DOKUZ EYLÜL UNIVERSITY GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

ANAEROBIC/AEROBIC SEQUENTIAL TREATMENT OF CHLORAMPHENICOLE AND STREPTOMYCIN ANTIBIOTICS USING ANAEROBIC BAFFLED AND AEROBIC SLUDGE REACTORS

by

Seçil TÜZÜN

October, 2009

İZMİR

ANAEROBIC/AEROBIC SEQUENTIAL TREATMENT OF CHLORAMPHENICOLE AND STREPTOMYCIN ANTIBIOTICS USING ANAEROBIC BAFFLED AND AEROBIC SLUDGE REACTORS

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by
Seçil TÜZÜN

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M.Sc. THESIS EXAMINATION RESULT FORM

We have read the thesis entitled "**ANAEROBIC/AEROBIC SEQUENTIAL TREATMENT OF CHLORAMPHENICOLE AND STREPTOMYCIN ANTIBIOTICS USING ANAEROBIC BAFFLED AND AEROBIC SLUDGE REACTORS**" completed by **SEÇİL TÜZÜN** 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 Master of Science.

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ANAEROBIC/AEROBIC SEQUENTIAL TREATMENT OF CHLORAMPHENICOLE AND STREPTOMYCIN ANTIBIOTICS USING ANAEROBIC BAFFLED AND AEROBIC SLUDGE REACTORS

ABSTRACT

In the context of this thesis, treatability of Streptomycin and Chloramphenicole, antibiotics which are toxic and non-degradable were experienced with the increasing dosages of Streptomycin and Chloramphenicole in a sequencing anaerobic baffled reactor (ABR)/aerobic continuous stirred tank reactor (CSTR) system. Furthermore, the effects of decreasing hyraulic residence time (HRT) on the performance of sequencing ABR/CSTR reactor system was investigated. COD, streptomycin and cloramphenicole removal efficiencies, total, metan gas productions, methane percentage, TVFA, Bic. Alk., TVFA/Bic.Alk. ratios were investigated in ABR reactor at increasing streptomycin and chloramphenicole concentrations and decreasing HRTs. The maximum chemical oxygen demand removal efficiency was between 89-95 percentage and 94-95 percentage at streptomycin and chloramphenicole concentrations varying between 100-200 mg/L and 50-130 mg/L in the ABR reactor. The maximum methane percentage of the biogas were 53and 58 percetages at a streptomycin concentration of 200 mg/L and chloramphenicole concentration of 130 mg/L. For maximum COD removal efficiency (94.5percentage) the optimum HRT was found as 19.2 days. The acute toxicity test results performed with *Daphnia magna* showed that the EC_{50} values decreased from influent 400 mg/L to 132 mg/L , and to 20 mg/L in the effluents of ABR , in aerobic reactor effluent at a HRT of 38.4 days. The total maximum streptomycin and chloramphenicole removal efficiency was 74 and 95 percentages in the sequential reactor system at an influent streptomycine(179.57 mg/L) and chloramphenicole(128mg/L) concentration at HRTs of 12.8 and 38,4 days, respectively. The kinetic constants found in the Monod and Grau kinetic models were found to be meaningfull for anaerobic degradation of streptomycin and chloramphenicol antibiotics.

Keywords: Anaerobic baffled reactor (ABR), Streptomycin, Chloramphenicole, Anaerobic treatment, Toxicity, Kinetic.

KLORAMFENİKOL VE STREPTOMİSİN ANTİBİYOTİKLERİNİN ARDIŞIK ANAEROBİK PERDELİ VE AEROBİK AKTİF ÇAMUR REAKTÖR SİSTEMLERDE ARITILMASI

ÖZ

Bu tez kapsamında toksik ve zor ayrışabilen antibiyotiklerden olan streptomisin ve kloramfenikolün arıtılabilirliği, ardışık Anaerobik Perdeli Reaktör (APR)/ Aerobik Sürekli Karıştırmalı Tank Reaktör (SKTR) sisteminde, artan streptomisin ve kloramfenikol dozlarında çalışılmıştır. Ayrıca, ardışık APR reaktörde/ SKTR reaktörde azalan hidrolik bekleme sürelerinin (HBS) etkileri incelenmiştir. APR reaktör de 100 ve 200 mg/L arasında değişen streptomisin ve 50-130 mg/L arasında değişen kloramfenikole konsantrasyonların da maksimum kimyasal oksijen ihtiyacı (KOİ) giderme verimi yüzde 89-95 ve yüzde 95-96 arasında sırasıyla değişmiştir. Streptomisin konsantrasyonu 0 dan 400 mg/L ye ve kloramfenikole konsantrasyonu 0 dan 340 mg/L ye arttırılırken, APR çıkışın da uçucu yağ asidi (UYA) / Bikarbonat Alkalinitesi (Bik. Alk.) oranı 0,368 ve 0,005 arasında değişmiştir ki bu APR reaktörün kararlılığını göstermektedir. Streptomisin 200 mg/L ve kloramfenicole 130 mg/L iken biogaz daki maksimum methane yüzdesi yüzde53 ve 58 dir. Maksimum KOİ giderme verimi (%94,5) için uygun HBS 19,2 gün olarak bulunmuştur. 38,4 gündeki HBS inde Daphniz magna (su piresi) lı akut toksiti de test sonuçları gösteriyor ki EC_{50} değerleri APR girişin deki 400 mg/L, APR çıkışın da 132 mg/L ve aerobik reaktör çıkışında 20 mg/L ye düşmektedir.12,8 günlük HBS inde girişte 179,57 mg/L lik streptomisin ve 128 mg/L lik kloramfenikol konsantrasyonlarında ardışık reaktör sisteminde, toplam maksimum streptomisin ve kloramfenikole giderme verimi yüzde 74 ve 95 dir. Bu çalışmada anlaşılmıştırki artakalan küçük miktardaki streptomisin ve kloramfenicol aerobik SKTR reaktör de giderilirken, büyük çoğunluğu aaaerobik APR reaktörde indirgenmiştir. Streptomisin ve kloramfenicole antibiyotikleri nin anaerobik indirgenme için en uygun kinetik sabiti Monod ve Grau kinetik modelleri olarak bulunmuştur.

Anahtar Kelimeler: Anaerobik perdeli reaktör (APR), Streptomisin, Kloramfenikole, Anaerobik arıtım, Toksisite, Kinetik.

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CHAPTER ONE
INTRODUCTION

1.1 Introduction

Antibiotics are an important group of pharmaceuticals in today's medicine. In addition to the treatment of human infections, they are also used in veterinary medicine such as streptomycin and chloramphenicole. Bacteria that are resistant to antibiotics are present in surface water (Kümmerer, 2009). Antibiotics are found in ground water at concentrations below than 10 μg/L. The source of antibiotics in ground water originating from the leaching the fertilized fields with animal slurry and from the waters passing through the sediments (Kümmerer, 2009).

The anaerobic treatability studies concerning the pharmaceuticals and antibiotics are limited with few studies: The performance of an upflow anaerobic filter (UAF) treating a chemical synthesis-based pharmaceutical wastewater was evaluated under various operating conditions (B Kasapgil Ince, A Selcuk and O Ince, 2002).The performance of an up-flow anaerobic stage reactor (UASR) treating pharmaceutical wastewater containing macrolide antibiotics was investigated (Shreeshivadasan Chelliapan, Thomas Wilby, Paul J. Sallis, 2006).The performance of a lab-scale hybrid up-flow anaerobic sludge blanket (UASB) reactor, treating a chemical synthesis-based pharmaceutical wastewater, was evaluated under different operating conditions. This study consisted of two experimental stages: first, acclimation to the Pharmaceutical wastewater and second determination of maximum loading rate(OLR) 1 kg COD/m^3d (Yalcin Aksin Oktem, Orhan İnce, Paul Sallis, Tom Donnelly, Bahar Kasapgil Ince, 2007).A four-compartment periodic anaerobic baffled reactor (PABR) was run in a 'clockwise sequential' switching manner continuously fed on chinese traditional medicine industrial wastewater (Xiaolei Liu, Nanqi Ren, Yixing Yuan, 2009).

The anaerobic baffled reactor (ABR) is high rate anaerobic reactor offering two-phase separation with a single vessel. The literature survey shows that there is a lack on the anaerobic treatment of streptomycin and chloramphenicole by ABR. In other

words, no study was found in the literature for the ABR reactor treating the wastewaters containing streptomycin and chloramphenicole.

1.2 The Objective and Scope of the Study

The general objective of this study was to evaluate the performance of the anaerobic baffled reactor (ABR) and to investigate the effect of their compartments on the treatment efficiency during various hydraulic retention time (HRT) and organic loading rates (OLR) using synthetic wastewater containing Streptomycin and Chloramphenicole, separately. The specific objectives of this study are as follows:

1. To determine the inhibition concentration of Streptomycin and Chloramphenicole which caused 50% decrease in the methanogenic activity (IC_{50}) in batch serum bottles. The batch studies gives information about the Streptomycin and Chloramphenicole doses will be used in the ABR reactor through continuous operation.

2. To determine the Streptomycin, Chloramphenicole and dissolved chemical oxygen demand (COD) removal efficiencies, total gas, methane gas productions, methane percentages in ABR reactor at increasing Streptomycin, and Chloramphenicole concentrations under constant flow rate (Q=2L/day) and hydraulic retention times (HRT=19,2days). Furthermore, to determine the effect of compartments, located in the reactors, on the total reactor performances (COD, Streptomycin, Chloramphenicole removal efficiencies, total volatile fatty acid (TVFA), bicarbonate alkalinity (Bic.Alk.) concentrations and TVFA/Bic.Alk. ratios at increasing Streptomycin and Chloramphenicole concentrations under constant flow and HRTs

3. To determine the total removal efficiency in sequential anaerobic ABR/ completely stirred tank rector (CSTR) system at increasing Streptomycin and Chloramphenicole concentrations under constant HRTs.

4. To determine the Streptomycin, Chloramphenicole and COD removal efficiencies, total gas, methane gas productions, methane percentages in ABR reactors at decreasing five HRTs (from 38,4 to 7,68 days) under constant Streptomycin (200 mg/L) and Chloramphenicole (130 mg/L) concentrations, separately. Furthermore, to determine the effect of compartments located in the reactor on the total reactor performances based on Streptomycin, Chloramphenicole, COD removal efficiencies, total volatile fatty acid, bicarbonate alkalinity (Bic.Alk.)concentrations and TVFA/Bic.Alk. ratios at decreasing five HRTs under constant Streptomycin and Chloramphenicole concentrations

5. To determine the total removal efficiency in sequential anaerobic ABR/ completely stirred tank rector (CSTR) system at decreasing hydraulic retention times (HRTs) under constant Streptomycin and Chloramphenicole concentration.

6. To determine the acute toxicity effect of Streptomycin through anaerobic/aerobic degradation in ABR/CSTR reactor system operated at constant Streptomycin concentration and different HRTs.

7. To determine the substrate (COD), Streptomycin and Cloramphenicole removal kinetics through continuous operation of the anaerobic ABR reactor.

In the first step of this study, the toxic effect of Streptomycin and Chloramphenicole on methane *Archaea* was investigated using anaerobic toxicity (ATA) test under batch conditions in the beginning of the study in order to determine the IC_{50} (The Streptomycin and Chloramphenicole concentrations which caused 50% decrease in the methanogenic activity) values of the Streptomycin and Chloramphenicole.

