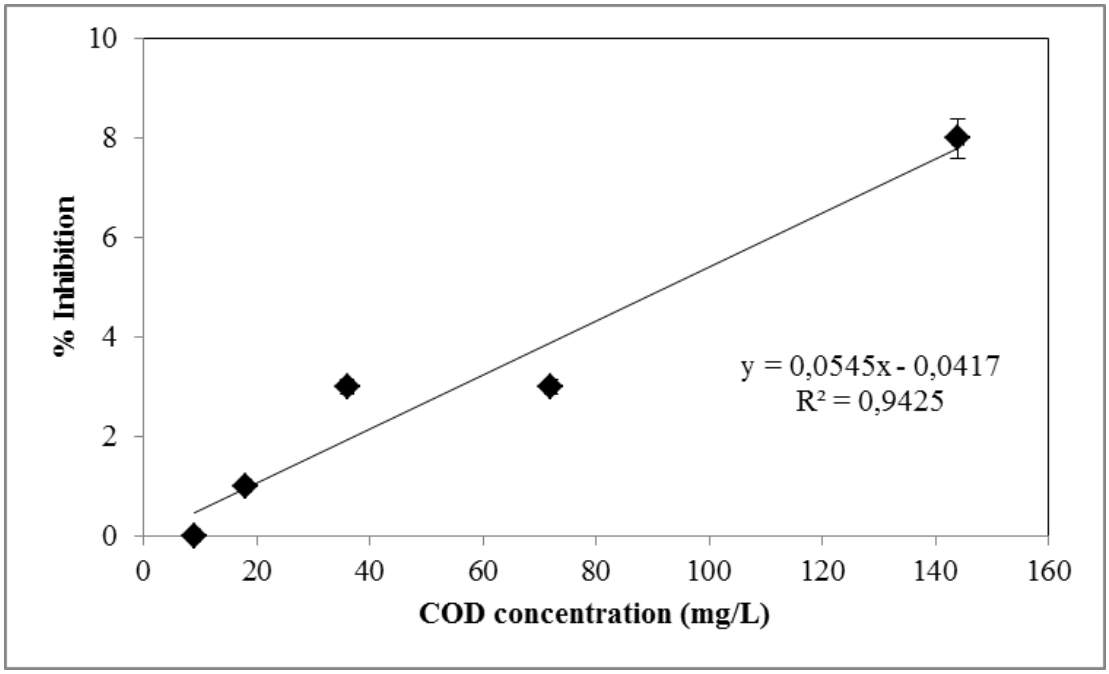

Figure 6.115 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of the anaerobic ITBR system with containing 75 mg/L RD (EC_{50}= 918 mg/L SRT=63 days, HRT= 2.75 days)

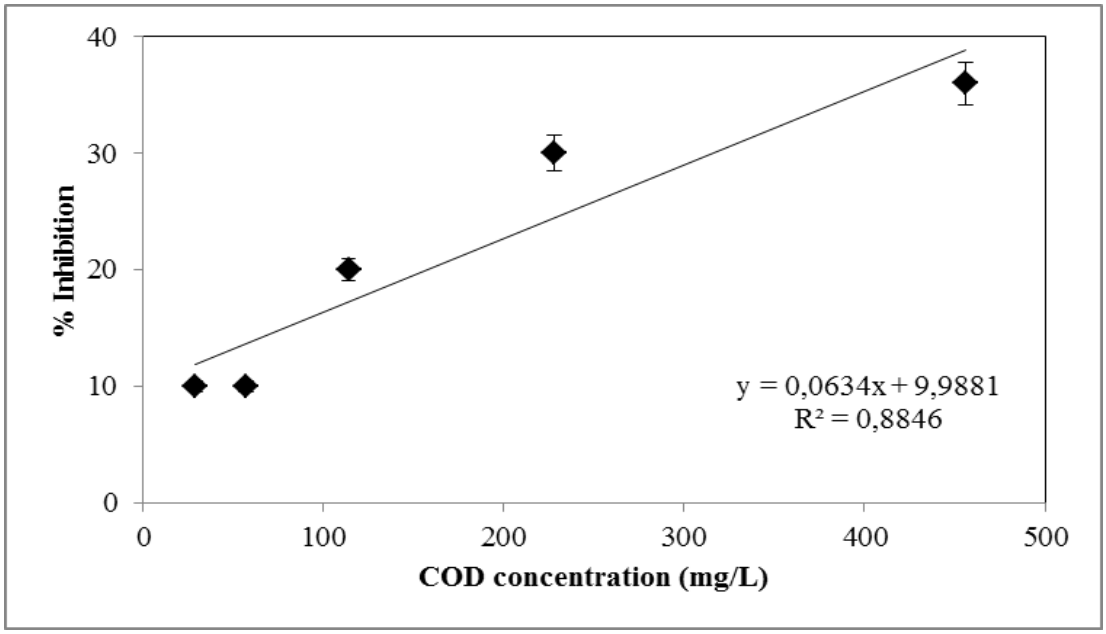

Figure 6.116 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system with containing 100 mg/L RD (EC_{50}= 631 mg/L SRT=63 days, HRT= 2.75 days)

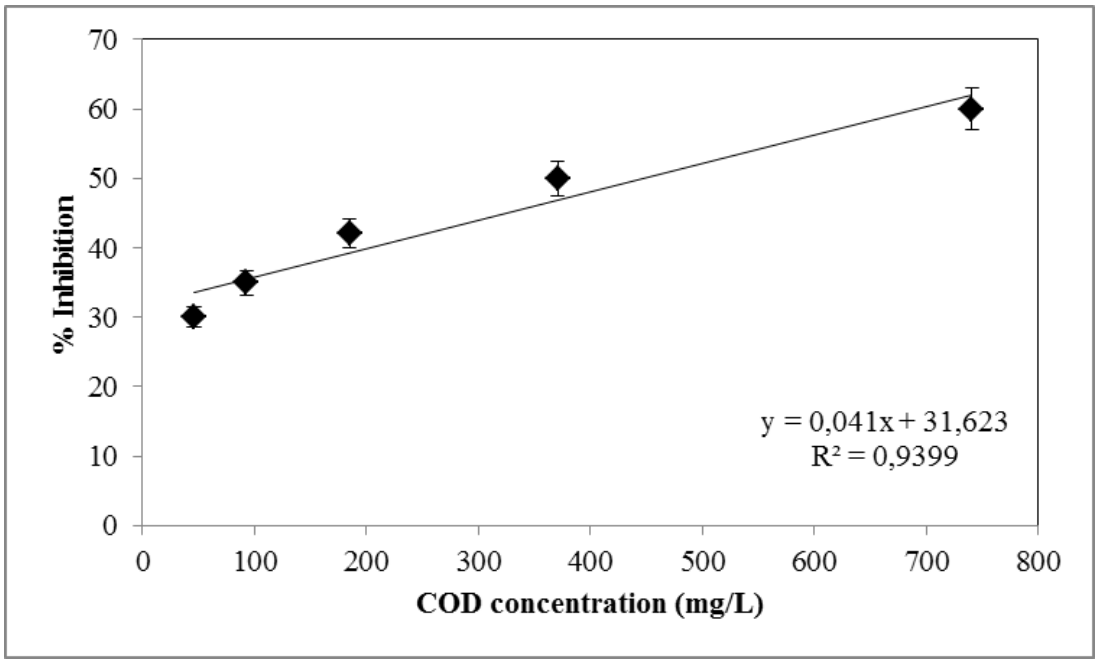

Figure 6.117 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of the anaerobic ITBR system with containing 150 mg/L RD (EC_{50}= 448 mg/L SRT=63 days, HRT= 2.75 days)

Table 6.56 The EC_{50} values in the influent and effluent of the anaerobic ITBR at increasing RD (COD_{dis}=2850 mg/L PAHs=292 ng/mL SRT=63 days, HRT= 2.75 days)

RD concentration (mg/L)	Influent EC_{50} value (mg/L)	Effluent EC_{50} value (mg/L)	*Vibrio fischeri* acute toxicity removal (%)
0	140	400	65
15	140	504	72
50	140	615	77
75	140	918	85
100	140	631	78
150	140	448	69

6.6.2.2 Effect of Increasing HRTs on the Acute Toxicity Removal with 75 mg/L RD in the Anaerobic ITBR System

6.6.2.2.1 Effect of Increasing HRTs on the Daphnia magna Acute Toxicity at 75 mg/L RD in the Anaerobic ITBR System. Figures 6.118-6.123 and Table 6.57 show the EC_{50} values in the influent and effluent of the anaerobic ITBR at increasing HRTs at a SRT of 59-79 days. The operational conditions for this study are summarized in Table 5.11 in the section Material and Methods. The EC_{50} value was obtained as 174 mg/L in the influent wastewater (Figure 6.118). As the HRTs were increased from 1.38 to 1.83 and 2.75 days, the EC_{50} values increased from 2109 to 2425 and to 2511 mg/L, respectively, in the effluent of the anaerobic ITBR system (Figures 6.119-6.121). The EC_{50} values decreased from 2511 to 677 and 689 mg/L when the HRT was increased from 2.75 to 5.5 and 11 days (Figures 6.122-6.123). The acute toxicity reductions in the anaerobic ITBR were 92%, 93%, 93%, 74% and 70% at HRTs 1.38, 1.83, 2.75, 5.5 and 11 days, respectively. In this study it was found that the EC_{50} value increased from174 mg/L to 2109 mg/L from the influent of the petrochemical wastewater to the effluent of the anaerobic ITBR system at a HRT of 1.38 days. As the HRTs were increased from 1.38 to 2.75 days, the EC_{50} values increased in the effluent of the ITBR system. At high EC_{50} value (2511 mg/L), the maximum acute toxicity removal (93%) was observed at 75 mg/L RD (Table 6.57).

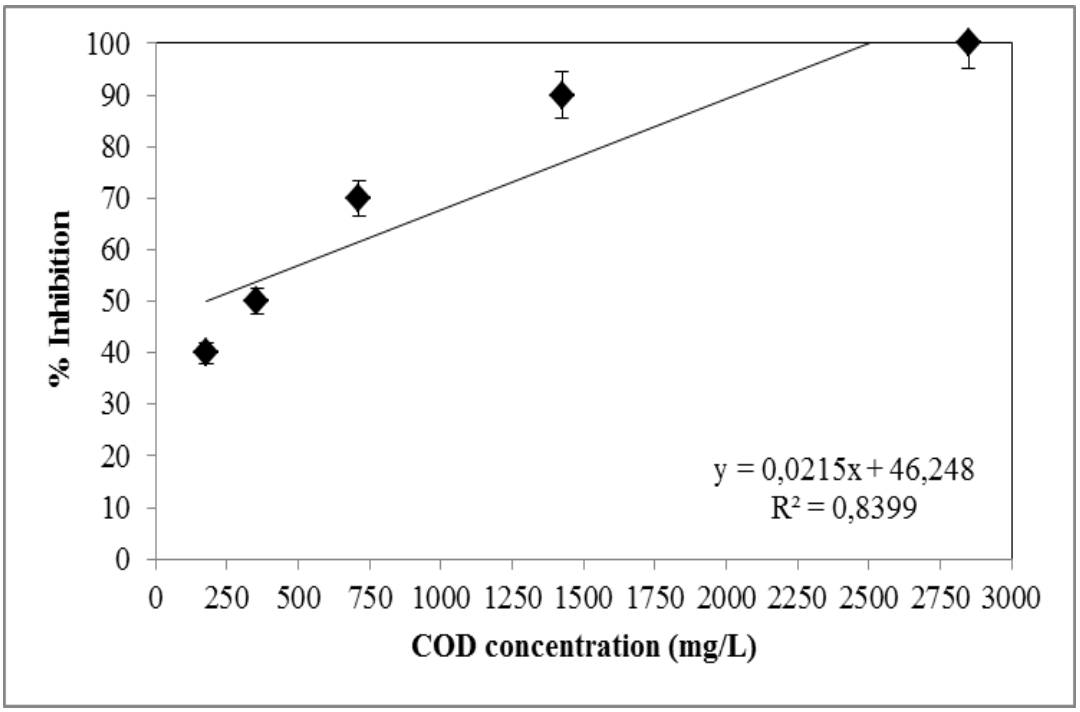

Figure 6.118 Variations of inhibition percentages versus COD_{dis} concentrations in the influent raw petrochemical wastewater (EC_{50}= 174 mg/L)

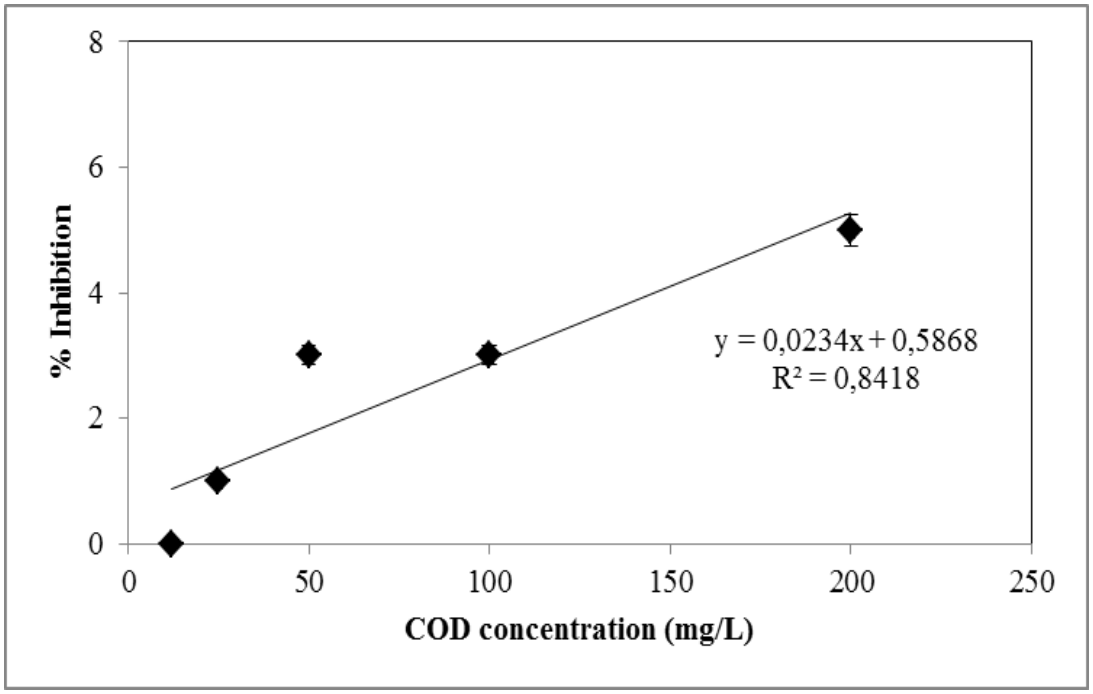

Figure 6.119 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system at a HRT of 1.38 days with 75 mg/L RD (EC_{50}= 2109 mg/L)

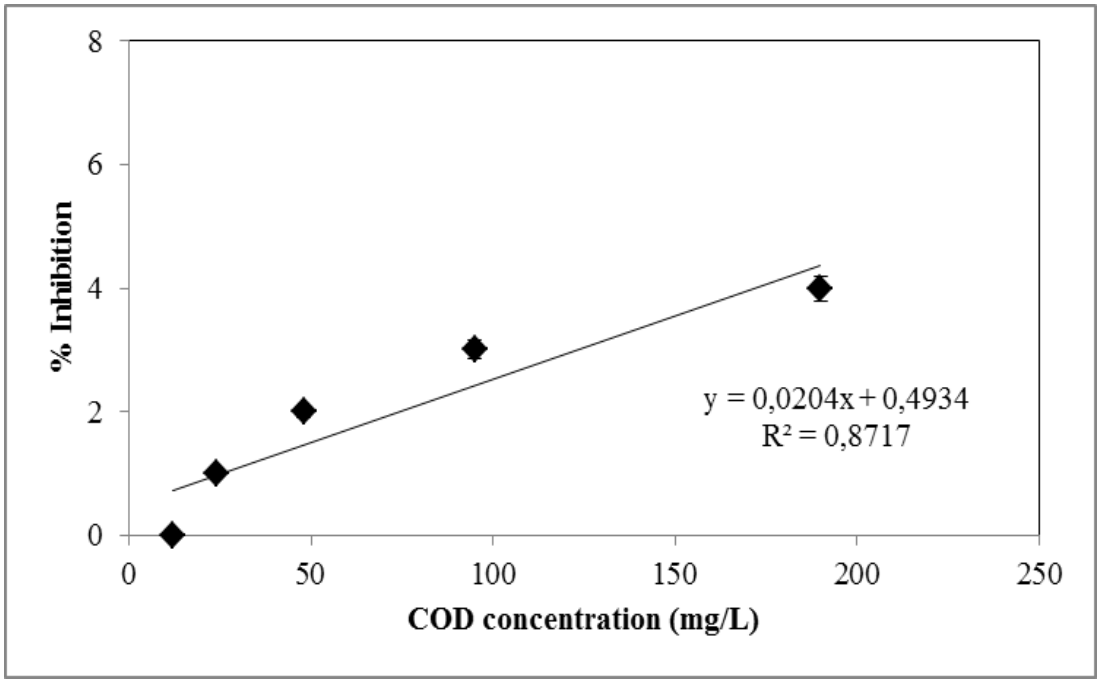

Figure 6.120 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system at a HRT of 1.83 days with 75 mg/L RD (EC_{50}= 2425 mg/L)

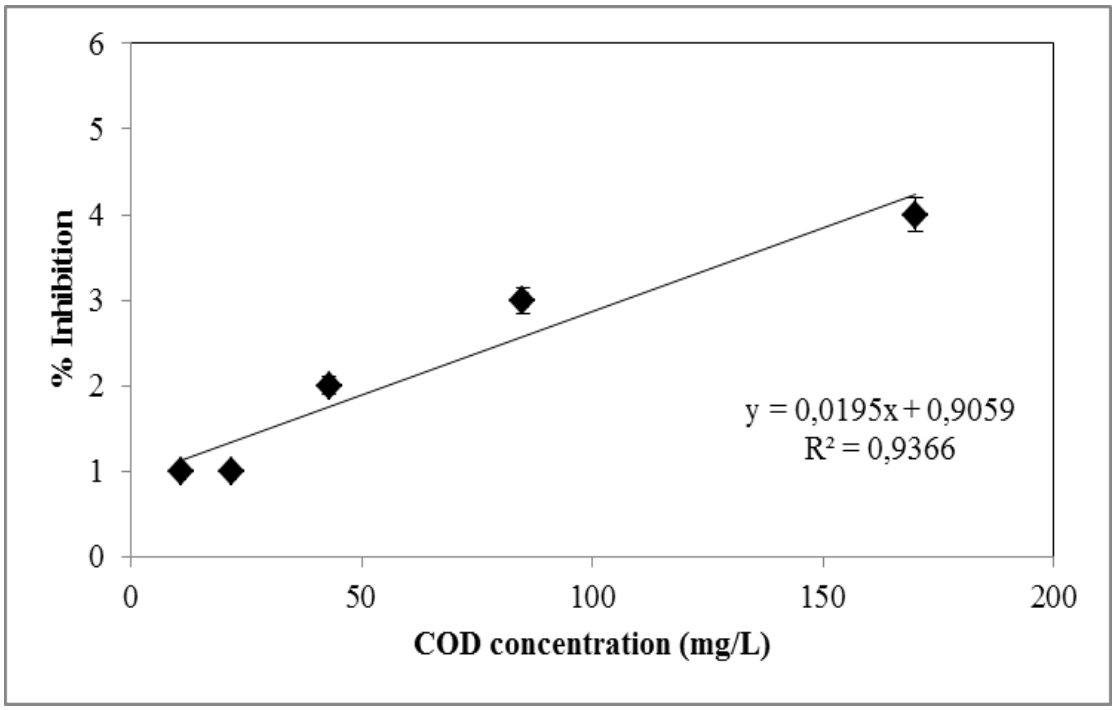

Figure 6.121 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system at a HRT of 2.75 days with 75 mg/L RD (EC_{50}= 2511 mg/L)

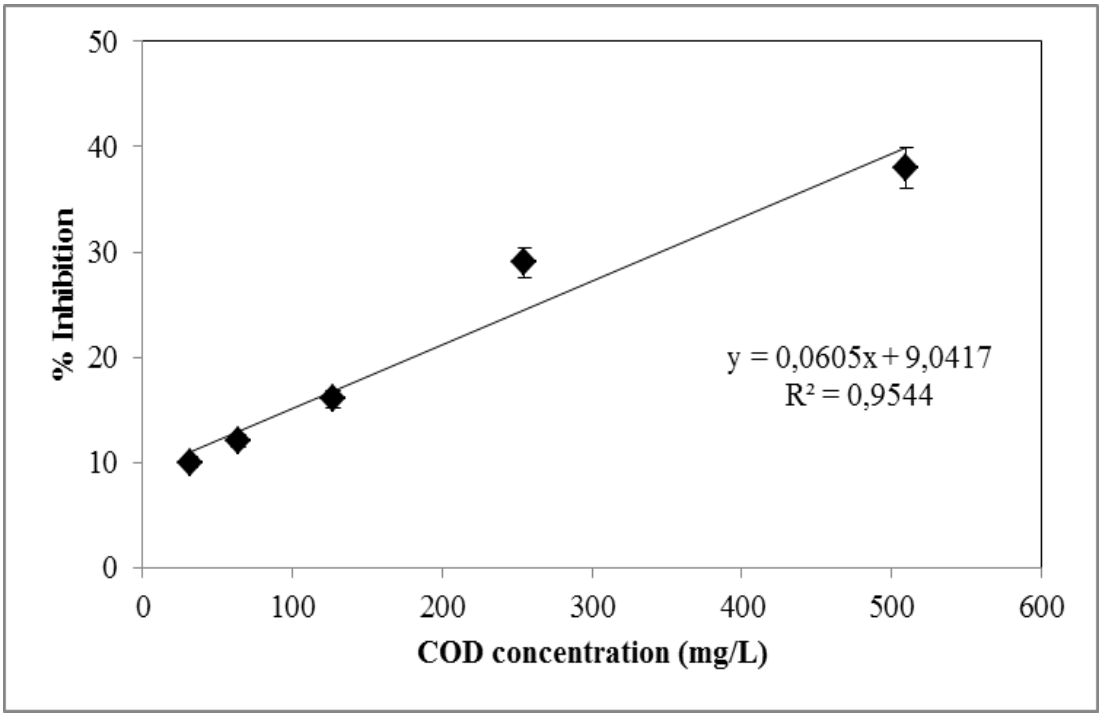

Figure 6.122 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system at a HRT of 5.5 days with 75 mg/L RD (EC_{50}= 677 mg/L)

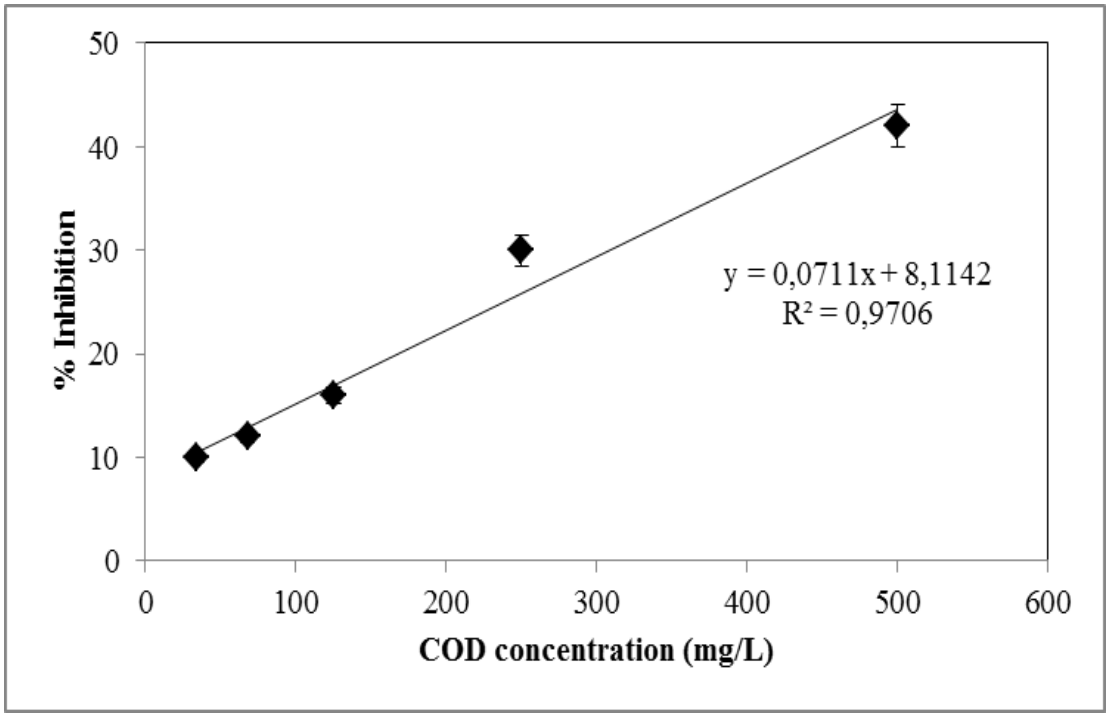

Figure 6.123 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system at a HRT of 11 days with 75 mg/L RD (EC_{50}= 589 mg/L)

Table 6.57 EC_{50} values in the influent and effluent of the ITBR with 75 mg/L RD (COD_{dis}=2850 mg/L PAHs=292 ng/mL, SRT=59-79 days)

HRT (days)	Influent EC_{50} value (mg/L)	Effluent EC_{50} value (mg/L)	*Daphnia magna* acute toxicity removal (%)
1.38	174	2109	92
1.83	174	2425	93
2.75	174	2511	93
5.5	174	677	74
11	174	589	70

6.6.2.2.2 Effect of ıncreasing HRTs on the Vibrio fischeri acute toxicity 75 mg/L RD in the anaerobic ITBR system. Figures 6.124-6.128 and Table 6.58 show the *Vibrio fischeri* acute toxicity results in the anaerobic ITBR effluent samples containing 75 mg/L RD at increasing HRTs from 1.38 days up to 11 days. The operational conditions for this study are summarized in Table 5.11 in the section Material and Methods. The EC_{50} value was found as 140 mg/L in the influent wastewater. The EC_{50} values increased from 728 to 765 and to 827 mg/L, at HRTs 1.38, 1.83 and 5 days, respectively, in the effluent of the anaerobic ITBR (Figures 6.124-6.126). When the HRTs were increased from 2.75 to 5.5 and 11 days, the EC_{50} values decreased from 827 to 411 and 369 mg/L at 75 mg/L RD (Figures 6.127-6.128). The acute toxicity removals in the anaerobic ITBR were 81%, 82%, 83%, 66% and 62% at HRTs 1.38, 1.83 2.75, 5.5 and 11 days, respectively. In this study it was found that the EC_{50} value increased from 728 to mg/L from the influent of the petrochemical wastewater to the effluent of the anaerobic ITBR system at a HRT of 1.38 days. As the HRTs were increased from 1.38 to 2.75 days, the EC_{50} values increased in the effluent of the anaerobic ITBR system. The maximum acute toxicity removal (83%) was observed at a HRT of 2.75 days (Table 6.58).