In the second step of this study COD, Streptomycin and Chloramphenicole treatabilities were studied in a sequential anaerobic ABR/aerobic completely stirred tank reactor (CSTR) reactor system at increasing Streptomycin and Chloramphenicole concentrations under constant flow rates. In this study, the COD, Streptomycin and Chloramphenicole removal efficiencies, total and methane gas productions, methane gas percentage were investigated at increasing Streptomycin and Chloramphenicole concentrations under constant flow rates. Furthermore the effects of compartments on the total reactor performances were determined with measuring Streptomycin, Chloramphenicole, COD, total volatile fatty acid, bicarbonate alkalinity (Bic.Alk.) concentrations and TVFA/Bic.Alk. ratios at increasing Streptomycin and Chloramphenicole concentrations and at constant HRTs.

In the third step of this study the COD, the Streptomycin and the Chloramphenicole treatabilities were studied in a sequential anaerobic ABR/aerobic CSTR reactor system at different HRTs under constant Streptomycin and Chloramphenicole concentrations. In this study, the COD, Streptomycin and Chloramphenicole removal efficiencies, total and methane gas productions, methane gas percentage were investigated at increasing flow rates. Furthermore the effects of compartments on the total reactor performances was determined with measuring Streptomycin, Chloramphenicole, COD, total volatile fatty acid, bicarbonate alkalinity (Bic.Alk.) concentrations and TVFA/Bic.Alk. ratios at decreasing HRTs and constant Streptomycin and Chloramphenicole concentrations. The acute toxic effect of synthetic wastewater containing Streptomycin was investigated, through anaerobic/aerobic degradation at decreasing HRTs using *Daphnia magna* test.

In the fourth step of this study, different kinetic models such as Monod, Contois, Stover-Kincannon, Grau-second order were applied to the experimental data obtained from the continuous operation of the ABR reactor to determine the suitable subsrate removal kinetic and relevant kinetic constants under different HRTs.

1.3 The Novelties of the Study

The novelties of the study can be summarized as follows:

1. The compartmentalisation structure of the ABR reactor increase the treatment efficiencies of THE anaerobic reactor. The first compartments play as acidogen phase while the subsequent compartments play as methanogen phases to treat the COD, TVFA, Streptomycin, Chloramphenicole and the intermediate products in the reactor.

2. The anaerobic substrate removal kinetics was investigated in the ABR reactor through Streptomycin and Chloramphenicole removals.

3. The addition of aerobic (CSTR) reactor on the effluent of the ABR reactor improve the removal efficiencies by removing the COD, Streptomycin, and Chloramphenicole remaining from the ABR aerobic, resulting in high removals in sequential anaerobic/aerobic reactor system.

4. Acute toxicity tests performed with Dalphnia magna to determine the responses of streptomycin antibiotic.

CHAPTER TWO
LITERATURE REVIEW

2.1 Antibiotics

An antibiotic is a substance or compound that kills bacteria or inhibits their growth. Antibiotics belong to the broader group of antimicrobial compounds, used to treat infections caused by microorganisms, including fungi and protozoa (Wikipedia, 2009).

The fate of antibiotics in the environment, and especially antibiotics used in animal husbandry, is subject to recent studies and the issue of this review. The scientific interest in antimicrobially active compounds in manure and soil, but also in surface and ground water, has increased during the last decade (Nicole Kemper, 2008).

Some antibiotics are characterized by a very narrow spectrum, whereas others possess a wide range of activity. Some are active only against certain bacteria and not against others, whereas some are active against fungi, and some against viruses. There is not only considerable qualitative variation in the activity of different antibiotics, but also wide quantitative differences. Antibiotics are produced by bacteria, fungi, actinomycetes, and, to a limited extent, by other groups of microorganisms (Science, New Series, 2009).

It is often assumed that hospitals are the most important source for the input of antibiotics and resistant into municipal waste water. The concentrations of antibiotics in municipal sewage and in sewage treatment plants are typically lower by a factor of 100 compared to hospital effluents (Klaus Kümmerer, 2009).Bacteria that are resistant to antibiotics are present in surface water. A correlation between resistant bacteria in rivers and urban water input has been found, as have antibiotic resistant genes (Kümmerer, 2009). Antibiotic- resistant bacteria were detected in drinking water as early as the 1980s and later in the 1990s. In agreement with these data,

increased phenotypic resistance rates were also detected at drinking water sampling points (Scoaris et al., 2007; K.Kümmerer, 2009).

Antibiotics are not completely eliminated in animal organisms, as they are bioactive substances, acting highly effectively at low doses and excreted after a short time of residence. Antibiotics are optimised with regard to their pharmacokinetics in the organisms: organic accumulation is, as in other pharmaceutics, objectionable and thus, they are excreted as parent compounds or metabolites (K.Kümmerer, 2009).The distribution of the veterinary antibiotics is given in Figure 2.1.

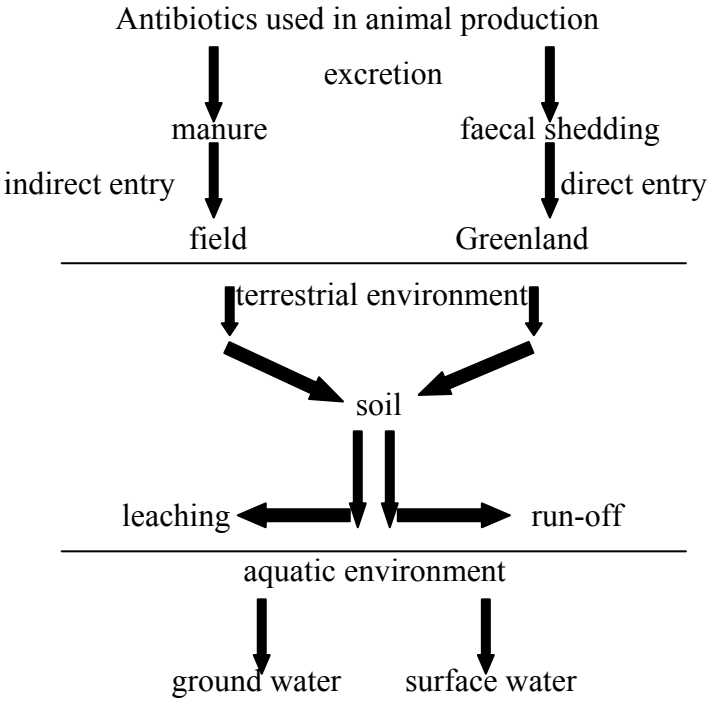


Figure 2.1. Veterinary antibiotics in the environment: anticipated exposure pathways.

2.1.1 Streptomycin

2.1.1.1 The Physical and Chemical Characteristics of the Streptomycin

Streptomycin is an antibiotic drug, the first of a class of drugs called amino glycosides to be discovered, and was the first antibiotic remedy for tuberculosis. Streptomycin was first isolated on October 19, 1943 by Albert Schatz, a graduate

student, in the laboratory of Selman Abraham Waksman at Rutgers University. The chemical identities of the streptomycin and physical and chemical characteristics of the streptomycin, in Tables 2.1 and 2.2, respectively (Wikipedia,2009).

Table 2.1 The chemical identities of the streptomycin (Wikipedia,2009).

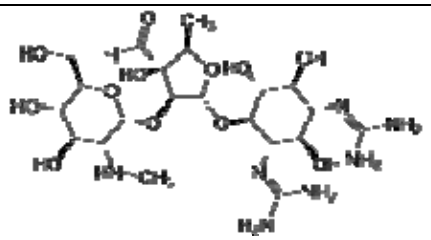
Characteristics	**Streptomycin**
Chemical name	Streptomycin
Chemical formula	$C_{21}H_{39}N_7O_{12}$
Chemical structure	

Table 2.2 The physical and chemical characteristics of the streptomycin (Wikipedia,2009).

Property	Streptomycin
Molecular weight	581.574 g/mol
Melt point	12 °C (54 °F)
Color	White
Half life	5 to 6 hours
Excretion	Renal
Bioavailability	84% to 88%
Routes	Intramuscular, intravenous

It has been known for more than six decades that certain fungi and bacteria are capable of producing chemical substances which have the capacity to inhibit the growth of, and even to destroy, pathogenic organisms. Only within the last twelve or thirteen years, however, have antibiotics begun to find extensive application as chemotherapeutic agents. Among these, penicillin and streptomycin have occupied a prominent place. Penicillin is largely active against gram-positive bacteria, gram-negative cocci, anaerobic bacteria, spirochetes and actinomycetes; streptomycin is

active against a variety of gram-negative and acid-fast bacteria, as well as against gram-positive organisms which have become resistant to penicillin.

Since the discovery of streptomycin, the production and clinical application of this antibiotic have had a phenomenal rise. The streptomycin producing strain of Streptomycin griseus was isolated in September, 1943, and the first public announcement of the antibiotic was made in January, 1944. Before the end of that year, streptomycin was already being submitted to clinical trial. Within 2 years, the practical potentialities of streptomycin for disease control were definitely established.

2.1.2 Chloramphenicole

2.1.2.1 The Physical and Chemical Characteristics of the Chloramphenicole

Chloramphenicole is a bacteriostatic antimicrobial originally derived from the bacterium *Streptomyces venezuelae*, isolated by David Gottlieb, and introduced into clinical practice in 1949. It was the first antibiotic to be manufactured synthetically on a large scale, and along side the tetracyclines, is considered the prototypical broad spectrum antibiotic (Wikipedia,2009).

Chloramphenicole is effective against a wide variety of Gram-positive and Gram-negative bacteria, including most anaerobic organisms. Due to resistance and safety concerns, it is no longer a first-line agent for any indication in developed nations and has been replaced by newer drugs in this setting, although it is sometimes used topically for eye infections. In low-income countries, chloramphenicole is still widely used because it is exceedingly inexpensive and readily available. The chemical identities of the chloramphenicole and physical and chemical characteristics of the chloramphenicole, in Tables 2.3 and 2.4, respectively (Wikipedia, 2009).

Table 2.3 The chemical identities of the chloramphenicole (Wikipedia,2009).

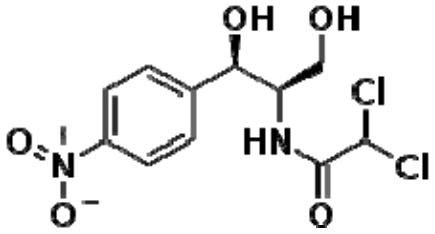
Characteristics	Chloramphenicole
Chemical name	Chloramphenicole
Chemical formula	$C_{11}H_{12}Cl_2N_2O_5$
Chemical structure	

Table 2.4 The physical and chemical characteristics of the chloramphenicole (Wikipedia,2009).

Property	Chloramphenicole
Molecular weight	323.132 g/mol
Melt point	151°C (303.8°F)
Color	Colorless to light yellow.
Half life	1.5–4.0 hours
Excretion	Renal
Bioavailability	75–90%
Odor	odorless
Taste	Bitter (strong)

2.2 Literature Review for the Treatment of Streptomycin

The study performed by Vanneste and co-workers (2008) showed that a pathogenic bacteria which is resistant to copper and streptomycin was isolated from the treated municipal wastewater. Therefore this wastewater could be not utilized for the irrigation of agriculture or horticulture (Wikipedia,2009).