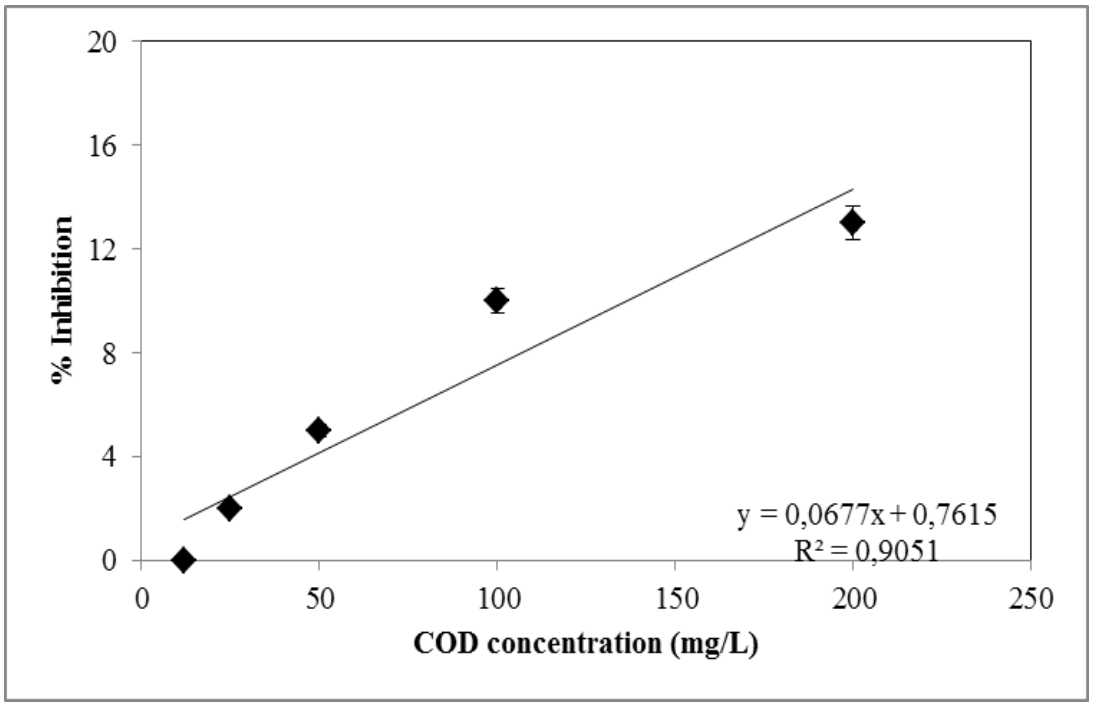

Figure 6.124 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system at a HRT of 1.38 days with 75 mg/L RD (EC_{50}= 728 mg/L)

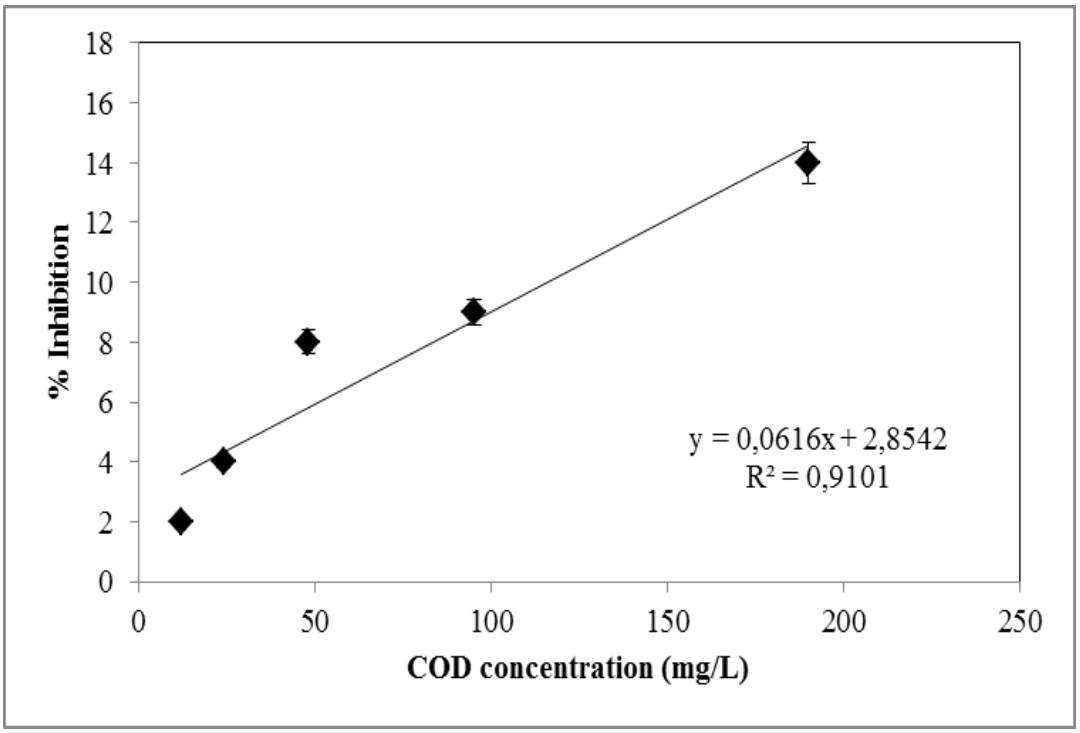

Figure 6.125 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system at a HRT of 1.83 days with 75 mg/L RD (EC_{50}= 765 mg/L)

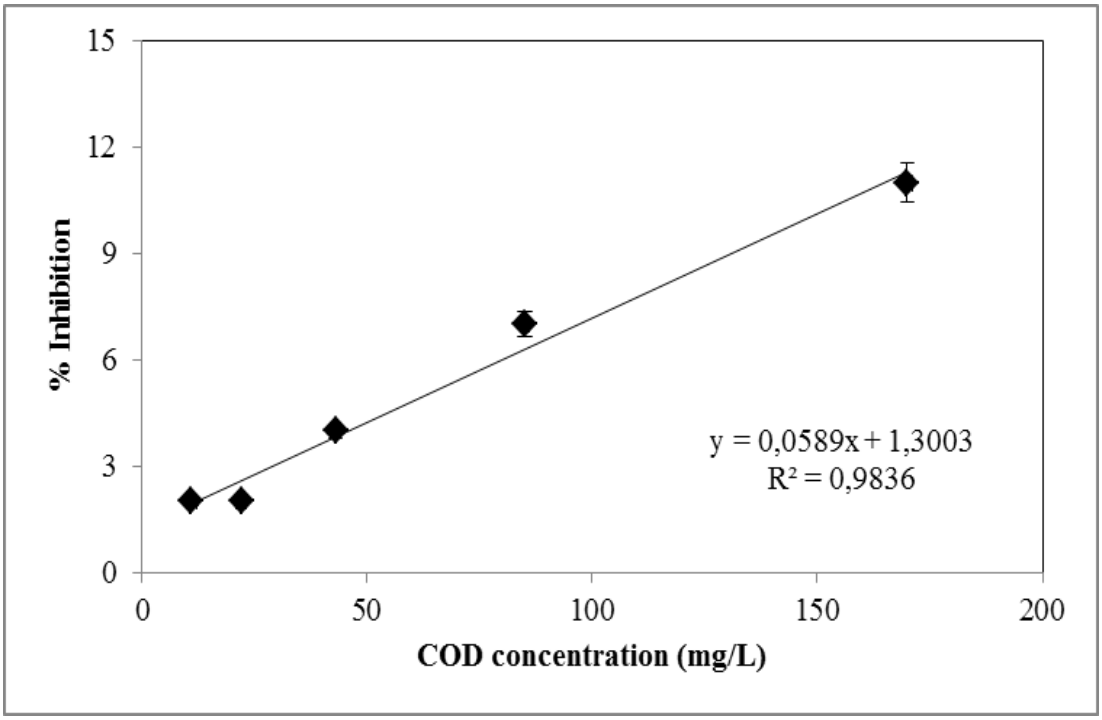

Figure 6.126 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system at a HRT of 2.75 days with 75 mg/L RD (EC_{50}= 827 mg/L)

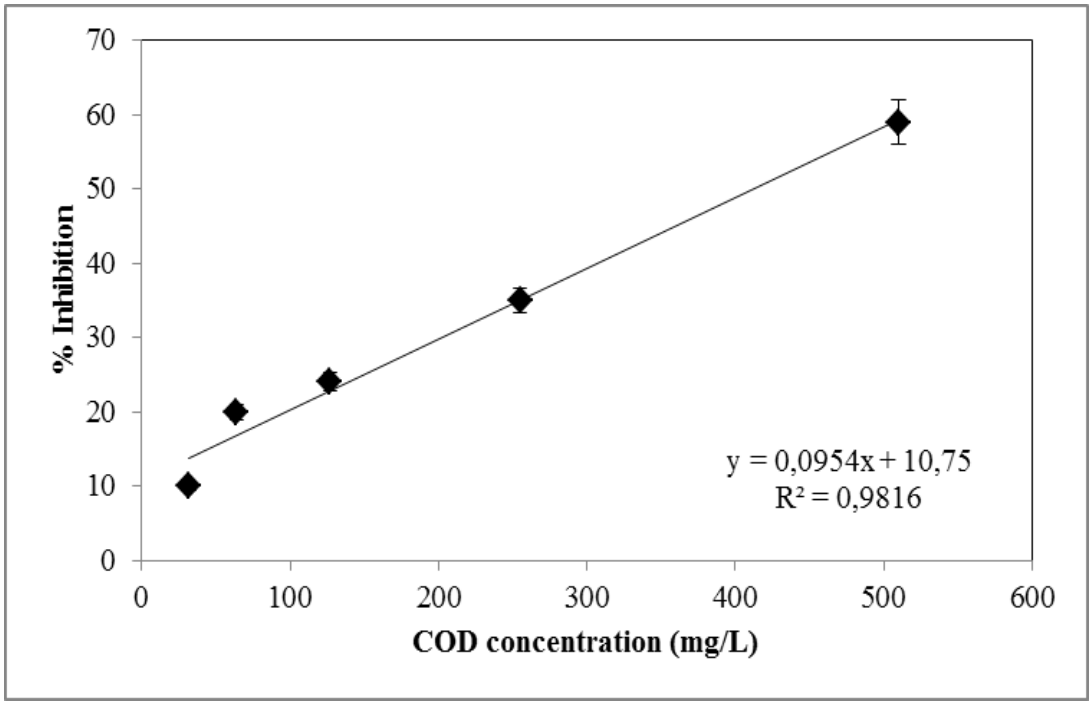

Figure 6.127 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system at a HRT of 5.5 days with 75 mg/L RD (EC_{50}= 411 mg/L)

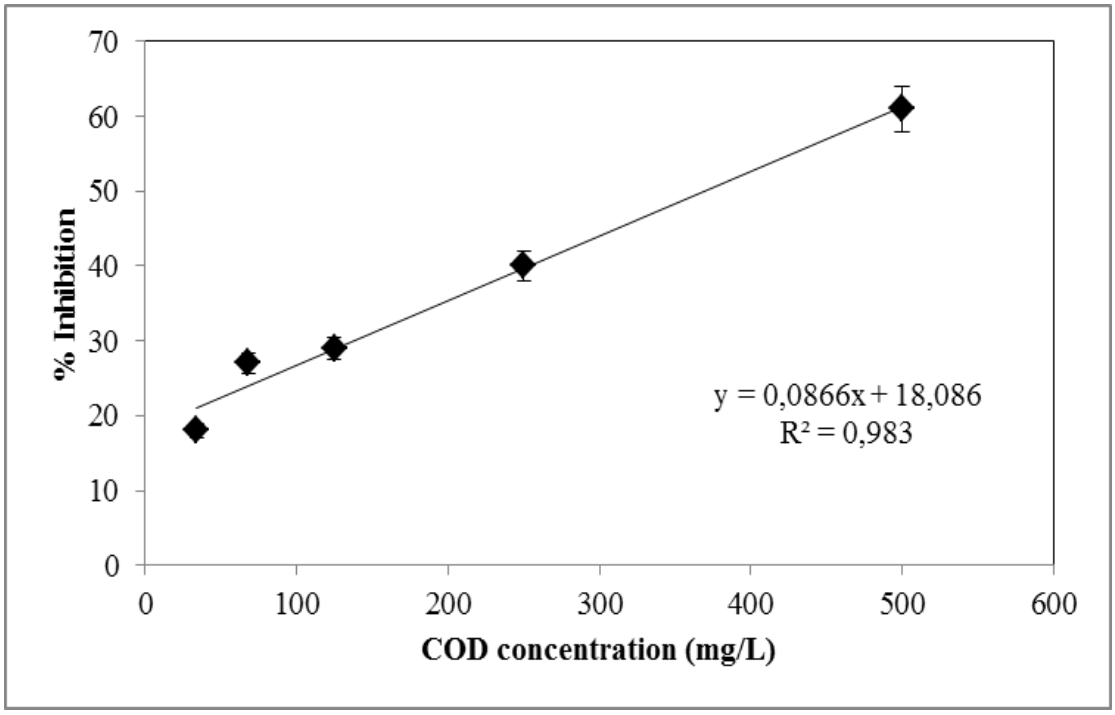

Figure 6.128 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of anaerobic ITBR system at a HRT of 11 days with 75 mg/L RD (EC_{50}= 369 mg/L)

Table 6.58 The EC_{50} values in the influent and effluent of the anaerobic ITBR with 75 mg/L RD (COD_{dis}= 2850 mg/L PAHs =292 ng/mL, SRT=59-79 days)

HRT (days)	Influent EC_{50} value (mg/L)	Effluent EC_{50} value (mg/L)	*Vibrio fischeri* acute toxicity removal (%)
1.38	140	728	81
1.83	140	765	82
2.75	140	827	83
5.5	140	411	66
11	140	369	62

6.6.3 Acute Toxicity Evaluations in the Petrochemical Industry Wastewater in the Sequential Anaerobic ITBR/Aerobic CSTR system with Daphnia magna and Vibrio fischeri

6.6.3.1 Acute Toxicity Evaluations in the Sequential Anaerobic ITBR/Aerobic CSTR System with Daphnia magna. The EC_{50} value was obtained as 174 mg/L in the influent wastewater of the anaerobic ITBR system **(**See Table 6.59). As the HRTs were increased from 1.38 to 1.83 and 2.75 days, the EC_{50} values increased from 2109 to 2425 and to 2511 mg/L, respectively, in the effluent of the anaerobic ITBR system (See Table 6.59). The EC_{50} values decreased from 2511 to 677 and 689 mg/L when the HRT was increased from 2.75 to 5.5 and 11 days (See Table 6.59). The acute toxicity reductions in the anaerobic ITBR were 92%, 93%, 93%, 74% and 70% at HRTs 1.38, 1.83, 2.75, 5.5 and 11 days, respectively. As the optimum HRT was found as 2.75 days, the EC_{50} value was 2511 mg/L in the effluent of the anaerobic ITBR system. The acute toxicity reduction was obtained as 93% at a HRT 2.75 days and at 75 mg/L RD in the anaerobic ITBR system. After anaerobic step the EC_{50} values increased from 174 to 3690 mg/L, in the effluent of the aerobic CSTR reactor at HRT of 1.38 days (See Table 6.59). As the HRTs were increased from 1.38 to 1.83 and 2.75 days, the EC_{50} values increased from 3690 to 4315 and to 4455 mg/L, respectively, in the effluent of the aerobic CSTR system (Figures 6.129-6.131). The EC_{50} values decreased from 4455 to 1064 and 880 mg/L when the HRT was increased from 2.75 to 5.5 and 11 days (Figures 6.132-6.133). The acute toxicity reductions in the aerobic CSTR were 43%, 44%, 44%, 36% and 33% at HRTs 1.38, 1.83, 2.75, 5.5 and 11 days, respectively.

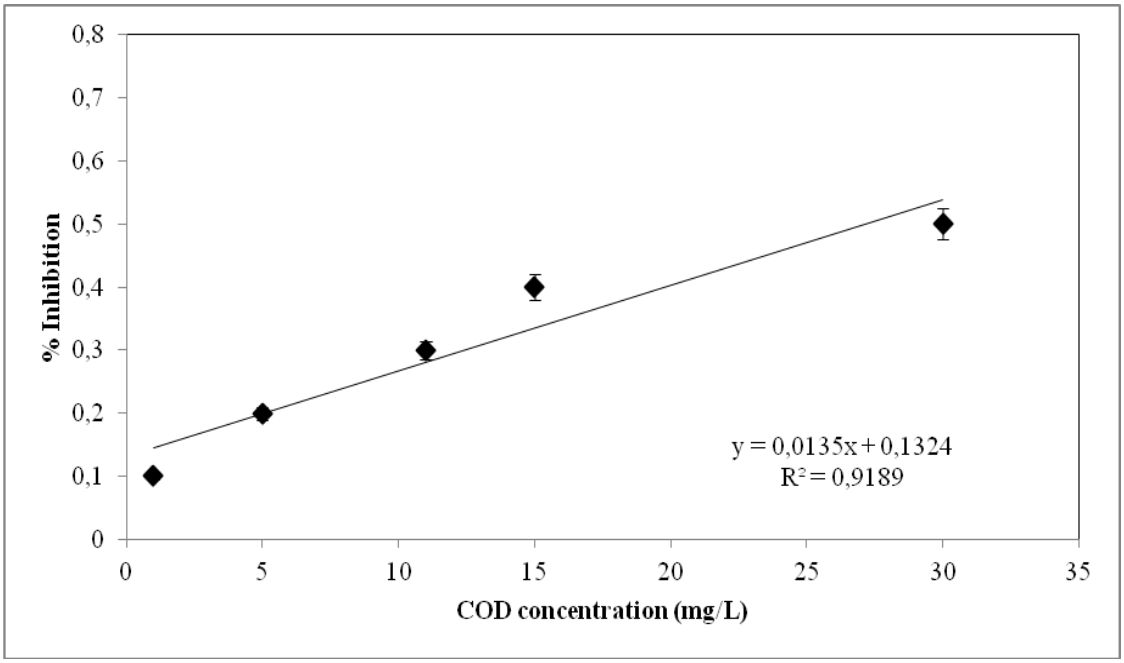

Figure 6.129 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of sequential anaerobic ITBR/aerobic CSTR system at a HRT of 1.38 days with 75 mg/L RD (EC_{50}= 3690 mg/L)

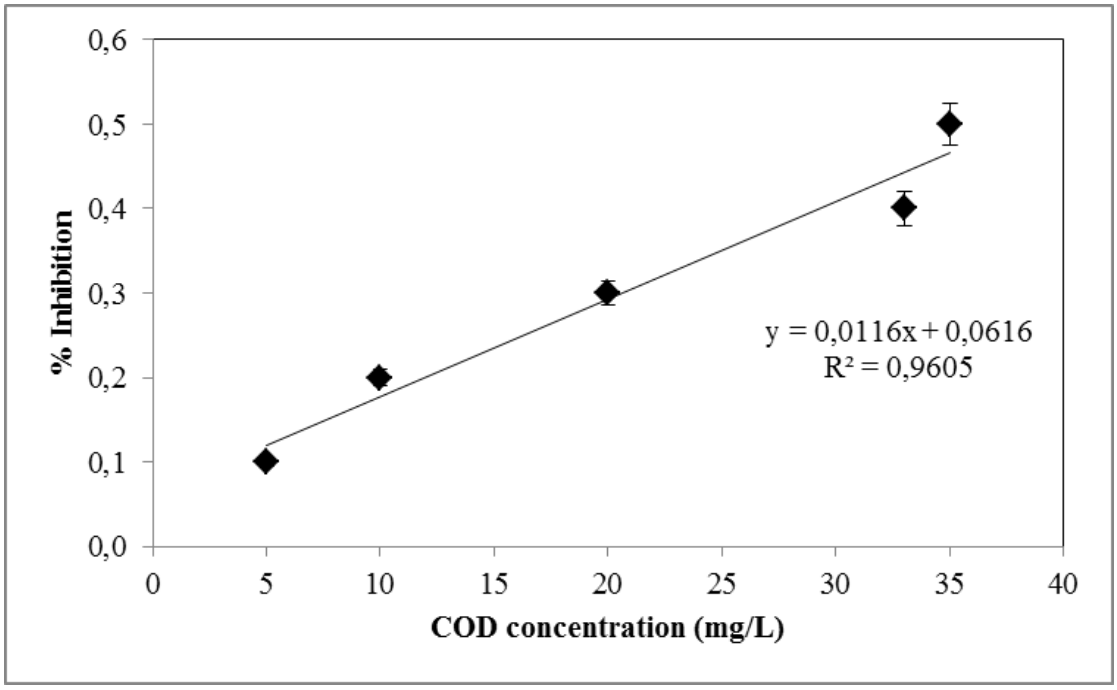

Figure 6.130 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of sequential anaerobic ITBR/aerobic CSTR system at a HRT of 1.83 days with 75 mg/L RD (EC_{50}= 4315 mg/L)

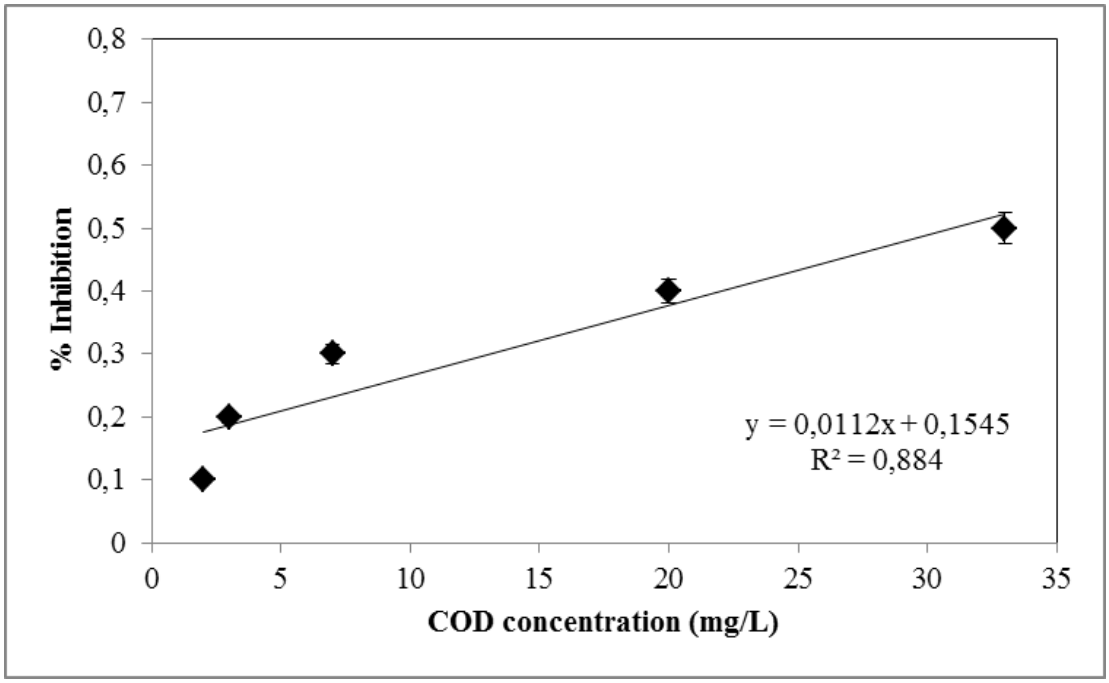

Figure 6.131 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of sequential anaerobic ITBR/aerobic CSTR system at a HRT of 2.75 days with 75 mg/L RD (EC_{50}= 4455 mg/L)

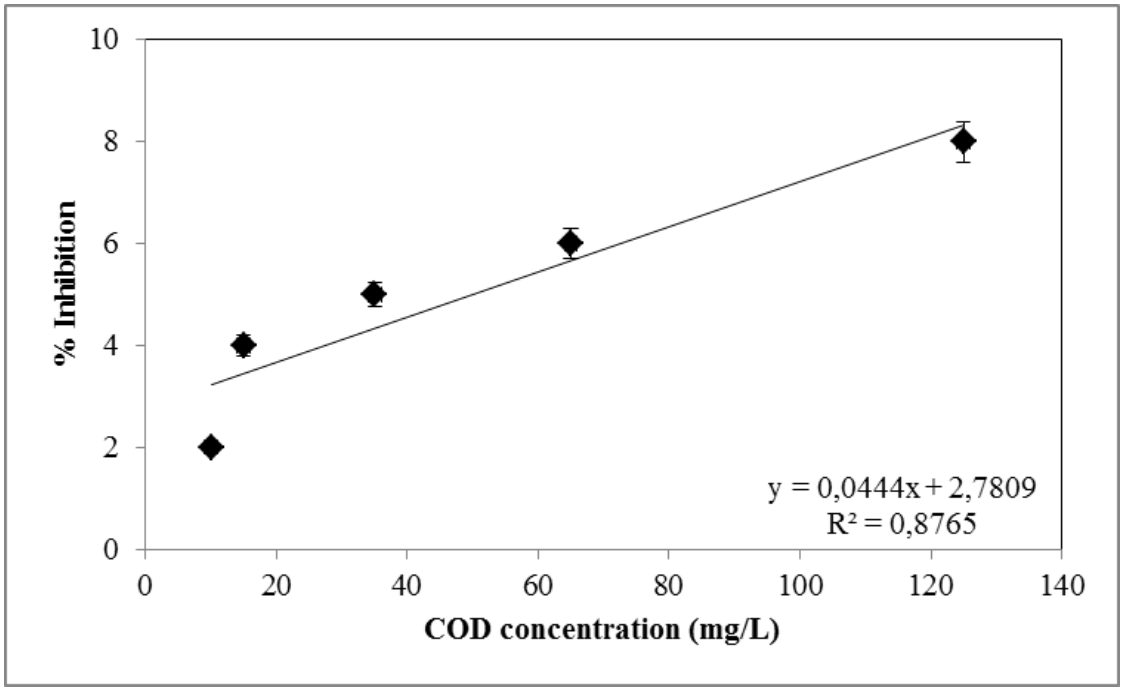

Figure 6.132 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of sequential anaerobic ITBR/aerobic CSTR system at a HRT of 5.50 days with 75 mg/L RD (EC_{50}= 1064 mg/L)

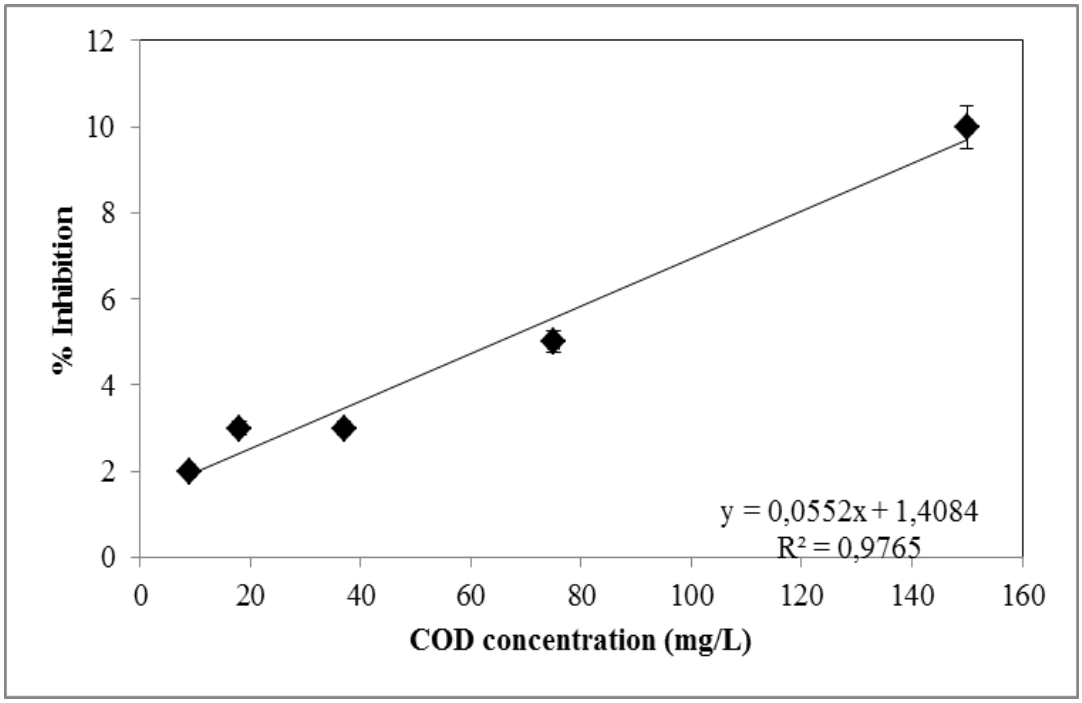

Figure 6.133 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of sequential anaerobic ITBR/aerobic CSTR system at a HRT of 11.00 days with 75 mg/L RD (EC_{50}= 880 mg/L)

The maximum acute toxicity yields were 92-93% and 43-44% in the effluents of the anaerobic ITBR and aerobic CSTR reactor, at HRT of 1.38, 1.83 and 2.75 days. The total *Daphnia magna* acute toxicity removal in the effluent of the sequential anaerobic ITBR/aerobic CSTR system was 96% at HRT of 1.83 and 2.75 days and at 75 mg/L RD. The results of the acute toxicity showed that the *Daphnia magna* acute toxicity of PAHs was removed in both anaerobic and aerobic sequential. The water flea in *Daphnia magna* acute toxicity test results demonstrated that the sequential anaerobic ITBR/aerobic CSTR system eliminated the inhibitory effect of the petrochemical industry wastewater containing PAHs on *Daphnia magna* in anaerobic and aerobic effluents.