B. Halling-Sùrensen, (2000) studied the growth inhibiting effects of eight antibiotics on two species of micro algae, Microcystis aeruginosa (freshwater cyanobacteria) and Selenastrum capricornutum (green algae). The effects of the antibiotics benzylpenicillin (penicillin G) (BP), chlortetracycline (CTC), olaquindox

(O), spiramycin (SP), streptomycin (ST), tetracycline (TC), tiamulin (TI) and tylosin (TY) were tested in accordance with the ISO 8692 (1989) standard protocol. Algal growth was measured as increase in chlorophyll concentration by extraction with ethanol followed by measurement of fluorescence. Results were quantifed in terms of growth rates using the Weibull equation to describe the concentration response relationship. The toxicity (EC_{50} value, mg/l) in alphabetic order were BP (0.006); CTC (0.05); O (5.1); SP (0.005); ST (0.007); TC (0.09); TI (0.003) and TY (0.034) for M. aeruginosa. BP (NOEC . 100); CTC (3.1); O (40); SP (2.3); ST (0.133); TC (2.2); TI (0.165) and TY (1.38) for S. capricornutum. In this investigation M. aeruginosa is found to be about two orders of magnitude more sensitive than S. Capricornutum (B. Halling-Sùrensen, 2000)

2.3 Literature Review for the Treatment of Chloramphenicole

Chloramphenicole (CAP), a broad-spectrum antibiotic, is a very effective veterinary drug and it is used to treat diseases of animal pathogen, which have become resistant to other commonly used antibiotics. In fish farming, CAP has been recommended for the treatment of Salmonella infections (D'Aoust, 1994). Although for CAP has no reported adverse effect on animal health, it has to be toxic to humans. CAP cause dose-related suppression of bone marrow, which results in many related diseased such as leucopenia. In view of the high toxic effects of CAP to humans, it has been subject to strict control in many countries around the world. Threfore In china, the use of chloramphenicole has been forbidden just now (Wang Weifen, Lin Hong, Xue Changhu, Khalid Jamil, 2004).

By Hong-Thih Lai , Jung-Hsin Hou, Chyong-Ing Su, Chun-Lang Chen,(2009) investigated the growth inhibition effects of three phenicol antibiotics on microalgae used in aquaculture. Different dose levels of chloramphenicole (CAP), florfenicol (FF), and thiamphenicol (TAP) were added to cultures of one freshwater green alga, Chlorella pyrenoidosa, and two marine algae, Isochrysis galbana and Tetraselmis chui. For the two marine algae, FF showed higher toxicity levels (EC_{50}, 1.3–8 mg/L) than CAP (4–41 mg/L) and TAP (38–158 mg/L). CAP was more toxic to the freshwater algae (EC_{50}, 14 mg/L) than FF (215 mg/L) and TAP (1283 mg/L). TAP

was the least toxic to the three algae, but it exhibited the highest stability during the test period. Among the tested algae, T. chui was the most sensitive species to the three antibiotics. This study demonstrated that all three phenicol antibiotics can inhibit growth of the three microalgae and should be carefully used in aquaculture.

In a study performed by Xianzhi Peng, Zhendi Wang, Wenxing Kuang, Jianhua Tan, Ken Li, (2006) the wastewater samples collected from two sewage treatment plants (STPs) in Guangzhou, China. The occurrence and the fate of antimicrobial compounds sulfadiazine (SDZ), sulfamethoxazole (SMX), ofloxacin (OFX) and chloramphenicol (CAP) were investigated. Antimicrobials have been detected at concentrations varying between 5.10–5.15, 5.45–7.91, 3.52–5.56 and 1.73–2.43 μg L^{-1} for SDZ, SMX, OFX and CAP in the raw sewages of the two STPs, respectively. The concentrations of antimicrobials do not show substantial changes after preliminary mechanical sedimentation. No quantifiable sulfonamides and chloramphenicol have been identified, and >85% of ofloxacin has been removed in the effluents after activated sludge treatment in the two STPs, indicating that activated sludge treatment is effective and necessary to removed the antimicrobial substances in municipal sewage.

2.4 Literature Revive for the Treatment of Anaerobic Baffled Reactor (ABR)

A review concerning the development, applicability and possible future application of the an aerobic baffled reactor for wastewater treatment is presented. The reactor design has been developed since the early 1980s. Anaerobic baffled reactor (ABR) is high-rate and compartmentalise reactor containing between 3 and 8 compartments (Barber & Stuckey, 1999).

ABR reactor consists of a series of baffles to forces the wastewater to flow from inlet to outlet. The flow is under and over the baffles. During upflow, wastewater contact with the active biomass. The ABR can be described as a series of upflow anaerobic sludge blanked reactor (UASB) (Barber & Stuckey, 1999).

The successful application of anaerobic technology to the treatment of industrial wastewaters is critically dependent on the development, and use, of high rate anaerobic bioreactors (Xiaolei Liu, Nanqi Ren, Yixing Yuan, 2009).

As the anaerobic baffled reactor (ABR) has been compared with traditional anaerobic reactors include higher resilience to hydraulic and organic shock loads, longer biomass retention times and lower sludge yields. There are no requirement unusual settling properties for biomass. The advantages of ABR reactor are summarized in Table 2,5 (Barber & Stuckey, 1999).

Table 2.4 Advantages associated with the anaerobic baffled reactor

Construction
1- Simple desing
2- No moving parts
3- No mechanic mixing
4- Inexpensive Construction
5- High void volume
6- Reducing clogging
7- Reduced sludge bed expension
8- Low capital and operating costs
Biomass
1- No requirement for biomass with unusual settling properties
2- Low sludge generetion
3- High solids retention times
4- Retention of biomass doses not require a solid-settling chamber
5- No special separation required for gas and sludge
Operation
1- Low HRT
2- Intermitten operation is possible
3- Extremely stable to hydraulic shock loads
4- Protection from toxic materials in influent
5- Long operation times without sludge wasting
6- High stability to organic shocks

There are several studies performed with ABR treating the different wastewaters such as palm oil mill effluent wastewater (Setiadi et al., 1996), swine wastes (Boopathy, 1998), pulp and paper mill black liquors (Grover et al., 1999), azodyes containing wastewater (Bell et al., 2000), landfill leachate (Wang and Shen, 2000), synthetic tannery wastewater containing sulfate and chromium(III) (Barber and Stuckey, 2000), treating whisky distillery wastewater (Akunna and Clark, 2000), nitrogen containing wastewaters (Bodik et al., 2003), sulfate containing wastewaters (Vossoughi et al., 2003), textile dye wastewater (Bell and Buckey, 2003), p-nitrophenol containing wastewaters (Kusçu and Sponza, 2005, 2006), and also domestic wastewaters (Dama et al., 2002).

Grover, Marwaha, & Kennedy, (1999) investigated the effect of different pH, temperatures, hydraulic retention times and organic loading rates on an anaerobic baffled reactor (ABR) treating black liquor from pulp and paper mills. A maximum COD reduction was found as 60% at HRT of 2 days.

The stability and performance of an anaerobic baffled reactor (ABR) treating an ice-cream wastewater was investigated at HRTs varied between 0.43 and 10 days (Uyanik, Sallis, & Anderson, 2002). COD removal efficiency was found as 99% at all HRTs. High COD removal efficiency in ABR came from its compartmentalized structure. The most of the influent COD was removed in compartment 1 (approximately 80%) through the study.

A four-compartment periodic anaerobic baffled reactor (PABR) was run in a ‘clockwise sequential’ switching manner continuously fed on chinese traditional medicine industrial wastewater under an alkalinity concentration between 1000 and 1500 mg $CaCO_3$/L of the feed with average organic loading rate (OLR) at about 1, 2, 4 and 6 kg COD/(m^3day) for 12, 24, 24 and 6 days, respectively. Hydraulic residence time as 2d, while switching period was 4d. As the average OLR increased to 6 kg COD/(m^3day), the time of the sharp fall in pH, chemical oxygen demand (COD) removal, gas production and methane percentage of the biogas of all the

compartments and the time of rapid volatile fatty acids accumulation in the effluent were investigated(Xiaolei Liu, Nanqi Ren, Yixing Yuan, 2009).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Experimental System

3.1.1 Anaerobic Baffled Reactor (ABR)/Completely Stirred Tank Reactor (CSTR) System

A schematic of the lab-scale sequential ABR and CSTR reactors used in this study are presented in Figure 3.1. The effluent of the anaerobic ABR reactor was used as the influent of aerobic CSTR reactor. The ABR reactor was rectangular box having the dimensions 20 cm wide, 60 cm long and 40 cm high. The ABR reactor with the active reactor volume (38.4 L) was divided into four equal compartments by vertical baffles. Each compartment was further divided into two by slanted edge (45◦C) baffles to encourage mixing within each compartment. Therefore, down-comer and up-comer regions were created. The liquid flow is alternatively upwards and downwards between compartment partitions. This provided effective mixing and contact between the wastewater and biomass at the base of each upcomer. In other words, during upflow, the waste flow contact with the active biomass and it is retained within the reactor providing a homogenous distribution of wastewater. An additional mixing was not supplied to the compartments of the reactor. The width of the downcomer was 4 cm and the width of the up-comer was 11 cm. The passage of the liquid from each compartment to another was through an opening with size 40 mm×10 mm which located about 80 mm from the top of each compartment. The liquid sampling ports were located at 40 mm back of the effluent opening of each compartment. The sludge sampling ports were also located in the center of the compartments and 80 mm above from the bottom of the each compartment. The influent feed was pumped using a peristaltic pump. The outlet of the ABR was connected to a glass U-tube for controlling the level of wastewater. The produced gas was collected via porthole in the top of the reactor. The operating temperature of the reactor was maintained constant at 37±1 ^{o}C by placing the ABR reactor on a heater. A digital temperature probe located in the middle part of the second compartment provided the constant operation temperature. This provided a homogenous temperature in whole compartments of ABR reactor. The aerobic CSTR reactor

consisted of an aerobic (effective volume=9 L) and a settling compartment (effective volume = 1.32 L).

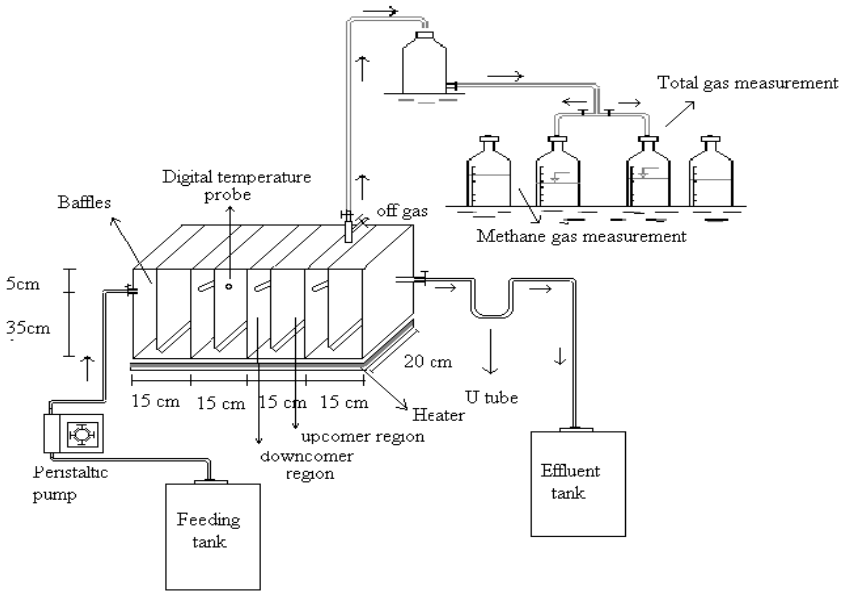


Figure 3.1 Schematic configurations of lab-scale anaerobic (ABR)/aerobic (CSTR) sequential reactor system.

3.2 Seed of Reactors

Partially granulated anaerobic sludge was used as seed in THE ABR reactor. The seed sludge was obtained from an anaerobic upflow anaerobic sludge blanket reactor containing acidogenic and methanogenic partially granulated biomass taken from the Pakmaya Yeast Beaker Factory in Izmir, Turkey. Activated sludge culture was used as seed for the aerobic CSTR reactor and it was taken from the activated sludge reactor of Pakmaya Yeast Beaker Factory in Izmir. The volatile suspended solid (VSS) concentration of seed sludge in ABR reactor was adjusted as 25 g/L. The mixed liquor solids concentration (MLSS) in the CSTR were adjusted between 3000 and 4000 mg/L.