Table 6.59 Variations of *Daphnia magna* acute toxicity values in the influent and effluents of the anaerobic ITBR, aerobic CSTR and sequential anaerobic ITBR/aerobic CSTR system (SRT=59-79 days for the ITBR, RD=75 mg/L and SRT=25 days, RD=15 mg/L for the aerobic CSTR reactor, influent PAHs=292 ng/mL, influent COD_{dis}=2850 mg/L)

HRTs (days)	Anaerobic ITBR reactor		Aerobic CSTR reactor	Acute toxicity removal in the reactor system		
	*EC_{50} value in the influent of the anaerobic ITBR (mg/L)	*EC_{50} value in the effluent of the anaerobic ITBR (mg/L)	* EC_{50} value in the effluent of the aerobic CSTR (mg/L)	Anaerobic ITBR (%)	Aerobic CSTR (%)	Anaerobic ITBR/aerobic CSTR (%)
1.38	174	2109	3690	92	43	95
1.83	174	2425	4315	93	44	96
2.75	174	2511	4455	93	44	96
5.5	174	677	1064	74	31	84
11	174	589	880	70	30	80

* EC_{50} values were calculated based on COD_{dis} (mg/L).

6.6.3.2 Acute Toxicity evaluations in the sequential anaerobic ITBR and aerobic CSTR system with Vibrio fischeri

The EC_{50} value was obtained as 140 mg/L in the influent wastewater of the anaerobic ITBR system (See Table 6.60). As the HRTs were increased from 1.38 to 1.83 and 2.75 days, the EC_{50} values increased from 728 to 765 and to 827 mg/L, respectively, in the effluent of the anaerobic ITBR system (See Table 6.60). The EC_{50} values decreased from 827 to 411 and 369 mg/L when the HRT was increased from 2.75 to 5.5 and 11 days (See Table 6.60). The acute toxicity reductions in the anaerobic ITBR were 81%, 82%, 83%, 66% and 62% at HRTs 1.38, 1.83, 2.75, 5.5 and 11 days, respectively. As the optimum HRT was found as 2.75 days, the EC_{50} value was 827 mg/L in the effluent of the anaerobic ITBR system. The acute toxicity reduction was obtained as 93% at a HRT 2.75 days and at 75 mg/L RD in the anaerobic ITBR system. After anaerobic step the EC_{50} values increased from 140 to 1452 mg/L, in the effluent of the aerobic CSTR reactor at HRT of 1.38 days (See Table 6.60). As the HRTs were increased from 1.38 to 1.83 and 2.75 days, the EC_{50} values increased from 1452 to 1545 and to 1773 mg/L, respectively, in the effluent of the aerobic CSTR system. The EC_{50} values decreased from 1773 to 699 and 683 mg/L when the HRT was increased from 2.75 to 5.5 and 11 days (Figures 6.134-6.138). The *Vibrio fischeri* acute toxicity reductions in the aerobic CSTR were 50%, 50%, 53%, 41% and 34% at HRTs 1.38, 1.83, 2.75, 5.5 and 11 days, respectively.

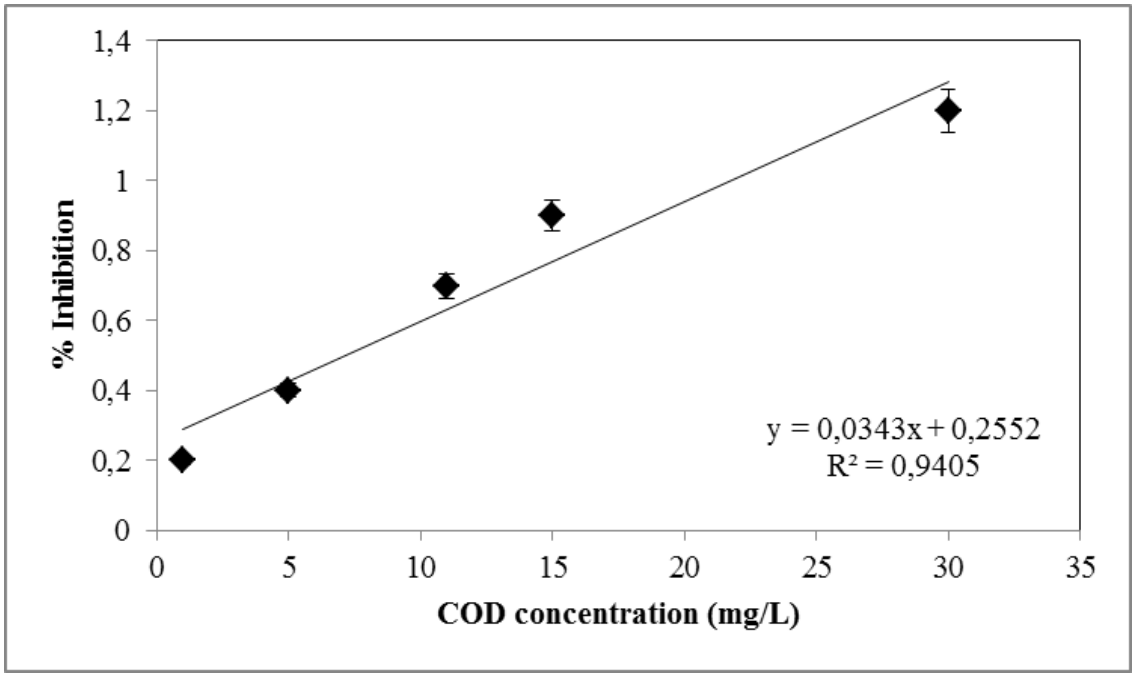

Figure 6.134 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of sequential anaerobic ITBR/aerobic CSTR system at a HRT of 1.38 days with 75 mg/L RD (EC_{50}= 1452 mg/L)

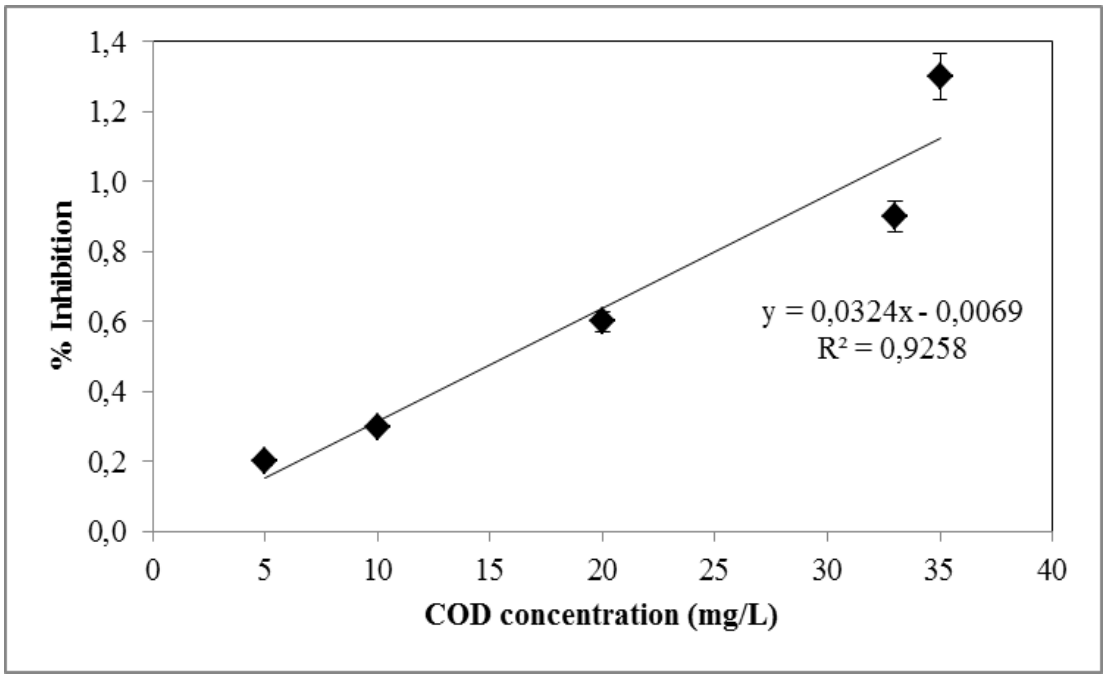

Figure 6.135 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of sequential anaerobic ITBR/aerobic CSTR system at a HRT of 1.83 days with 75 mg/L RD (EC_{50}= 1545 mg/L)

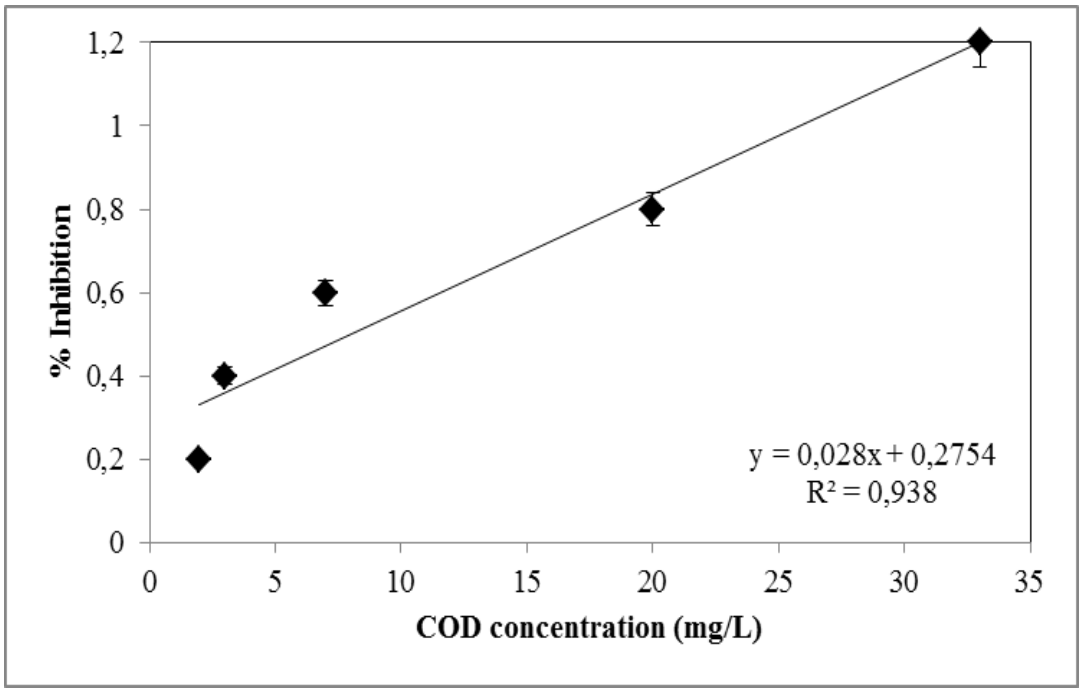

Figure 6.136 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of sequential anaerobic ITBR/aerobic CSTR system at a HRT of 2.75 days with 75 mg/L RD (EC_{50}= 1773 mg/L)

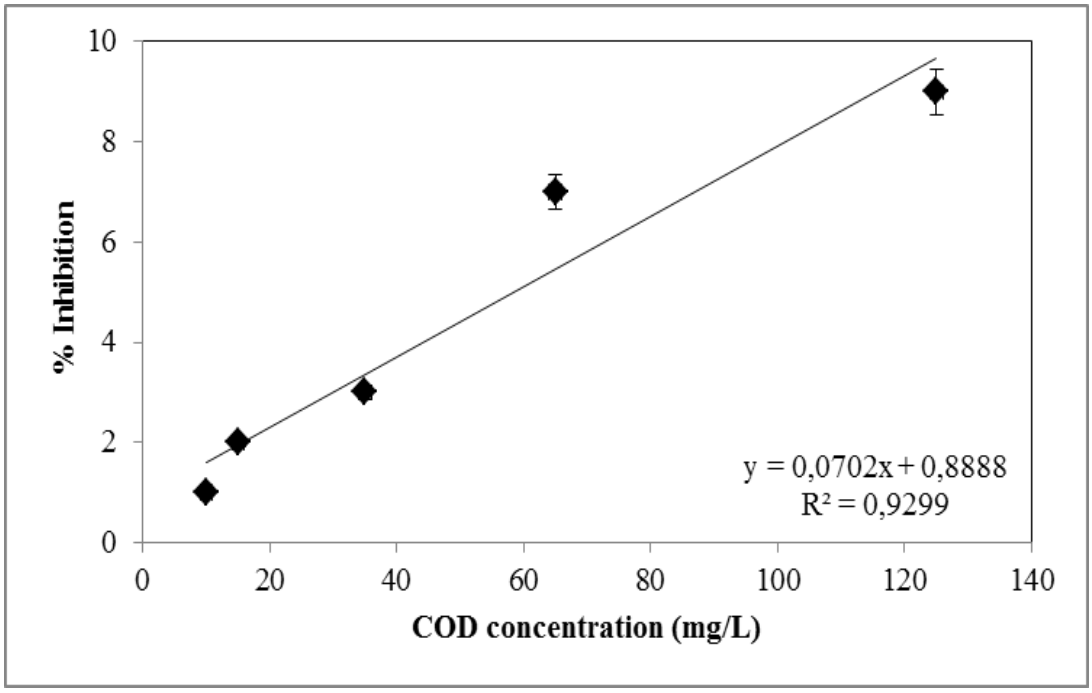

Figure 6.137 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of sequential anaerobic ITBR/aerobic CSTR system at a HRT of 5.50 days with 75 mg/L RD (EC_{50}= 699 mg/L)