3.3 Composition of Synthetic Wastewater

Streptomycin concentration varying between 25 and 400 mg/L and Chloramphenicole concentration varying between 50 and 340 mg/L were used

through continuous operation of the ABR reactor. Glucose was used as primary substrate giving a COD concentration of 3000 ± 100 mg/L. Vanderbilt mineral medium was used in synthetic wastewater as mineral source. This mineral medium consisted of the following inorganic composition (in mg/l): NH_4Cl, 400; $MgSO_4.7H_2O$, 400; KCl, 400; $Na_2S.9H_2O$, 300; $(NH_4)_2HPO_4$, 80; $CaCl_2.2H_2O$, 50; $FeCl_3.4H_2O$, 40; $CoCl_2.6H_2O$, 10; KI, 10; $(NaPO_3)_6$, 10; L-cysteine, 10; $AlCl_3.6H_2O$, 0.5; $MnCl_2.4H_2O$, 0.5; $CuCl_2$, 0.5; $ZnCl_2$,0.5; NH_4VO_3, 0.5; $NaMoO_4.2H_2O$, 0.5; H_3BO_3, 0.5; $NiCl_2.6H_2O$, 0.5; $NaWO_4.2H_2O$, 0.5; Na_2SeO_3, 0.5 (Speece, 1996). The anaerobic conditions were maintained by adding 667 mg/l of Sodium Thioglycollate (0.067 %) which is proposed between 0,01-0,2% (w/w) for maintaining the strick anaerobic conditions (Speece, 1996). The alkalinity and neutral pH were adjusted by addition of 5000 mg /L $NaHCO_3$.

3.4 Analytical Methods

3.4.1 Dissolved Chemical Oxygen Demand (DCOD) Measurement

The dissolved COD was measured calorimetrically by using closed reflux method (APHA AWWA, 1992). Firstly the samples were centrifuged 10.0 min at 7000 rpm. Secondly, 2.5 ml samples were mixed with 1.5 ml 10216 mg/l $K_2Cr_2O_7$, 33.3 g/l $HgSO_4$ and 3.5 ml 18 M H_2SO_4 containing 0.55% (w/w) Ag_2SO_4. Thirdly the closed sample tubes were stored in a heater with a temperature of 148°C for two hours. Finally, after cooling, the samples were measured at a wave-length of 600 nm with a Pharmacia LKB NovaPec II model spectrophotometer. The COD values given in Tables and in Figures are measured as dissolved COD (DCOD).

3.4.2 Gas Measurements

Gas productions were measured with liquid displacement method. The total gas was measured by passing it through a liquid containing 2% (v/v) H_2SO_4 and 10% (w/v) NaCl (Beydilli, Pavlosathis & Tincher, 1998). Methane gas was detected by using a liquid containing 3% NaOH to scrub out the carbon dioxide from the biogas (Razo-Flores et al., 1997). The methane gas percentage in biogas was also determined by Dräger Pac®Ex methane gas analyzer. The H_2S gas was measured

using Dräger (Stuttgart, Germany) kits in a Dräger H_2S meter. H_2 gas was measured using (Dräger Pac®Ex) H_2 meter. N_2 gas was measured by discarding of the sum of $CH_4 + H_2S + H_2$ gases from the total gas.

3.4.3 Mixed Liquor Suspended Solids (MLSS), Mixed Liquor Volatile Suspended Solids (MLVSS), Suspended Solids (SS) and Volatile Suspended Solid (VSS) Measurements

Biomass was measured as total suspended solid (TSS) and volatile suspended solid (VSS) in anaerobic reactors. Biomass in aerobic tank was measured as mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS). Assays were performed according to Standard Methods for Examination of Water and Wastewater (APHA AWWA, 1992).

3.4.4 Total Bicarbonate Alkalinity (Bic.Alk.) and Total Volatile Fatty Acid (TVFA) Measurements

Bicarbonate alkalinity (Bic.Alk.) and total volatile fatty acid (TVFA) concentrations were measured simultaneously using titrimetric method proposed by Anderson & Yang, (1992). The test was carried out as follows: firstly the pH of the sample was measured, secondly the sample was titrated with standard sulphuric acid (0.1 N) through two stages (first to pH=5.1, then from 5.1 to 3.5), and finally the VFA and Bic.Alk. concentrations were calculated with a computer program by solved the Eqs (3.1) and (3.2)

$$A_1=\frac{[HCO_3^-]*([H]_2-[H]_1)}{[H]_1+K_C}+\frac{[VA]*([H]_2-[H]_1)}{[H]_2+K_{VA}} \quad (3.1)$$

$$A_2=\frac{[HCO_3^-]*([H]_3-[H]_1)}{[H]_3+K_C}+\frac{[VA]*([H]_3-[H]_1)}{[H]_3+K_{VA}} \quad (3.2)$$

where A_1 and A_2 are the molar equivalent of the standard acid consumed to the first and second end points; $[HCO_3^-]$ the bicarbonate concentration; $[VA]$ the volatile

fatty acid ion concentration; $[H]_{1,2,3}$ the hydrogen ion concentrations of the original sample and at the first and the second end points; K_c is the conditional dissociation constant of carbonic acid; K_{VA} is the combined dissociation constant of the volatile fatty acids (C_2–C_6), this pair of constants was assumed, being 6.6×10^{-7} for bicarbonate and 2.4×10^{-5} for volatile acids.

3.4.5 Anaerobic Toxicity Assay (ATA) and Specific Methanogenic Activity (SMA)

ATA test was performed at 35°C using serum bottles with a capacity of 150 ml as described by Owen, Stuckey, Healy, Young, McCarty, (1979) and Donlon et al. (1996). Serum bottles were filled with 2000 mg VSS/L of biomass, 3000 mg /l of glucose-COD, suitable volume from the Vanderbilt mineral medium, 667 mg /l of sodium thioglycollate providing the reductive conditions and 5000 mg /l of $NaHCO_3$ for maintaining the neutral pH. Before ATA test, the serum bottles were batch operated until the variation in daily gas production was less than 15% at least for 7 consecutive days. After observing the steady-state conditions, increasing concentration streptomycin and chloramphenicole were administered to serum bottles asmslug-doses from concentrated stock solutions of these chemicals. The effects of Streptomycin and Chloramphenicole on methane gas production were compared with the control samples. Inhibition was defined as a decrease in cumulative methane compared to the control sample. IC_{50} value indicates the 50% inhibition of methane gas production in serum bottles containing toxicant. This value shows the presence of toxicity. This value shows the toxicant concentration caused 50% inhibition in the methane gas production.

The SMA test was conducted in 150 ml serum bottles at 35 °C under anaerobic conditions. Serum bottles were filled with 3000 mg/l of glucose-COD, with suitable amount of Vanderbilt mineral medium, 667 mg/l of sodium thioglycollate for to provide the reductive conditions and 5000 mg/l of $NaHCO_3$ for maintaining the neutral pH and 2000 mg VSS/L of biomass. Maximum specific methanogenic activity was calculated from the total methane production through 3 days with the method proposed by Owen et al., (1979) as follows:

$$\text{SMA } (gCH_4\text{-COD/g VSS day}) = \frac{\text{produced methane volume (ml) x 395 ml/ 1 gCOD}}{\text{sample (ml) x incubation time (day) x biomass concetration (g/l)}}$$

3.4.6 Toxicity Measurements

3.4.6.1 Daphnia Magna Toxicity Test

Toxicity was tested using 24 h born *Daphnia magna* as described in Standard Methods (2005). Test animals were obtained from the Science Faculty in Aegean University in Izmir. 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. Young *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 *Daphnias.* The immobile animals which were not able to move were determined as the death of *Daphnias.*

3.4.7 Antibiotics Measurements

3.4.7.1. Streptomycin Measurement

Preparation of 1000 mg/L Streptomycin stock standard; 0,5 g streptomycin is weighted in a beaker, it was put into a 500 ml of volumetric flask and it was filled with HPLC grade deionized water. 5, 50, 100, 150, 300 mg/L standard Streptomycin solutions were prepared from the 1000 mg/L of Streptomycin Stock Standard.

3.4.7.1.1 HPLC Equipment Specifications. HPLC Degasser (Agilent 1100), HPLC Pump (Agilent 1100), HPLC Auto-sampler (Agilent 1100), HPLC Column Oven (Agilent 1100), HPLC Diode-Array-Detector (DAD) (Agilent 1100).

3.4.7.1.2 HPLC Conditions for Streptomycin Analysis. A C-18 250x4,6 mm. (id), column (ACE) was used The mobile phase consisted of the HPLC grade

Acetonitrile at pH=3, Sodium Phosphate Buffer + Sodium 1-Hexanesulphonic Acid ratio was (8:92), The flow rate was 1ml/min, the column temperature was 20 ^{o}C, the wave length was 195 nm (UV) and the injection volume was 10 microliter.

3.4.7.1.3 Extraction Prosedure. 1 L sample was centrifuged using a filter with a pore size of 0,20 micrometer. The vials was filled with 2 ml of centifuged sample and it was injected into sampling portes of the HPLC (Kurosawa, N.; Kuribayashi, S.; Owada, E.;et all 1985).

3.4.7.2 Chloramphenicole Measurement

Preparation of 1000 mg/L Chloramphenicole Stock Standard; 0,5 g chloramphenicole is weighted in a beaker, it was put into a 500 ml of volumetric flask and it was filled with HPLC grade deionized water. 5, 50, 100, 150, 300 mg/L standard Chloramphenicole solutions were prepared from the 1000 mg/L of Chloramphenicole Stock Standard.

3.4.7.1.1 HPLC Equipment Spesifications. HPLC Degasser (Agilent 1100), HPLC Pump (Agilent 1100), HPLC Auto-sampler (Agilent 1100), HPLC Column Oven (Agilent 1100), HPLC Diode-Array-Detector (DAD) (Agilent 1100).

3.4.7.1.2 HPLC Conditions for Chloramphenicole Analysis. A C-18 250x4,6 mm. (id,) column (ACE) was used the mobile phase consisted of the HPLC grade Water: HPLC grade Methanol ratio was (40:60), the flow rate was 1mL/min, the column temperature was 30 ^{o}C, the wave length was 280 nm (UV) and the injection volume was 10 microliter.

3.4.7.1.3 Extraction Prosedure. 1 L sample was centrifuged using a filter with a pore size of 0,20 micrometer. The vials were filled with 2 ml of centifuged sample and it was injected into sampling portes of the HPLC (E.H. Allen, J.Assoc.Off.Anal.Chem., (1985)).

3.5 Operational Conditions

3.5.1 Start-up Period

The adaptation period is very important since the bacterial population used as seed is going to be exposed to the Streptomycin and Chloramphenicole in an anaerobic environment of the ABR reactor. In order to acclimation the partially granulated biomass in the ABR reactor, the anaerobic reactor was operated with synthetic wastewater through 92 and 12 days without streptomycin and chloramphenicole for reach to steady-state conditions. HRT and OLR were 19,2 days and 156,25 kgCOD/ m^3 days, respectively.

3.5.2 Operation Parameters of Anaerobic Baffled Reactor (ABR) and Aerobic Reactor

3.5.2.1 Sludge Retention Time (SRT, Θ_C)

Sludge retention time (SRT, θ_C) is the total quantity of active biomass in the reactor divided by the total quantity of active biomass withdrawn daily. Since no sludge wasting was applied for granule formation in the ABR reactor, SRT in this reactor was determined using equations (3.3) and (3.4) (Metcalf & Eddy, 1991)

$$SRT = \frac{V_r * X_r}{Q_e * X_e + Q_w * X_w} \tag{3.3}$$

Q_w and X_w were defined as flow rate and microorganism concentrations, respectively in wasted sludge stream. The term $Q_w * X_w$ only makes sense if there is a waste sludge stream. Since no sludge wasting was applied in the ABR reactor, SRT can be expressed as follows:

$$SRT = \frac{V_r * X_r}{Q_e * X_e} \tag{3.4}$$

The sludge wasting in a conventional CSTR reactor occurred from the settling tank and the solids in the effluent (X_e) were taken into consideration. Therefore, SRT in this reactor was calculated by using equation (3.6) with rearranged equation (3.5).

$$SRT = \frac{V_r * X_r}{Q_e * X_e + Q_w * X_w} \tag{3.5}$$

V_r and X_r are effective volume of reactor and microorganism concentration in the aeration tank. Qe and Xe were defined as flow rate and microorganism concentration measured in the settling tank. Q_w and X_w are the flow rate and microorganism concentration wasted from the reactor. The CSTRs used in this study are recycled reactors. In other words, the sludge was recycled 100% from the settling tank to the aeration tank. If the concentration of microorganism in the effluent of the settling tank is low, X_e is negligible (Metcalf & Eddy, 1991). In this study, the activated sludge was withdrawn from the inside of the aeration stage, the microorganism concentration in the reactor (X_r) was equal to the wasted microorganism concentration (X_w). Therefore, in this study the SRT in CSTR was calculated using equation (3.6).

$$SRT = \frac{V_r}{Q_w} \tag{3.6}$$

In this study, SRT (θc) in the CSTR reactor was adjusted as 20 days by discarding a certain amount of sludge volume from the aeration stage of the CSTR reactor. HRT in anaerobic reactors and CSTR were calculated using equation (3.7).

$$HRT = \frac{V_r}{Q} \tag{3.7}$$

Vr and Q were defined as reactor volume (l) and influent flowrate (L/day), respectively.

In the first step study OLR, HRT, streptomycin and chloramphenicole consentrations were 0,156 kg COD/m^3 days, 19,2 days, 25–400 mg/L and 50–340 mg/L, respectively.

In the second step study OLR, HRT, streptomycin and chloramphenicole concentration were 0,078- 0,156 – 0,234 – 0,312 – 0,391 kg COD/m^3 days, 38,4 – 19,2 – 12,8 – 9,60 – 7,68 days, 200 mg/L and 130 mg/L, respectively.

3.6 Kinetic Approaches in Anaerobic Continuous Studies

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 models are used to determine the importance of the relationships between variables to guide the experimental design and to evaluate the experimental results. These models also used to control and predict the treatment plant operation performance and to optimize the plant design and the results of scale-up pilot studies (Iza, Colleran, Paris, & Wu, 1991).

3.6.1 Application of Kinetic Model for ABR Reactor

3.6.1.1 Substrate Removal Kinetics

3.6.1.1.1 Application of Monod Kinetic: For a completely mixing ABR reactor with no biomass recycle, microbial and substrate mass balance can be expressed using Eq.3.8 and Eq.3.9.

A microbial mass balance for the reactor can be described as follows:
(Microbial Change Rate) =
(microbial input rate)+(microbial growth rate)-(microbial death rate)-(microbial output rate) (3.8)
Mathematically, Eq (3.8) can be written as Eq (3.9).

$$\frac{dx}{dt} = \frac{Q}{V} * X_i + \mu * X_r - k_d * X - \frac{Q}{V} * X_e \quad (3.9)$$

Where

V, Q , Xi , Xr , Xe are defined as the reactor volume (L), the flow rate (L/day), the concentration of biomass in the influent (g/L), the concentration of biomass in the reactor (g/L) and the concentration of biomass in the effluent (g/l). μ and kd are specific growth rate (day^{-1}) and the endogenous decay coefficient (day^{-1}).

The concentration of biomass in the influent is very small and can be neglected (Xi = 0). Also, there is no change in the microbial mass at steady state conditions (dX/dt = 0). Therefore, Eq (3.9) can be written as Eq (3.10).

$$\mu - k_d = \frac{Q}{V} * \frac{X_e}{X_r} \tag{3.10}$$

Since no sludge wasting was applied in the anaerobic reactors, sludge retention time (SRT=θ_c) was calculated from the Eq (3.11) based on both MLVSS concentration into reactor and MLVSS concentration in the effluent of reactor.

$$\theta_C = \frac{V}{Q} * \frac{X_r}{X_e} \tag{3.11}$$

Equation (3.11) can be rearenged as follows:

$$\mu - k_d = \frac{1}{\theta_C} \tag{3.12}$$

Where; (μ-k_d) is the net specific growth rate,day^{-1}. Equation (3.12) indicates that the net microbial growth decreases as the sludge retention time (SRT=θ_c) increases. The relationship between the specific growth rate and the rate limiting substrate concentration can be expressed by the Monod equation (3.13):

$$\mu = \frac{\mu_{\max} * S}{K_S + S} \tag{3.13}$$

Eq (3.13) can be rearenged as follows.

$$\frac{\mu_{\max} * S_I}{K_S + S_I} = \frac{1}{\theta_C} + k_d \tag{3.14}$$

$$\frac{\theta_c}{1 + \theta_c * k_d} = \frac{K_S}{\mu_{MAX}} * \frac{1}{S_I} + \frac{1}{\mu_{MAX}} \tag{3.15}$$

The value of maximum specific growth rate (μ_{max}) (day^{-1}) and half saturation concentration (K_s) (mg/l) could be determined by plotting the Eq (3.15). The value of μ_{max} can be calculated from the intercept of the straight line while K_S can be obtained from the slope of the line.

Substrate Mass Balance:

A substrate mass balance for the reactor can be described as Eq (3.16)

(substrate Change Rate)=
(substrate input rate)-(substrate utilization rate)-(substrate output rate) (3.16)

Mathematically, Eq (3.16) can be written as Eq (3.17).

$$\frac{dS}{dt} = \frac{Q}{V} * S_i - (\mu - k_d) * \frac{X_r}{Y} - \frac{Q}{V} * S_e \tag{3.17}$$

dS/dt is defined as the rate of substrate removal (g/L day). S_i and S_e are influent substrate concentration (g/L) and the effluent substrate concentration (g/L), respectively. Y is defined the growth yield coefficient (mass cell produced mass substrate utilized) (g VSS/g COD).

At steady rate dS/dt is 0. Thus, substrate balance at equilibrium can be rewritten as Eq (3.18).

$$\frac{(S_i - S_e)}{\theta_h} = (\mu - k_d) * \frac{X_r}{Y} \tag{3.18}$$

The equation given above can be reduced to equation (3.19)

$$\frac{(S_i - S_e)}{\theta_h} = \frac{X_r}{Y} * \left(\frac{1}{\theta_C} + k_d \right) \tag{3.19}$$

The kinetic parameters Y (g VSS / g COD), k_d can be obtained by rearranging Eq (3.19) as shown below:

$$\frac{(S_i - S_e)}{\theta_h * X_r} = \frac{1}{Y} * \left(\frac{1}{\theta_C} \right) + \left(\frac{1}{Y} \right) * k_d \tag{3.20}$$

The values of Y and k_d can determined by plotting ($1/\theta_c$) versus (S_i -S_e)/(Xr* θ_h). The value of k_d can be calculated from the intercept of the straight line while Y can be obtained from the slope of the line.

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

$$\mu = \frac{\mu_{\max} * S}{\beta * X_r + S} \tag{3.21}$$

Where

β is the contais kinetic parameter (g COD/g biomass).

By substituting Eq (3.21), instead of the Monod equation, into Eq (3.9) can be obtained Eq (3.22) can be obtained.

$$\frac{\mu_{\max} * S_i}{\beta * X + S_i} = \frac{1}{\theta_C} + k_d \tag{3.22}$$

If Eq (3.22) is rearranged, Eq (3.23) is obtained

$$\frac{\theta_c}{1 + \theta_c * k_d} = \frac{\beta}{\mu_{MAX}} * \frac{X_r}{S_i} + \frac{1}{\mu_{MAX}} \tag{3.23}$$

Similarly, the values of μ_{max} and β can be obtained by plotting the Eq (3.23). The value of μ_{max} can be calculated from the intercept of the straight line and finally, β can be obtained from the slope of the line.

3.6.1.1.3 Grau Second- Order Multicomponent Substrate Removal Model. The general equation of a Grau second-order kinetic model is illustrated in Eq (3.24) (Grau, Dohanyas, & Chudoba, 1975, Öztürk, Altinbas, Arikan, & Demir, 1998)

$$\frac{ds}{dt} = k_S * X_r * \left(\frac{S_e}{S_i} \right)^2 \tag{3.24}$$

If Eq (3.24) is integrated and then linearilized, Eq (3.25) will be obtained:

$$\frac{\left(S_i * \theta_h\right)}{S_i - S} = \theta_h + \frac{S_i}{K_S * X} \tag{3.25}$$

If the second term of the right part of Eq (3.25) is accepted as a constant, the Eq (3.26) will be obtained.

$$\frac{(S_I * \theta_h)}{S_I - S_e} = b * \theta_h + a \tag{3.26}$$

k_s is second-order substrate removal rate constant (L/day). If Eq (3.25) re-arranged, Eq (3.26) will be obtained. This equation could be used to predict the effluent COD and antibiotics concentrations.

$$S_e = S_I\left(1 - \frac{1}{(b + a/\theta_h)}\right) \tag{3.27}$$

Where;

a is equal S_i / (k_s *X) (day) and b are constant (dimensionless). $(S_i\text{-}S_e)/S_e$ expresses the substrate removal efficiency and is symbolized as E (efficiency). S_e and S_i are effluent and influent COD concentrations (mg COD/L). X_e and X_i are effluent and influent antibiotics concentrations (mg COD/L). X_r is the average biomass concentration in the reactor (mg VSS/L). θ_h is hydraulic retention time (day).

3.6.1.1.4 Modified Stover-Kincannon Model. In this model, the substrate utilization rate is expressed as a function of the organic loading rate by monomolecular kinetic for biofilm reactors such as rotating biological contactors and biological filters. A special feature of Modified Stover-Kincannon model is the utilization of the concept of total organic loading rate as the major parameter to describe the kinetics of an anaerobic filter in terms of organic matter removal and methane production. A modified Stover-Kincannon model could be used for ABR reactor as follows (Yu, Wilson, & Tay, 1998):

$$\frac{ds}{dt} = \frac{R_{\max} * (Q * S_I / V)}{K_B + (Q * S_I / V)} \tag{3.28}$$

Where; d*S*/d*t* is defined in Eq. (3.27):

$$\frac{ds}{dt} = \frac{Q}{V} * (S_i - S_e) \tag{3.29}$$

Eq (3.30) obtained from the linearization of Eq (3.29) as follows:

$$\frac{V}{Q*(S_i - S_e)} = \frac{K_B * V}{R_{\max} Q * S_i} + \frac{1}{R_{\max}} \tag{3.30}$$

If the maximum utilization rate (R_{max}) (g/Lday) and the saturation value constant (K_B) (g/L.day) values obtained for COD was substituted in Eq (3.30), Eq (3.31) and (3.32) could be used to predict the effluent COD concentrations, respectively. (QS_i/V) explain the organic loading rate (OLR) applied to the reactor. Q and V are the in flow rate (L/day) and the volume of the anaerobic reactor (L), respectively.

$$\frac{Q(S_i - S_e)}{V} = \frac{R_{\max}(QS_i / V)}{K_B + (QS_i / V)} \tag{3.31}$$

$$S_e = S_i - \frac{R_{\max} S_i}{K_B + (QS_i / V)} \tag{3.32}$$

CHAPTER FOUR
RESULT AND DISCUSIONS

4.1 Batch Studies

4.1.1 Anaerobic Toxicity Assay (ATA) Results for Streptomycin and Chloramphenicole

The streptomycin and chloramphenicole concentrations caused 50% decreases in the methanogenic activity (decrease of methane gas production) were calculated as IC_{50} value. The IC_{50} value for streptomycin and chloramphenicole were found to be 292.06 mg/L and 252.49 mg/L, respectively as shown in the figures 4.1 and 4.2.