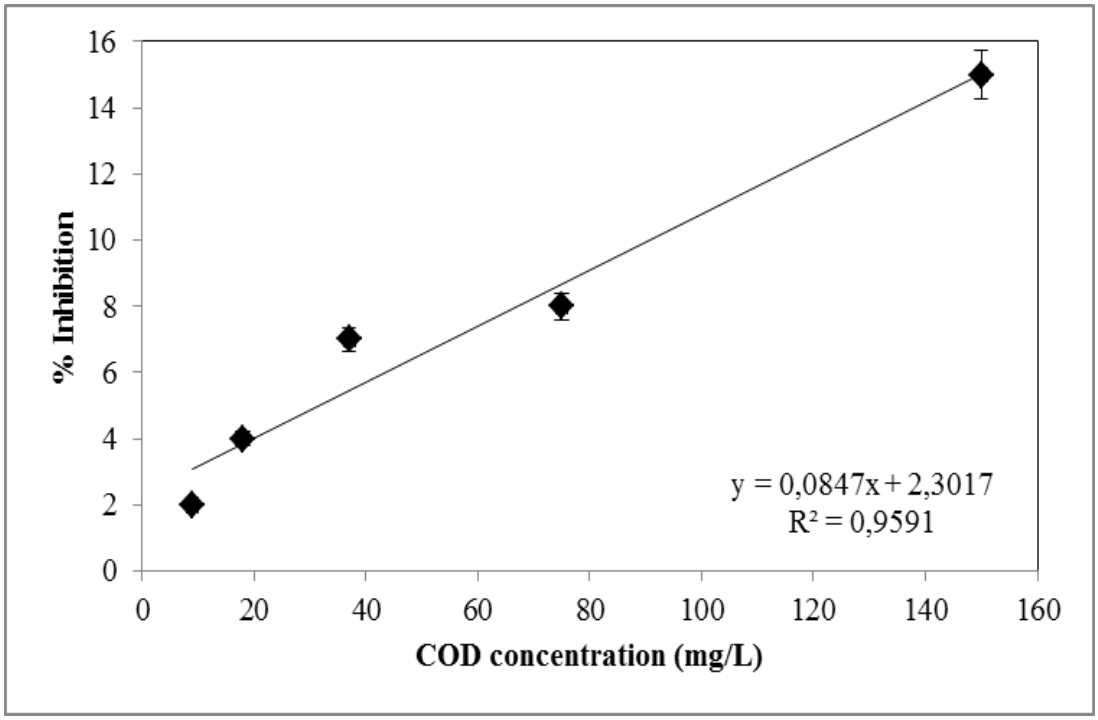

Figure 6.138 Variations of inhibition percentages versus COD_{dis} concentrations in the effluent of sequential anaerobic ITBR/aerobic CSTR system at a HRT of 11.00 days with 75 mg/L RD (EC_{50}= 563 mg/L)

The maximum acute toxicity yields were 90-92% and 50-53% in the effluents of the anaerobic ITBR and aerobic CSTR reactor, respectively, at HRT of 1.38, 1.83 and 2.75 days. The total *Vibrio fischeri* acute toxicity removal in the effluent of the sequential anaerobic ITBR/aerobic CSTR system was 92% at a HRT of 2.75 days. The results of the acute toxicity showed that the *Vibrio fischeri* acute toxicity of PAHs was removed in both anaerobic and aerobic sequential. The water flea in *Vibrio fischeri* acute toxicity test results demonstrated that the sequential anaerobic ITBR/aerobic CSTR system eliminated the inhibitory effect of the petrochemical industry wastewater containing PAHs on *Vibrio fischeri* in anaerobic and aerobic effluents.

Table 6.60 Variations of *Vibrio fischeri* acute toxicity values in the influent and effluents of the anaerobic ITBR, aerobic CSTR and sequential anaerobic ITBR/aerobic CSTR system (SRT=59-79 days for the ITBR, RD=75 mg/L and SRT=25 days, RD=15 mg/L for the aerobic CSTR reactor, influent PAHs=292 ng/mL, influent COD_{dis}=2850 mg/L)

HRTs (days)	Anaerobic ITBR		Aerobic CSTR	Acute toxicity removal in the reactor system		
	*EC_{50} value in the influent of the anaerobic ITBR (mg/L)	*EC_{50} value in the effluent of the anaerobic ITBR (mg/L)	* EC_{50} value in the effluent of the aerobic CSTR (mg/L)	Anaerobic ITBR (%)	Aerobic CSTR (%)	Anaerobic ITBR/aerobic CSTR (%)
1.38	140	728	1452	81	50	90
1.83	140	765	1545	82	50	91
2.75	140	827	1773	83	53	92
5.5	140	411	699	66	41	80
11	140	369	683	62	34	80

* EC_{50} values were calculated based on COD_{dis} (mg/L).

Limited data has been obtained in literature for *Vibrio fischeri* toxicity studies carried out through anaerobic treatment of petrochemical industry wastewater containing PAHs: Salizzato et al. (1997) found that the EC_{50} values in the influent were between 0.43 and 1.76 μg/mL in an anaerobic sludge reactor treating petrochemical industry wastewater at an influent total PAH concentration of 45 μg/mL. The acute toxicity removal was found to be 65%. In this study, the decrease in acute toxicities for *Vibrio fischeri* is comparably higher than those obtained by Salizzato et al. (1997). Perez et al. (2001) evaluated the toxicity of 16 PAHs (total concentration varied from 17 to 2030 μg/kg) in sludge extracts using *Vibrio fischeri* from an anaerobic treatment plant. They found that PAHs concentrations in the sewage sludge extracts were high and cause toxic effects to *Vibrio fischeri.* The EC_{50} values increased from 101 to 201 ng/mL resulting in a toxicity removal of 50% which is comparatively lower than that our acute toxicity removal. This could be attributed to the resistance of anaerobic sludge to PAHs. Acute toxicity of total 13 PAHs and their toxic effects after an anaerobic reactor were studied with *Vibrio fischeri* by Mantis et al. (2005). The EC_{50} values increased from 1.61 to 12.20 ng/mL, respectively, and the acute toxicity removal efficiency was 63% in treated effluents. In our study the EC_{50} values of the PAHs in the effluent of the anaerobic ITBR system exhibited higher toxicity removals than Mantis et al. (2005). In a study performed by Sanchez-Avila et al (2009) the total PAHs removal yields was 72% in a municipal wastewater treatment plants treating different contaminants like phthalates, polychlorinated biphenyls (PCBs), polybromodiphenyl ethers (PDBs) and PAHs.

6.6.4 Sensitivities of Daphnia magna and Vibrio fischeri Acute Toxicity Test Results

6.6.4.1 Sensitivities in the CSTR System

To verify the relationships between the acute toxicity data of two organisms the EC_{50} values of *Vibrio fischeri* were correlated to those of *Daphnia magna* by statistical analysis using their EC_{50} values in the influent of the petrochemical

industry wastewater. A score of *"1"* was assigned to the most sensitive test for each sample down to *"2"* for the least sensitive organism representing two trophic levels which were classified according to the acute toxicity test results (Sponza and Kuscu, 2011). Table 6.61 shows the sensitivity ranking and the EC_{50} values of the petrochemical industry wastewater for *Vibrio fischeri* and *Daphnia magna* in the influent and in the effluent of the CSTR reactor samples at a SRT of 25 days, at a HRT of 5 days and at 15 mg/L RD. The sensitivity scores were 5 and 10 in *Vibrio fischeri* and *Daphnia magna*, respectively, through operation of the aerobic CSTR reactor system during 5 sampling cases (Table 6.61).

The EC_{50} values were 100 mg/L for *Vibrio fischeri* and 143 mg/L for *Daphnia magna* in the influent of aerobic CSTR system (Table 6.61). The ANOVA test statistics showed that a weak correlation was found between the acute toxicities of *Vibrio fischeri* and *Daphnia magna* and this correlation was not significant (R^2 = 0.91, F = 8.04, p = 0.001) in the influent of the CSTR samples.

The EC_{50} values were 596 mg/L for *Vibrio fischeri* and 1447 mg/L for *Daphnia magna* in the effluent of the aerobic CSTR system. A weak correlation was found between the acute toxicities of effluent wastewater to *Vibrio fischeri* and to *Daphnia magna* and this correlation was not significant in the effluent of the CSTR samples (R^2=0.67, F=9.74, P=0.001). This indicated that the water fleas exhibited less acute toxicity to petrochemical industry wastewater. The differences in test sensitivities can be attributed to the differences in the responses of the two different trophic organisms in the wastewater. As a conclusion, this study showed that the *Vibrio fischeri* acute toxicity test is more sensitive than that *Daphnia magna* acute toxicity test in the effluent of the CSTR system.

Table 6.61 Sensitivity ranking and EC_{50} values of the petrochemical wastewater in *Daphnia magna* and *Vibrio fischeri* acute toxicity tests in the influent- effluent of aerobic CSTR system (SRT=25 days, HRT= 5 days RD=15 mg/L) (95% Confidence limits between brackets, mg/L; n=3 mean values)

	Daphnia magna				*Vibrio fischeri*			
Sample cases	Sensitivity ranking in the influent	Influent EC_{50} values (mg/L; 95% Confidence limits)	Effluent EC_{50} values (mg/L; 95% Confidence limits)	Sensitivity ranking in the effluent	Sensitivity ranking in the influent	Influent EC_{50} values (mg/L; 95% Confidence limits)	Effluent EC_{50} values (mg/L; 95% Confidence limits)	Sensitivity ranking in the effluent
1	2	141* (140-142)**	1449* (1447-1451)**	2	1	98* (91-105)**	595* (591-599)**	1
2	2	142* (141-143)**	1445* (1441-1449)**	2	1	99* (97-101)**	596* (594-598)**	1
3	2	144* (142-146)**	1446* (1440-1452)**	2	1	100* (97-103)**	596* (593-599)**	1
4	2	145* (142-148)**	1443* (1440-1446)**	2	1	101* (99-103)**	597* (595-599)**	1
5	2	143* (141-145)**	1452* (1449-1455)**	2	1	102* (100-104)**	596* (593-599)**	1
	TSS=10	Mean=143	Mean=1447	TSS=10	TSS=5	Mean=100	Mean=596	TSS=5

TSS: Total sensitive score; *: mean values of EC_{50} (mg/L); **: 95% Confidence limits of EC_{50} (mg/L)

6.6.4.2 Sensitivities in the Anaerobic ITBR System

To verify the relationships between the cute toxicity data of two organisms the EC_{50} values of *Vibrio fischeri* were correlated to those of *Daphnia magna* by statistical analysis using their EC_{50} values in the influent wastewater. The EC_{50} values were obtained as 140 mg/L and 174 mg/L for *Vibrio fischeri* and *Daphnia magna* acute toxicity test in the influent of anaerobic ITBR system samples, respectively (Table 6.62). The ANOVA test statistics showed that a weak correlation was found between the acute toxicities of *Vibrio fischeri* and *Daphnia magna* and this correlation was not significant (R^2=0.90, F=8.72, P=0.001) in the influent of the ITBR samples.

The *Vibrio fischeri* bacteria had lower EC_{50} values than *Daphnia magna*. This result verifies the sensitivity of *Vibrio fischeri* and the resistance of *Daphnia magna* to the influent wastewater. This indicated that the *Daphnia magna* exhibited less acute toxicity to the influent wastewater. The differences in test sensitivities can be attributed to the differences in the responses of the two different trophic organisms in the petrochemical industry wastewater. This study showed that the *Vibrio fischeri* acute toxicity test is more sensitive than that *Daphnia magna* to the response to petrochemical industry wastewater before anaerobic treatment. The EC_{50} values were found as 918 mg/L and 3181 mg/L for *Vibrio fischeri* and *Daphnia magna* acute toxicity test in the effluent of anaerobic ITBR system at a SRT of 63 days, at a HRT of 2.75 days and at 75 mg/L RD (Table 6.62). The ANOVA test statistics showed that a weak correlation was found between the acute toxicities of *Vibrio fischeri* and *Daphnia magna* and this correlation was not significant (R^2=0.78, F=14.72, P=0.001) in the effluent of the ITBR.

Table 6.62 Sensitivity ranking and EC_{50} values of the petrochemical wastewater in *Daphnia magna* and *Vibrio fischeri* acute toxicity test in the influent- effluent of anaerobic ITBR system (SRT=63 days, HRT= 2.75 days RD=75 mg/L) (95% Confidence limits between brackets, mg/L; n=3 mean values)

	Daphnia magna				*Vibrio fischeri*			
Sample cases	Sensitivity ranking in the influent	Influent EC_{50} values (95% Confidence limits; mg/L)	Effluent EC_{50} values (95% Confidence limits; mg/L)	Sensitivity ranking in the effluent	Sensitivity ranking in the influent	Influent EC_{50} values (95% Confidence limits; mg/L)	Effluent EC_{50} values (95% Confidence limits; mg/L)	Sensitivity ranking in the effluent
1	2	174* (173-175)**	3180* (3177-3183)**	2	1	138* (136-140)**	921* (918-924)**	1
2	2	175* (174-176)**	3180* (3176-3184)**	2	1	139* (137-141)**	918* (916-920)**	1
3	2	172* (170-174)**	3181* (3180-3182)**	2	1	142* (140-144)**	917* (911-923)**	1
4	2	175* (174-176)**	3182* (3179-3182)**	2	1	141* (139-143)**	918* (914-922)**	1
5	2	174* (171-177)**	3182* (3180-3184)**	2	1	140* (138-142)**	916* (914-918)**	1
	TSS=10	Mean=174	Mean=3181	TSS=10	TSS=5	Mean=140	Mean=918	TSS=5

TSS: Total sensitive score; *: mean values of EC_{50} (mg/L); **: 95% Confidence limits of EC_{50} (mg/L)

The results showed that *Daphnia magna* had higher EC_{50} values and lower sensitivity scores than *Vibrio fischeri.* This indicated that the water fleas exhibited less acute toxicity to the effluent wastewater. The differences in test sensitivities can be attributed to the differences in the responses of the two different trophic organisms in the effluent wastewater. As a conclusion, this study showed that the *Vibrio fischeri* acute toxicity test is more sensitive than that *Daphnia magna* in the effluent of anaerobic ITBR.

In other words, the *Daphnia magna* was found to be resistant compared to *Vibrio fischeri*. From the acute toxicity tests it can be seen that different organisms were affected differently by the influent and effluent wastewaters. It should be pointed out, however, that the *Vibrio fischeri* and *Daphnia magna* acute tests are reference standards used world-wide for toxicity testing and represent one of the trophic level tests required in toxicity evaluation. The increment in the EC_{50} values indicated the reduction in acute toxicity from the influent and effluent of the anaerobic ITBR system. The total sensitivity scores were 5 and 10 for *Vibrio fischeri* and *Daphnia magna*, respectively, indicating that *Vibrio fischeri* is more sensitive than *Daphnia magna* (Table 6.62).

As a conclusion, this study showed that the *Vibrio fischeri* acute toxicity test is more sensitive than that *Vibrio fischeri* to the petrochemical industry wastewater in the influent and effluent of the ITBR samples**.**

6.7 Cost Analysis in the Aerobic CSTR, Anaerobic ITBR and Sequential Anaerobic ITBR/ Aerobic CSTR System

Cost estimation was done for the sequential anaerobic ITBR/aerobic CSTR system throughout the treatment of the COD_{dis} and PAHs from petrochemical wastewaters. The overall costs are represented by the sum of the capital costs (reactor design with instruments such as aerator and pump), energy costs for aeration-and pumping, chemical (rhamnolipid, $NaHCO_3$ and sodium thioglycolate) costs, labor costs and analysis costs. For the full scale system these costs strongly depend on the concentrations of the pollutants, on the flow rate of the influent and on the configuration of the reactor.

6.7.1 Chemical Costs

The chemical costs include the RD biosurfactant (0.30 €/year) expenses, the $NaHCO_3$ (0.25 €/year) and the sodium thioglycolate (0.26 €/year) expenses. The total chemical cost for the anaerobic treatment of the real petrochemical industry wastewater in sequential anaerobic/aerobic reactor system was calculated as 0.81 €/year (Table 6.63).

Table 6.63 Chemical costs for the real petrochemical industry wastewater treatment in sequential anaerobic/aerobic reactor system

Chemicals consumption	**Quantity (kg/year)**	**Unit Price (€/kg)**	**Cost (€/year)**
$NaHCO_3$	0.05	5	0.25
Sodium thioglycolate	0.02	13	0.26
RD	0.03	10	0.30
Total Cost			0.81

6.7.2 Analysis Costs

The analysis costs for the anaerobic treatment of the real petrochemical industry wastewater in the sequential anaerobic/aerobic reactor system are shown in Table

6.64. The total analysis costs was calculated as 780 €/year for COD_{dis} and PAHs analysis in the laboratory for the sequential anaerobic/aerobic system.

Table 6.64 Analysis costs for the real petrochemical industry wastewater treatment in the sequential anaerobic/aerobic system

Analysis consumption	**Sample frequency (sample/week)**	**Sample frequency (sample/year)**	**Unit Price (€/sample)**	**Cost (€/year)**
COD_{dis}	1	52	5	260
PAHs	1	52	10	520
Total Cost				780

6.7.3 Labor Costs

The labor costs for the treatment of real petrochemical industry wastewater in the sequential anaerobic/aerobic reactor system are shown in Table 6.65. The total labor cost was calculated as 780 €/year for one person.

Table 6.65 Labor costs for the real petrochemical industry wastewater treatment in sequential anaerobic/aerobic system

Labor consumption	**Labor hours (hours/week**	**Labor hours (hours/year)**	**Labor cost (€/hours)**	**Cost (€/year)**
1 person	3	156	5	780
Total Cost				780

6.7.4 Capital Costs

The capital costs including the costs of anaerobic and aerobic reactors and the apparatus (air and peristaltic pumps and heater) used in this study is shown in Table 6.66.

Table 6.66 Capital costs in the sequential anaerobic/aerobic system

Type of apparatus	**Capacity (L)**	**Quantity**	**Material**	**Cost (€)**
Anaerobic reactor	4.5	1	Stainless steel	500
Aerobic reactor	9	1	Stainless steel	100
Air pump	-	1	-	50
Peristaltic Pump	-	1	-	50
Heater	-	1	-	25
Total Cost				725

The total capital costs including the anaerobic/aerobic reactors (600 €), the pumps (air and peristaltic pump) (100 €) and the heater (25 €) was calculated as 725 € (Table 6.66). The pay-back period of the capital investment is estimated at around 4 years.

6.7.5 Electricity Expenses in the Sequential Anaerobic/ Aerobic System

The peristaltic pump consumes 0.005 kWh of electric energy per hour. The air pump in the aeration tank of the CSTR reactor consumes 0.01 kWh of electric energy per hour. The heater in the sequential anaerobic/aerobic reactor system consumes 0.01 kWh of electric energy per hour. The electric energy consumed by the total sequential anaerobic/aerobic reactor is equal to 9.13 kWh per year (Table 6.67) while the total electricity cost was 0.90 €/year.

Table 6.67 Electricity consumption costs for the apparatus used in the sequential anaerobic/ aerobic reactor system the treating the real petrochemical industry wastewater

Type of Consumption	**Consumption (kWh)**	**Consumption (kWh/year)**	**Unit price (€/kWh)**	**Total cost (€/year)**
Air pump	0.01 (only aerobic CSTR)	3.65	0.098	0.36
Peristaltic pump	0.005	1.83	0.098	0.18
Heater	0.01	3.65	0.098	0.36
Total electricity consumption	0.025	9.13	0.098	0.90

6.7.6 Electric Energy Obtained from the Methane Gas and Electricity Equivalent of Methane Gas

The CH_4 produced from the anaerobic ITBR system treating COD_{dis} and PAHs at a HRT of 2.75 days is 0.0021 m^3CH_4/day. The annual methane production was 0.77 m^3CH_4/year. The electricity generation through CH_4 utilization was 2.90 kWh/m^3CH_4 (Ozdemir et al., 2006**)**. The total generated electricity was found as 2.23 kwh/m^3 CH_4 by multiplying the electricity equivalent of CH_4 (kWh/m^3CH_4) in the anaerobic ITBR system (Table 6.68).

Table 6.68 The electricity equivalent of CH_4 production in the anaerobic ITBR system (HRT=2.75 days, SRT=63 days, RD=75 mg/L)

CH_4 production (m^3CH_4/day)	0.0021
Annual CH_4 production (m^3CH_4/year)	0.77
Electricity equivalent of CH_4 (kWh/m^3CH_4)	2.90
Total generated electricity equivalent of CH_4 (kWh/m^3CH_4)	2.23
Electricity sale price (€/kWh)	0.098
Total electricity income from CH_4 (€/year)	0.22

If the energy obtained from the anaerobic ITBR system is compared with the consumed energy; the electricity obtained from the methane is 2.23 kWh/year (See Table 6.68) while the consumed energy was 9.13 kWh/ year (See Table 6.67) for the anaerobic ITBR system.

The total cost consisted of the chemical, of the analysis (COD_{dis} and PAHs), of the labor, of the capital, of the electricity expenses in the sequential anaerobic/aerobic reactor system (See Table 6.69).

Table 6.69 Total (chemicals, COD_{dis} and PAHs analysis, labor, capital and electricity) costs for the sequential anaerobic/aerobic system

Type of costs	**Information for Consumption**	**Cost (**€/year**)**
Chemical	$NaHCO_3$, Sodium thioglycolate, RD	0.81
Analysis	Analysis consumption for COD_{dis} and PAHs	780
Labor	The labor consumption for 1 person	780
Capital	Anaerobic, aerobic reactors and apparatus	725
Electricity	Air-peristaltic pump and heater	0.90
Total Cost		2287

It can be said that the total cost of the sequential anaerobic/aerobic reactor consisted of the capital cost including the chemical costs (0.81 €/year), the analysis costs (780 €/year), the labor costs (780 €/year), the capital cost (725 €/year) and the electricity costs (0.90 €/year). The total estimated cost was 2287 €/year for treating the real petrochemical industry wastewaters containing 292 ng/mL PAHs (See Table 6.69). The electric energy obtained from the methane in the anaerobic reactor was 182 kWh/year (See Table 6.68) while the total electric energy consumed in the sequential anaerobic/aerobic system was 9.13 kWh/year. 24% of the energy expenses could be recovered from the CH_4 production as follows:

$$\text{The energy recovered from the } CH_4 \text{ production}(\%) = \frac{production}{consumption} \times 100$$

$$\text{The energy recovered from the } CH_4 \text{ production}(\%) = \frac{2.22}{9.13} \times 100 = 24$$

$$\text{Total unit treatment cost} = \frac{\text{operation costs (chemicals and electricity consumption) (€/year)}}{\text{total flow rate } (m^3/\text{year})}$$

Unit cost for 1 m^3 petrochemical industry wastewater was calculated [(1.71(€/year) /0.73 (m^3/year)] 2.34 €/m^3 wastewater at optimum operation conditions in the sequential anaerobic/aerobic reactor for treating the real petrochemical industry wastewater.

CHAPTER SEVEN
CONCLUSIONS

7.1 The Performance of Aerobic CSTR Treating the Real Petrochemical Industry Wastewater

Although the contribution of biosurfactants [(Rhamnolipid (RD), Emulsan (EM) and Surfactin (SR)] used in this study are significantly affect the removals of COD_{dis} and total PAH the maximum yields was obtained at RD concentration of 15 mg/L for real petrochemical industry wastewater. Among the SRTs used (5-10-25 and 40 days) it was found 25 days was the optimum SRT for maximum total PAHs and COD_{dis} yields. The maximum total PAH (96%) and COD_{dis} (97%) yields were obtained at this RD concentration at a SRT and HRT of 25 and 5 days, respectively, in the CSTR for real petrochemical industry wastewater containing 65.32 ng/mL and 1200 mg/L COD_{dis} concentration. In other words the administration of 15 mg/L increased the total PAH yields to 97% from 74% in the comparison to the CSTR free of RD. In this study 15 PAHs namely acenaphthene (ACT), fluorene (FLN), phenanthrene (PHE), anthracene (ANT), carbazole (CRB), fluoranthene (FL), pyrene (PY), benz[a]anthracene (BaA), chrysene (CHR), benz[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (IcdP), dibenz[a,h]anthracene (DahA) and benzo[g,h,i]perylene (BghiP) are effectively biodegraded in the CSTR. In the presences of 15 mg/L RD the PAHs with high benzene rings [(BkF, BaP, IcdP, DahA and BghiP)] (95-96% removal efficiencies) were removed as high as PAHs with low benzene rings [(ACT, FLN, PHE, ANT and CRB)] (94-97% removal efficiencies) at a HRT and SRT of 5 and 25 days, respectively. In the study it was found that the RD biosurfactant improved excessively the PAH bioconversion process by increasing the numbers of PAH degrading bacteria. It was found that probably a significant uptake of the PAHs by PAH degrading bacteria together with biosurfactant occurred as a preferential adsorption of biosurfactants on the bacterial cell membrane to prevent the PAH uptake was observed at a RD of 15 mg/L.

The numbers of PAH degrading bacteria (*Pseudomonas aeruginosa* and *Zoogloea ramigera*) increased from $6x10^2$ colony/100mL (control, without RD) and $2x10^2$ colony/100mL (control, without RD) up to $6x10^8$ colony/100mL and $8x10^7$ colony/100mL in the presence of 15 mg/L RD in the CSTR treating real petrochemical industry wastewater. The presence of RD supported and stimulated the cell growth with respect to the control, whereas EM an SR biosurfactant did not favor the growth of the *Zoogloea ramigera* and *Pseudomonas aeruginosa* significantly. These biosurfactants are probably utilized by the *Zoogloea ramigera* and *Pseudomonas aeruginosa* bacteria as an additional carbon source in the CSTR.

The metabolites produced from the ACT and FLN were NAP, 8-dihyrox-1,4-naphthoquinone; 9-hydroxyflourene, respectively in aerobic CSTR at a HRT and SRT of 5 and 25 days in the presence of 15 mg/L RD . Similarly the metabolites produced from PHE, BaP, IcdP and DahA were 9-phenantrol, pyrene-8-hydroxy-7-carboxylic acid; 5,8-dihyrox-1,4-naphthoquinone; BaA-7,12 dione and benzenedicarboxaldehyde, respectively. 29.43 ng/mL ACT, 9.39 ng/mL FLN and 15.01 ng/mL PHE converted to 17.27 ng/mL NAP; 3.89 ng/mL 5,8-dihyrox-1,4-naphthoquinone; 7.27 ng/mL 9-hydroxyflourene and 12.98 ng/mL 9-phenantrol respectively, throughout aerobic treatment at 15 mg/L RD concentration and at SRT and HRT of 25 and 5 days, respectively, in the CSTR. 0.07 ng/mL BaP, 0.13 ng/mL IcdP and 0.27 ng/mL DahA, converted to 0.06 ng/mL pyrene-8-hydroxy-7-carboxylic acid; 0.11 ng/mL 5,8-dihyrox-1,4-naphthoquinone; 0.004 ng/mL BaA, 0.002 ng/mL BaA-7,12 dione and 0.001 ng/mL 1,2-benzenedicarboxaldehyde, respectively, throughout aerobic treatment at 15 mg/L RD concentration in the CSTR at the same HRT and SRT. These metabolites were removed with high yields varying between 95% and 96% in the CSTR. The effluent ACT (1.17 ng/mL), FLN (0.28 ng/mL), PHE (0.6 ng/mL) BaP (0.0042), IcdP (0.0052) and DahA (0.011) concentration were 0.11, 0.21, 0.25, 0.004, 0.004, and 0.011 ng/mL respectively. These concentrations and the concentrations of all other PAHs in the effluent of the CSTR are low according to limits given by the U.S. Environmental Protection Agency (EPA, 2004) and the European Union (EU, 2008) (Total PAH limit in the effluent to the surface water is 1 ng/mL). Total PAHs were not including in the

Turkish Water Pollution and Control Regulation for Treated Wastewaters discharges (Water Pollution and Control Regulation, 2004) and in the Regulation for Control of Pollution Causing by the Toxic Substances around Water and Environment (Regulation for Control of Pollution Causing by the Toxic Substances around Water and Environment, 2005). In the last regulation the names of ANT, CHR, FL, BaP, BbF, BghiP and IcdP PAHs were observed however it was not found limitations for the aforementioned PAHs.

The main removal mechanisms of the total PAHs and COD_{dis} was found to be biodegradation in the CSTR at a RD concentration of 15 mg/L, at a SRT and HRT of 25 and 5 days, respectively, in the CSTR treating real petrochemical industry wastewater. 94–97% of the total PAHs was biodegraded, 1.1–1.5% and 0.7–1.2% of the total PAHs were accumulated in the sludge and in the mixed liquor of the aerobic reactor, respectively, while 0.9–1.3% of the total PAHs were in the effluent of the CSTR at 15 mg/L RD, at a SRT of 25 days and at a HRT of 5 days.