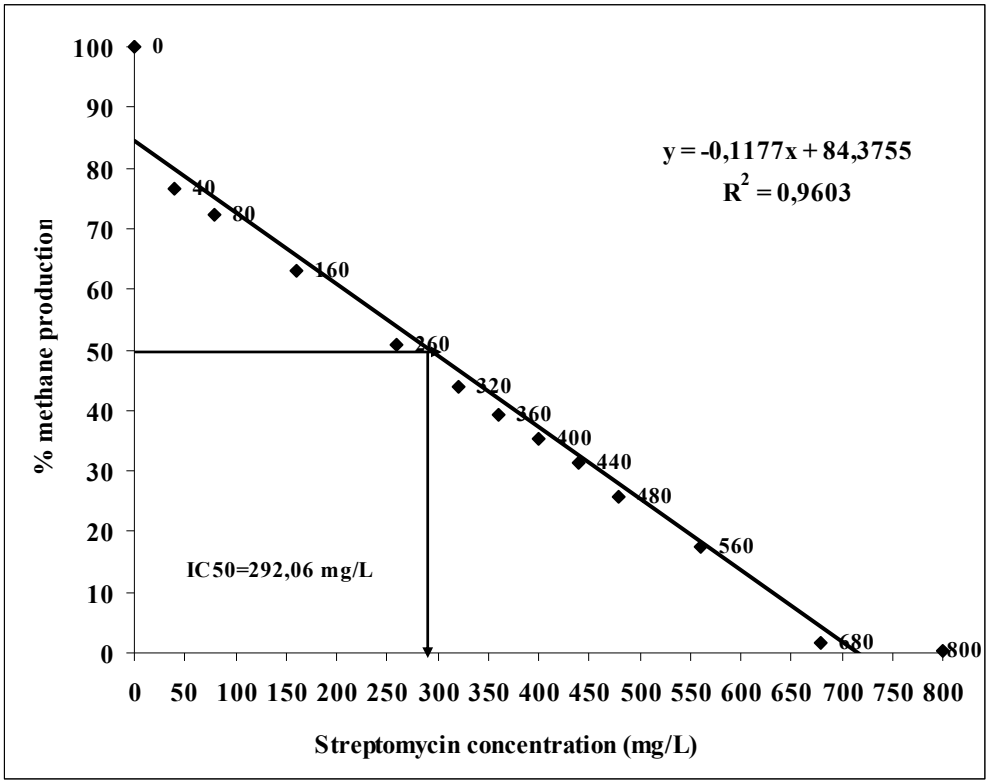


Figure 4.1 IC_{50} value for streptomycin (IC_{50}= 292,06 mg/L)

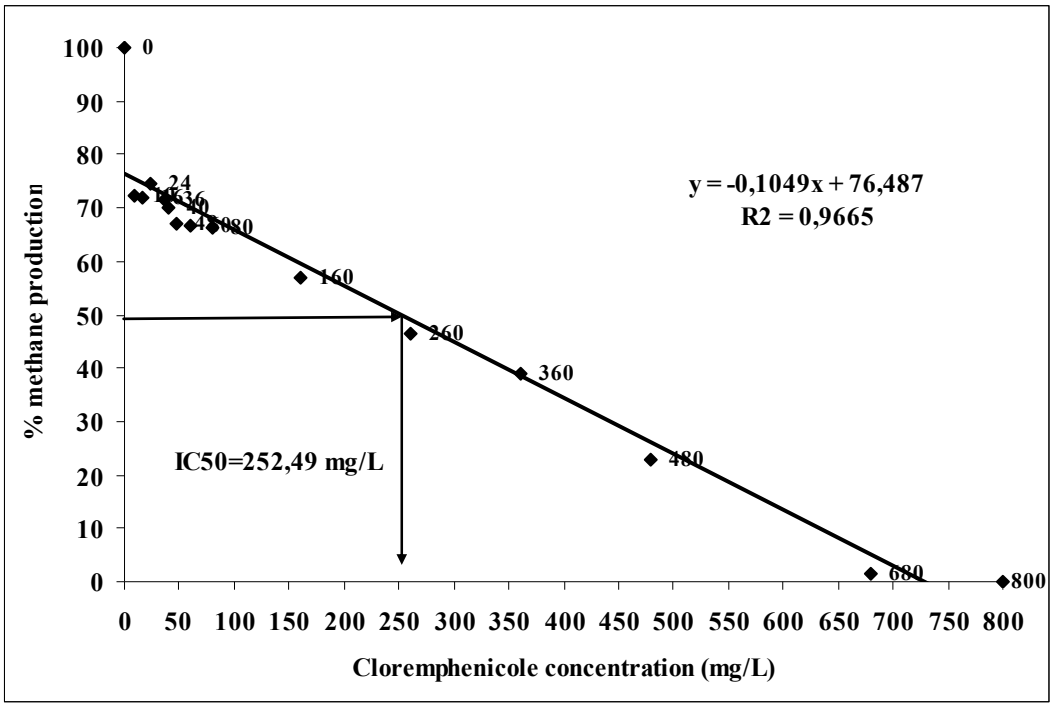


Figure 4.2 IC_{50} value for chloramphenicole (IC_{50}= 252,49 mg/L)

4.2 Continuous Studies

4.2.1 The removal of streptomycin in Anaerobic Baffled Reactor (ABR) and Sequential ABR/CSTR Reactor System

4.2.1.1 Start-up of Anaerobic Baffled Reactor (ABR)

The ABR reactor was operated through 92 days without streptomycin under steady-state conditions to acclimate the granular sludge to ABR reactor. Figure 4.3 shows the COD removal efficiencies in the ABR during the start-up period. The COD removal efficiency was 10% at the operation time of 4 days. The COD removal efficiency was 70% at an operation time of 71 days. The COD removal efficiencies remained stable 82% after an operation period of 85 days. Figure 4.4 shows the methane gas percentages in the ABR during the start-up period. The methane gas production and methane percentage reached 69,12 L/day and 45% , respectively at operation time of 44 days at an organic loading rate of 0,16 Kg COD / m^3 day. The daily methane gas production and methane percentage remained stable at 9,6 L/day and 56%, respectively, after 64 days of the start-up period. Figure 4.5 shows the total

gas percentages in the ABR during the start-up period. The total gas production and methane percentage reached 100,8 L/day and 45%, respectively at operation time of 44 days. The daily total gas production and methane percentage remained stable at 187,2 L/day and 56%, respectively, after 64 days of the start-up period.

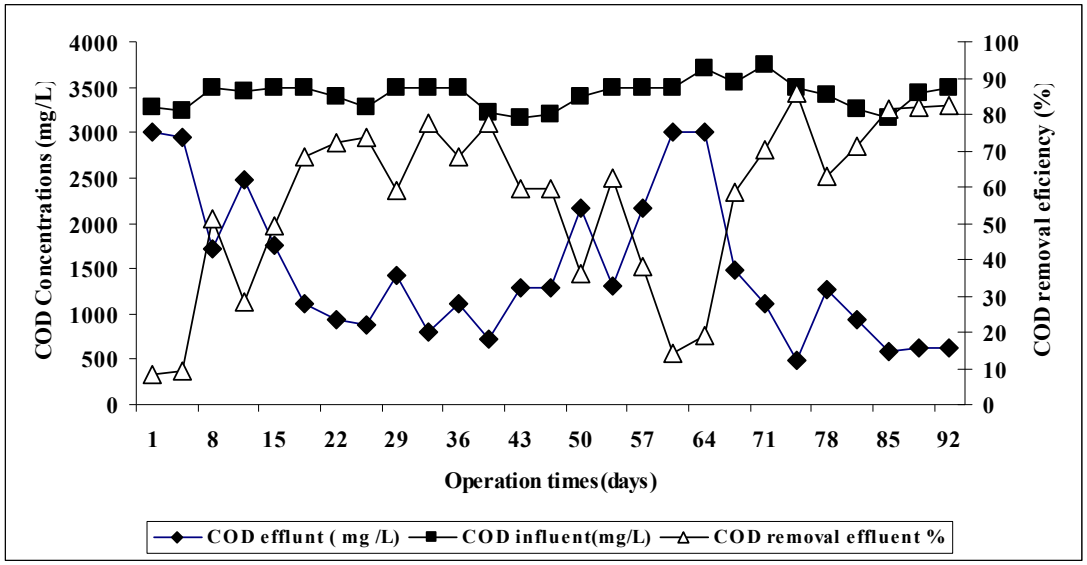


Figure 4.3 COD removal efficiencies in the ABR during the start-up period in ABR

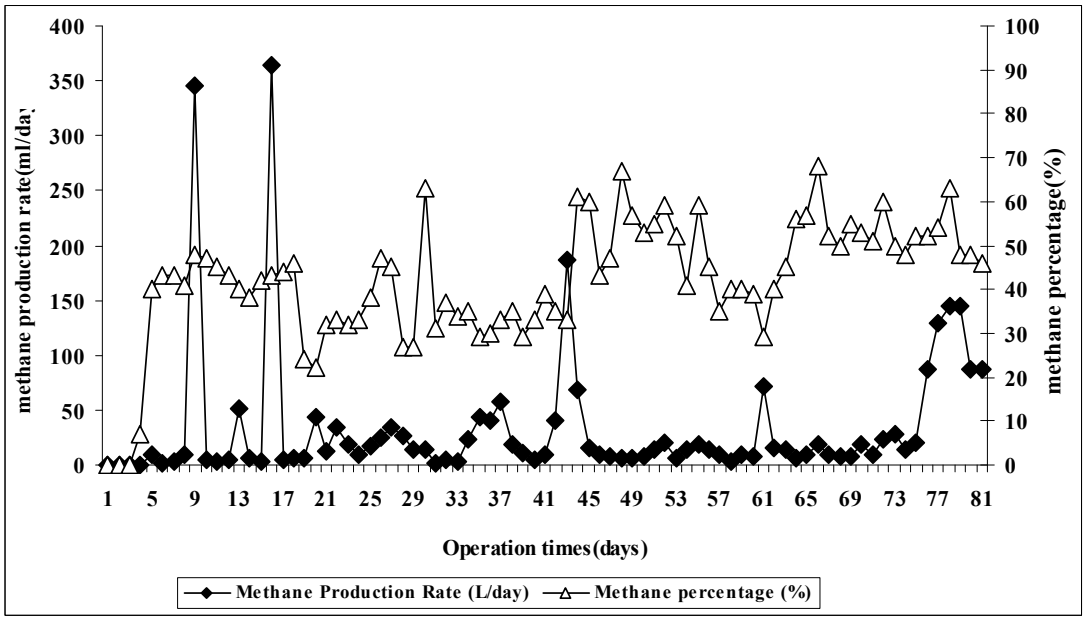


Figure 4.4 Methane gas production and methane percentages in the ABR during the start-up period in ABR.