The concentrations of RD in the aerobic activated sludge and in the effluent were in the range of 0.25–0.40 mg/L and 0.20–0.38 mg/L, respectively, in the end of aerobic treatment in the CSTR at an initial RD concentration of 15.01–15.09 mg/L. As results it can be said that 14.31–14.61 mg/L RD was biodegraded throughout 94–97% total PAH treatment in the CSTR at a SRT of 25 days and at a HRT of 5 days.

15 mg/L RD increased the removal efficiencies of the readily degradable COD (COD_{rd}) and slowly degradable COD (COD_{sd}) from 78% and 2% up to 91% and 74% respectively, compared to the control CSTR without RD at a SRT of 25 days and at a HRT of 5 days. Similarly 15 mg/L RD increased the inert COD (COD_i) and metabolic products COD (COD_{mp}) removal efficiencies from zero up to 82% and 99% respectively, compared to the control CSTR reactor without RD at a SRT of 25 days and at a HRT of 5 days. The high COD subcategories yields found in the study could be attributed to the uptake of inert COD to the bacteria cell together with hydrolyzed slowly degradable organics through fast PAH diffusion with RD. On the other hand the slowly degradable COD in the CSTR is transformed into readily

degradable COD. Probably the readily degradable COD converted into stored material in bacteria through endogenous phase. The stored compounds are probably used as a carbon and energy source for growth purposes.

15 mg/L RD increased BOD_5/COD_{dis} ratio from 0.23 to 0.37 indicating biodegradability of the effluent wastewater compared to CSTR without RD. This RD concentration increased Oxygen Utilization Rate (OUR) from 70 to 198 mg/L.h compared to CSTR containing no RD. Increasing the temperature from 21 to 45 ^{o}C elevated the total PAHs yield from 95% up to 99% at a RD of 15 mg/L. Positive ORPs such as +45 mV and +145 mV oxygenated environments (DO=4-5 mg/L) increase the yields of total PAHs. Electron acceptor such as NO_3^{-1} and SO_4^{-2} did not change the PAHs yields in the CSTR while the optimum pH values for maximum PAHs yields varied between 7 and 8.

The maximum acute toxicity removal was 90% at a SRT of 25 days at a HRT of 5 days in the presence of 15 mg/L RD in the aerobic CSTR in the bioassays test performed by *Daphnia magna*. The maximum acute toxicity removal performed by *Vibrio fischeri* was 79% at a SRT of 25 days at a HRT of 5 days in the presence of 15 mg/L RD in the aerobic CSTR reactor. The sensitivity test results showed that the *Vibrio fischeri* is more sensitive than the *Daphnia magna* to the raw and to the treated petrochemical industry wastewater in the CSTR.

Among the biodegradation kinetic models (Monod, zero order, half order, first order and second order) used in the treatment of real petrochemical industry wastewater in the CSTR it was found that Monod kinetic model was more appropriate than the other kinetic models to describe the biodegradation of PAHs and COD_{dis} in the aerobic CSTR at a SRT of 25 days in the presence of 15 mg/L RD at HRT of 5 days and SRT of 25 days. The K_s value decreased from 110.59 mg/L to 56.34 mg/L in the presence of 15 mg/L RD indicating the utilization of COD_{dis} by dominated bacteria in the CSTR reactor. On the other hand μ_{max} and Y kinetic constants increased from 0.10 1/day and from 0.45 mg VSS/mg up to 0.17 1/day and up to 0.86 mg VSS/mg in the presence of 15 mg/L RD.

Among the inhibition kinetics used in the CSTR (competitive, noncompetitive, uncompetitive and Haldane) it was observed that the biodegradation of PAHs in the petrochemical wastewaters were inhibited by competitive inhibition at RD, SR and EM concentrations >15 mg/L. Substantial inhibitory effect on K_s value was observed for PAHs, as evidenced by a decrease in inhibition constant (K_{ID}). This inhibition can be characterized as a system with low affinity to PAHs and indicates the difficulties in the metabolizability of PAHs.

The total annual cost in the aerobic CSTR was calculated as 1761 €/year including the capital (100 €/year) apparatus (air and peristaltic pump) (100 €/year), chemical (RD) (0.30 €/year), analysis (780 €/year) labor (780 €/year) and electricity (0.54 €/year) costs through biodegradation PAHs and COD_{dis} with maximum yields of 96% and 97%, respectively.

7.2 The performance of anaerobic ITBR system treating the real petrochemical industry wastewater

Among the HRTs used (1.38-1.83-2.75-.5.5-11 days) it was found that the optimum HRTs for maximum total PAH (88%) and COD_{dis} yields (94%) varied between 1.38 and 2.75 days. Among the RD concentrations (15-50-75-100-150 mg/L) used in the anaerobic ITBR system 75 mg/L RD increased the COD_{dis} and the total PAHs yields from 72% to 94% and from 71% to 88% in comparison to RD free ITBR reactors at a HRT of 2.75 days, at a SRT of 63 days, at an OLR of 1.04 g COD_{dis}/m^3.day (COD_{dis} concentration=2850 mg/L) and at a OLR of 106.18 ng PAH/mL.day (total PAH concentration=292 ng/mL) in the anaerobic ITBR system.

The VSS analysis showed that the anaerobic ITBR performance was directly related with the VSS accumulated surrounding of the Omega U-Spheres W as carrier material. The contribution of VSS in the mixed liquor of the ITBR to the total PAH and COD_{dis} yields was found to be minor. The properties of the carriers used in the

ITBR provided a big performance on the treatment petrochemical industry wastewater containing the PAHs, since the accumulation of biomass around carriers has a great importance.

The maximum total gas, methane gas productions and the methane percentage were measured as 3.10 L/day, 2.10 L/day and 67%, respectively, at 75 mg/L RD at a HRT of 2.75 days, at a SRT of 63 days, at a OLR of 1.04 g COD_{dis}/m^3.day and at a OLR of 106.18 ng PAH/mL.day in the anaerobic ITBR system at an initial PAHs concentration of 292 ng/mL. The PAHs and the COD_{dis} in real petrochemical wastewaters were ultimately biodegraded to H_2O and CO_2 in the methanogenesis phase of the anaerobic treatment in the ITBR with high methane gas yields. The Bic. Alk. and TVFA concentrations and TVFA/Bic. Alk. ratios were between the limit values know for anaerobic reactor although it was studied it at a toxic wastewater with a PAH concentration of 292 ng/mL.

The maximum acute toxicity removals were 93% and 83% for *Daphnia magna* and *Vibrio fischeri* acute toxicity tests, respectively, at a SRT of 63 days, at a HRT of 2.75 days in the presence of 75 mg/L RD in the anaerobic ITBR system. The acute toxicity results showed that the *Daphnia magna* is more resistant than *Vibrio fischeri* to the petrochemical industry wastewater.

Among the kinetic models applied to the experimental data obtained from the continuous operation of the anaerobic ITBR system it was found that the Modified Stover-Kincannon kinetic model was more appropriate than Monod and to the Contois kinetic models to the describe the anaerobic biodegradation of COD_{dis} and PAHs at 75 mg/L RD and at five HRTs varying between 1.38 and 11 days, in the comparison of the value of kinetic constants and the regression coefficient (R^2). K_B and R_{max} was 44.91 mgCOD_{dis}/L.day and 13.37 mgCOD_{dis}/L.day, respectively with high regression coefficient (R^2=0.99) for COD_{dis}. Similarly, the K_B and R_{max} values for PAHs were obtained as 20.12 ngPAH/mL.day and 12.28 ng/mL.day, respectively, with high regression coefficient (R^2=0.99).

Among the biogas kinetic models applied to the ITBR it was found that the Modified Stover-Kincannon was more appropriate to determine the total and methane gas productions compared to the Van der Meer & Heertjes kinetic model. The maximum methane gas production rate (M_{max}) and gas kinetic constant (M_B) were calculated as 495 mL/L.day and 0.42 mg/L.day, respectively. The following equation could be used to estimate the methane gas productions in the anaerobic ITBR reactor treating petrochemical wastewater with 292 ng/mL total PAH concentration at a SRT of 63 days, at a HRT of 2.75 days yielding 88% PAHs.

$$\frac{1}{M} = \frac{0.42}{495}\frac{1}{OLR} + \frac{1}{495} \tag{6.2}$$

The electricity funds obtained from the methane gas obtained in the anaerobic ITBR system is 2.11 kWh/year while the consumed total energy was 9.13 kWh/year including the air pump, heater and peristaltic pumps for the anaerobic ITBR system. The total cost spending throughout operation of ITBR consisted of capital cost (625 €/year), chemical costs (0.81 €/year), labor cost (780 €/year), analysis (780 €/year) and electricity cost (0.54 €/year). As a result the total cost was calculated as 2186 €/year for ITBR treating petrochemical wastewaters a PAHs concentration of 292 ng/mL yielding with 88% PAHs. It was found that 24% of the energy expenses could be recovered from the CH_4 gas production.

7.3 The performance of sequential anaerobic ITBR/aerobic CSTR system treating the real petrochemical industry wastewater

The maximum COD_{dis} and total PAHs yield were 99.8% and 99.5% respectively in the sequential anaerobic ITBR/aerobic CSTR system in the presence of 75 mg/L RD at a total SRT 88 days, at a total HRT of 7.75 days, at a total OLR of 1.039 g COD_{dis}/m^3.day and at a total OLR of 113.19 ngPAH/mL.day. The PAHs and COD_{dis} in the real petrochemical industry wastewater were mainly removed in the anaerobic of the sequential anaerobic/aerobic system. From 292 ng/mL initial total PAHs concentration of 256.96 ng/mL PAH were biodegraded (88% PAH yield) in

the anaerobic ITBR. Since the effluent of the ITBR was used as feed of the CSTR the PAH concentration (35.04 ng/mL) remaining from the anaerobic reactor was removed with a yield of 96% in the CSTR ending with a PAH concentration of 1.40 ng/mL in the effluent of CSTR. From 2850 mg/L initial COD_{dis} 2679 mg/L was biodegraded in the anaerobic ITBR similarly to the PAH. The effluent of ITBR containing 171 mg/L COD_{dis} was removed in the CSTR with a yield of 97% resulting in COD_{dis} effluent concentration of 5.13 mg/L.

The contribution of aerobic reactor to the removal of PAH in the sequential system was the biodegradation of PAHs in the effluent of the anaerobic reactor (35.04 ng/mL) and to mineralize the PAH metabolites produced from the ITBR. Although the COD_{dis} concentration (171 mg/L) in the effluent of the ITBR is between the discharge limits for COD given by the Turkish Water Pollution and Control Regulation (2004) the aerobic reactor provides the ultimate mineralization of PAHs and of carbonaceous organic metabolites.

The maximum acute toxicity yield in the sequential anaerobic ITBR/aerobic CSTR system effluent was 96% for *Daphnia magna* at a total HRT of 7.75 days, at a total SRT of 88 days at a total RD concentration of 90 mg/L. The maximum acute toxicity yield in the sequential anaerobic ITBR/aerobic CSTR system effluent was 92% for *Vibrio fischeri* at a total HRT of 7.75 days, at a total SRT of 88 days at a total RD concentration of 90 mg/L.

In this study, the total annual cost for sequential anaerobic ITBR/aerobic CSTR system was calculated as 2287 €/year. The electric energy obtained from the methane in the anaerobic reactor can be used to recover a part of the total electricity spends for sequential reactor. 24% of the energy expenses could be recovered from the CH_4 production in the anaerobic reactor.

7.4 Recommendations

Activated sludge is the most widely used biological wastewater treatment process to treat petroleum refinery industry wastewaters in Turkey. However, the removal efficiencies of PAHs are low in the conventional aerobic activated sludge reactor system treating this wastewater. Since the Turkish Water Pollution Control Regulation has no limitation for PAHs concentrations in the effluent discharges the PAHs could be sending to the receiving environments before treating effectively. As a result, the greater parts of the PAHs are sent to the receiving bodies without treatment and accumulate in the aquatic ecosystem. The results of this study showed that the biosurfactants can significantly improve the individual 15 PAHs (ACT, FLN, PHE, ANT, CRB, FL, PY, BaA, CHR, BbF, BkF, BaP, IcdP, DahA and BghiP), dissoleved COD (COD_{dis}), inert COD (COD_i), inert metabolite product COD (COD_{imp}), slowly degradable COD (COD_{sd}) and readily degradable COD (COD_{rd}) yields. The results of this study showed that the sequential anaerobic/aerobic system with biosurfactants can be used effectively to treat the toxic petrochemical industry wastewaters containing PAHs. Furthermore, it can be recommended to treat the chemical industrial wastewater containing PAHs, coal gasification wastewaters, iron and steel foundries wastewaters and carbon electrode production wastewaters.

The utilization of anaerobic ITBR reactor provides energy recovery from methane gas production. Among the anaerobic reactors (anaerobic fluidized beds, anaerobic expanded bed, up-flow anaerobic sludge blanket and anaerobic baffled reactors), in the ITBR floating particles are fluidized by a down flow current of liquid. The main advantages of the ITBR were: the down-flow configuration enables over coated particles to be recovered in the bottom of the bed. Moreover, the liquid and the biogas are flowing in opposite directions, which help for bed expansion the expansion of a floating carrier is also possible under an up-flow current of gas only. This phenomenon called pseudo-fluidization. The gas bubbles generate downward liquid motions and apparent bed expansion. As a result in the anaerobic ITBR system the PAHs and the COD_{dis} were removed with high yields.

From the appropriate biodegradation kinetic models the obtained kinetic constants could be used to estimate the biodegradation yields in similar industries under anaerobic and aerobic conditions. In the excess biosurfactant concentration the obtained inhibition kinetic can be used to evaluate the hindering substance in the aerobic reactor. The biodegradation and inhibition kinetic models can be recommended for similar industry wastewaters containing PAHs. Among the two bioassay tests used in this study it was found that *Vibrio fischeri* acute toxicity test could be recommended to determine the toxicity of the petrochemical wastewater as a sensitive organism.

The results of this laboratory scale study can be used to apply the data in a pilot scale or in a land space as follows:

a- In the first suggestion the optimum biosurfactant concentration can be administered to the present aerobic conventional activated sludge process treating petrochemical industry wastewaters, in the absence of suitable land and in the case of insufficient money.

b- In the second suggestion an anaerobic inverse turbulent bed reactor (ITBR) can be placed to the front of the aerobic conventional activated sludge process as pre-treatment step. The energy obtained from the anaerobic reactor could be used to recover the expenses.

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