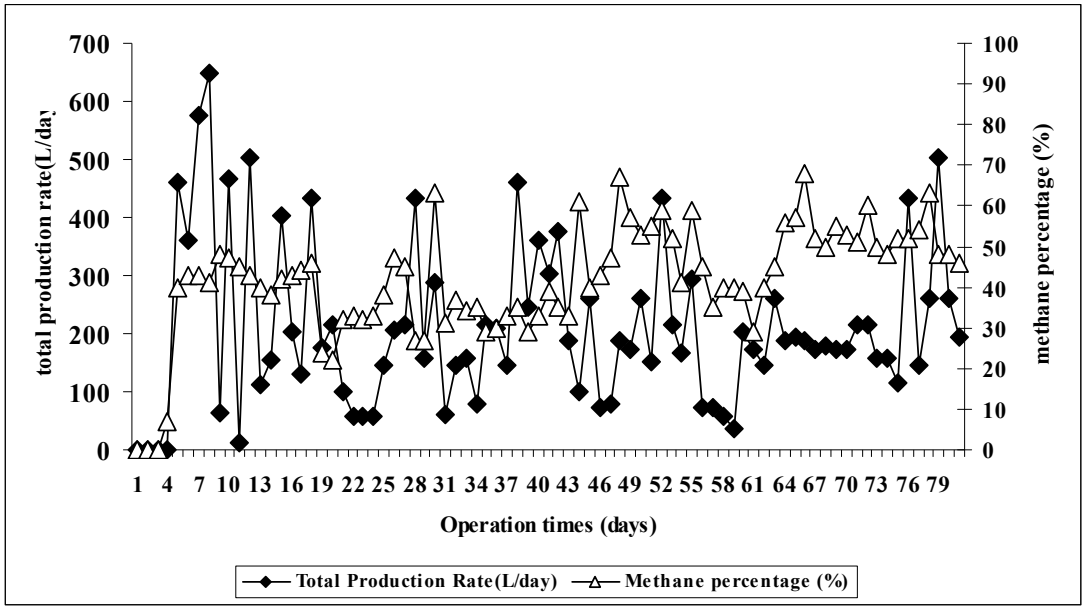


Figure 4.5 Total gas production and methane percentages in the ABR during the start-up period in ABR

4.2.1.2 Effect of Increasing Streptomycin Concentration on the COD Removal Efficiencies in ABR Reactor

In this study, the effect of increasing streptomycin concentrations on COD removal efficiencies was investigated in ABR. The operation of the ABR with streptomycin was started at an influent streptomycin concentration of 25 mg/L, and then streptomycin concentration was subsequently increased from 25, 50, 75, 100, 150, 175, 200, 240, 280, 320, to 400 mg/L (At OLRs from 0.188 to 0,156 kg COD/m^3 day). The effect of streptomycin concentration on the COD removal efficiencies in ABR was shown in Figure 4.6. Although the influent COD concentration was kept constant at 3000 mg/L with glucose, the influent COD concentrations increased with increasing streptomycin concentration since streptomycin give additional COD to synthetic wastewater. The influent COD concentration was 3660 mg/L at a streptomycin concentration of 25 mg/L while it was measured as 2990 mg/L at a streptomycin concentration of 400 mg/L. The COD removal efficiency was 90,72% at an initial streptomycin concentration of 25 mg/L introduced to ABR. In a study performed by Liu at al., (2009) the COD removal efficiency was found as 82.47% at a organic loading rate of (ORL) 2 kg COD/m^3*day on Chinese traditional medicine industrial wastewater. The COD

removal efficiency found in this study is comparable higher than that aforementioned study. The COD removal efficiency was measured approximately as 81,96 % at a streptomycin concentration of 320 mg/L. The maxsimum COD removal efficiency was between 89-95 % at streptomycin are concentration of 100-150 mg/L. When the streptomycin concentration was increased to 400 mg/L a the COD removal efficiency was measured as 67,55 % (Figure 4.6.)

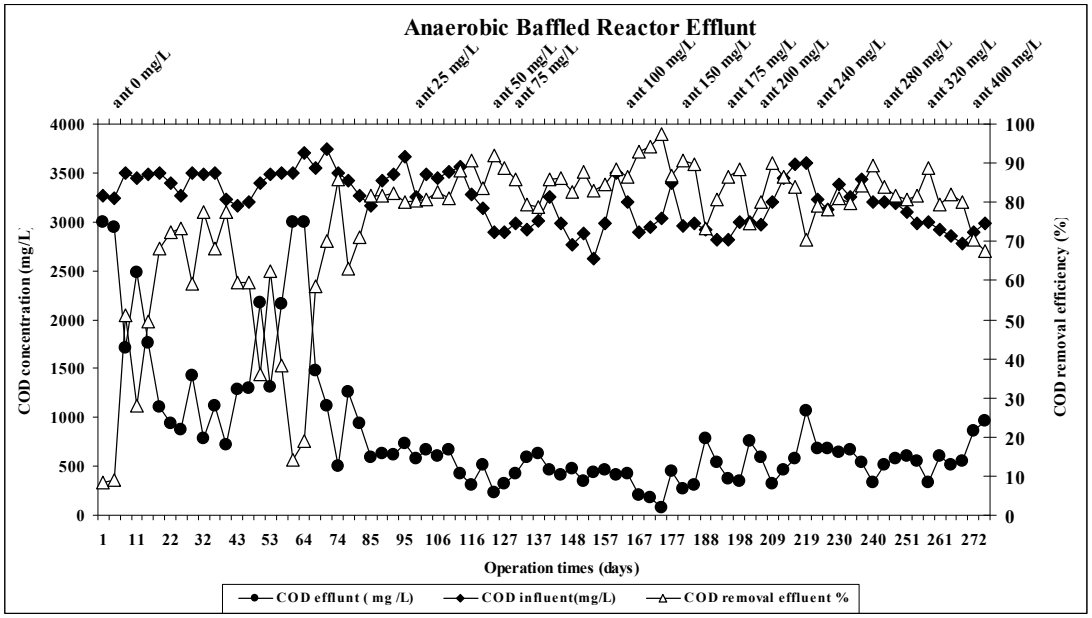


Figure 4.6 Effect of streptomycin concentration on COD removal efficiencies in ABR reactor

4.2.1.3 Effect of Increasing Streptomycin Concentration on the VFA, Bicarbonate Alkalinity (Bic.Alk.) concentrations and VFA/Bic.Alk. ratio in ABR Reactor

Figure 4.7 shows the variations in VFA concentrations and VFA/Bic.Alk. ratios in the ABR reactor at increasing streptomycin concentrations. As the streptomycin concentrations increased from 25mg/L to 400 mg/L the VFA concentration increased from 0 mg/L to 191 mg/L. Figure 4.8 shows the variations of Bic.Alk. concentrations through 268 days of operation period. Their concentrations were approximately 3600-1900 mg/l in the effluent. The Bic.Alk. concentrations decreased in the effluent, step by step. VFA/ Bİc.Alk. ratios varied between 0,368 and 0,005 in the effluent of ABR reactor at increasing streptomycin concentration(from 0 mg/L up to 400 mg/L).This showed that the ABR reactor operated under steady-state conditions

since the VFA/ BİC.Alk. ratios were lower than 0.5(Behling et al., 1997).The HCO_3 alkalinity also remained between 1250 and 2500 mg/L indicating the buffer capacity of the ABR reactor for methanogenesis (Speece, 1996)

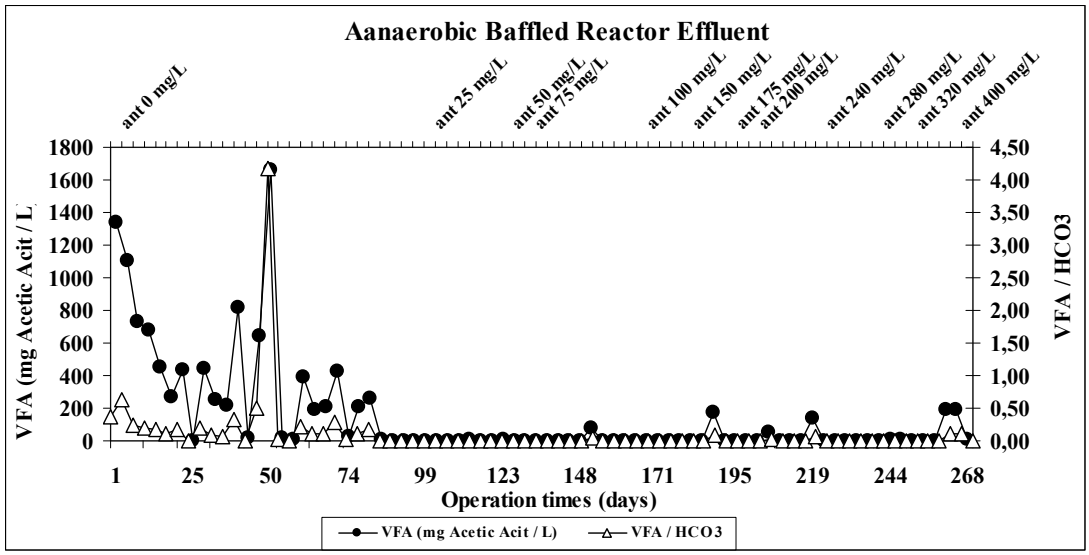


Figure 4.7 The variations of VFA in ABR at increasing streptomycin concentrations.

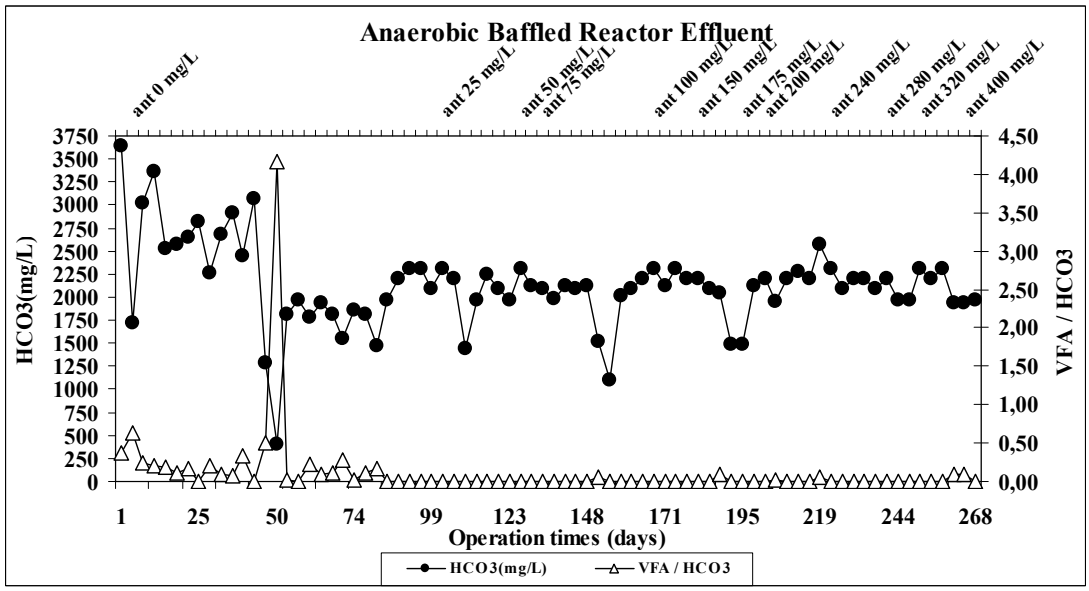


Figure 4.8 The variations of HCO_3 in ABR at increasing streptomycin concentrations.

4.2.1.4 The Variations of COD Removal Efficiency in Compartments of the ABR Reactor at Increasing Streptomycin Concentrations

In this study, the effect of increasing streptomycin concentrations on COD removal efficiencies was investigated in four compartments of the ABR reactor. COD removal efficiencies were high (98%) in compartment IV compared to the other compartments. The COD removal efficiency increased from 8,5% to 96,9% until a streptomycin concentration of 200 mg/L. Then the COD removal efficiency decreased from 96,9% to 82% after 200 mg/L at compartment IV (see 4.9.(d)).As shown in fig.4.9 (a), the COD removal efficiency values in the compartment I was lower than the other compartments. The COD removal efficiency values in the first compartment varied between 8,29% and 32,69% at all streptomycin concentrations. The COD removal efficiency values increased to 67,10 and to 88,10 in compartments II and III. Figure 4.9. (b),(c), shows the COD removal efficiencies in the compartment II (varied between 8,29% and 68,85%)and compartment III (varied between 8,29% and 80,72%).

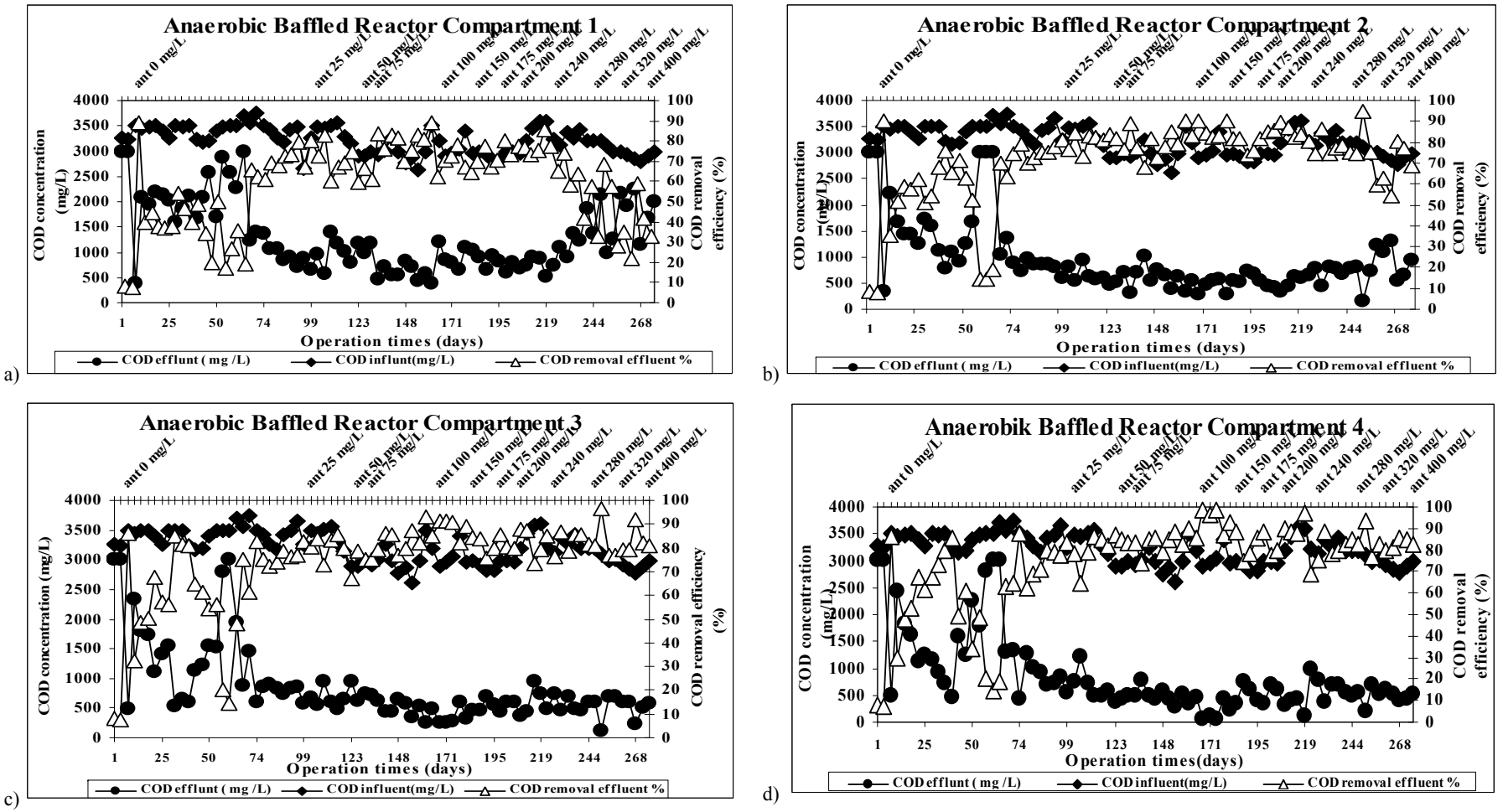


Figure 4.9 Effect of streptomycin concentration on COD removal efficiencies in all compartments. (a- compartment 1, b-compartment 2, c-compartment 3, d- compartment 4)

4.2.1.5 The Variations of VFA, Bicarbonate Alkalinity (Bic.Alk.) and VFA/Bic.Alk. ratio in Compartments of the ABR Reactor at Increasing Streptomycin Concentrations

Figure 4.10 shows the VFA, VFA/Bic.Alk. ratio variations in the ABR reactor at increasing streptomycin concentrations from 0 mg/L up to 400 mg/L. VFA concentrations were high in the compartment I compared to the other compartments, because in compartment I the activity of acidogens was a maximum rate (See 4.10. (a)). VFA concentrations decreased from 1200 mg/l to 208 mg/l as the streptomycin concentrations increased from 25 mg/L up to 400 mg/L in first compartment. VFA concentrations decreased in compartments II, III and IV. VFA concentrations decreased from 1341 mg/L to 157 mg/L until a streptomycin concentration of 25 mg/L while the VFA concentration was zero until a streptomycin concentration of 400 mg/L in compartment IV. The VFA concentrations zero at a streptomycin concentration of 400 mg/L in compartment III (See 4.10. (c)).The VFA concentrations decreased from 794 mg/L to 191 mg/L until a streptomycin concentration of 25 mg/L while the VFA concentrations were zero in compartment II at all streptomycin concentration (See 4.10. (b)). VFA concentrations decreased from 1341 mg/L to 112 mg/L until a streptomycin concentration of 25 mg/L and the VFA concentration were zero until a streptomycin concentration of 400 mg/L in compartment IV. The VFA concentrations decreased from 191 mg/l to 0 mg/l at a streptomycin concentration of 400 mg/L in compartment IV (See 4.10. (d)).

The Bicarbonate Alkalinity (HCO_3) and VFA/Bic.Alk. ratio variations in all compartments of the ABR reactor at increasing streptomycin concentrations (from 0 mg/L up to 400 mg/L) were shown in Figure 4.11. Figure (4.11.(a)) indicates a low concentration of HCO_3 concentration from 3803 mg/L down to 1817mg/L was present in the compartment I when the ABR reactor was operated at streptomycin concentration in the range 0 mg/L - 400 mg/L. However, in compartment IV, the HCO_3 alkalinity concentrations increased to 1931 mg/L at a streptomycin concentration 400 mg/L (see figure 4.11. (d)). HCO_3 concentrations decreased from 3771 mg/L to 1858 mg/L at a streptomycin concentration of 400 mg/L in compartment II. After that HCO_3 concentrations decreased from 3648 mg/L to 1858

mg/L at a streptomycin concentration of 400 mg/L in compartment III. The HCO_3 concentrations in the compartment IV is higher than the others compartments in the ABR reactor.

Generally it was found that in the first compartment of ABR reactor the acidogenesis is the major step of the anaerobic treatment. The third and fourth compartments are the major removal steps for methanogenesis. Therefore the VFA concentrations were high in while the HCO_3 alkalinities were low in the first compartment of ABR.

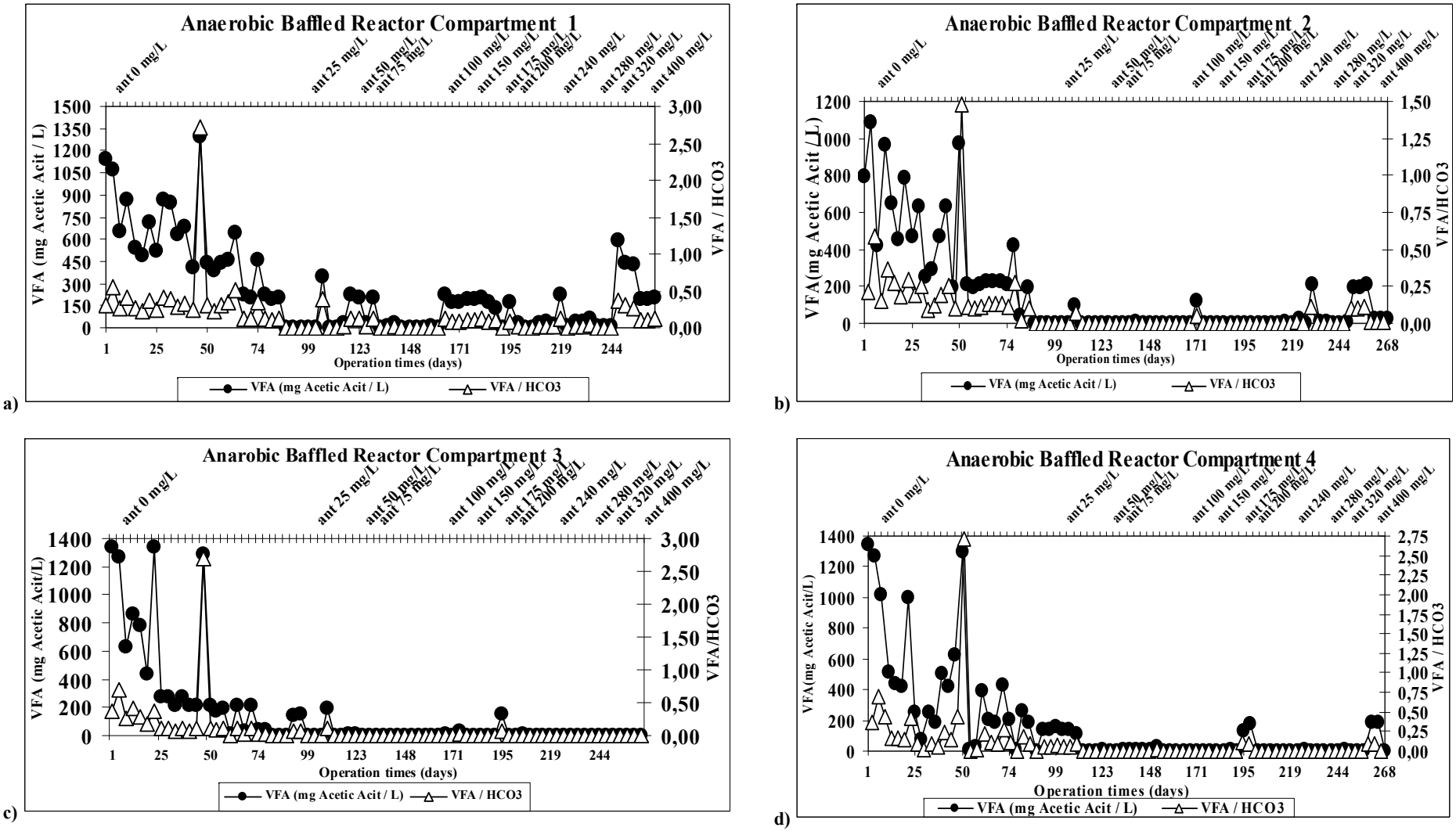


Figure 4.10 The variations of VFA in ABR at increasing streptomycin concentrations in the all compartments. (a- compartment 1, b-compartment 2, c-compartment 3 d- compartment 4)

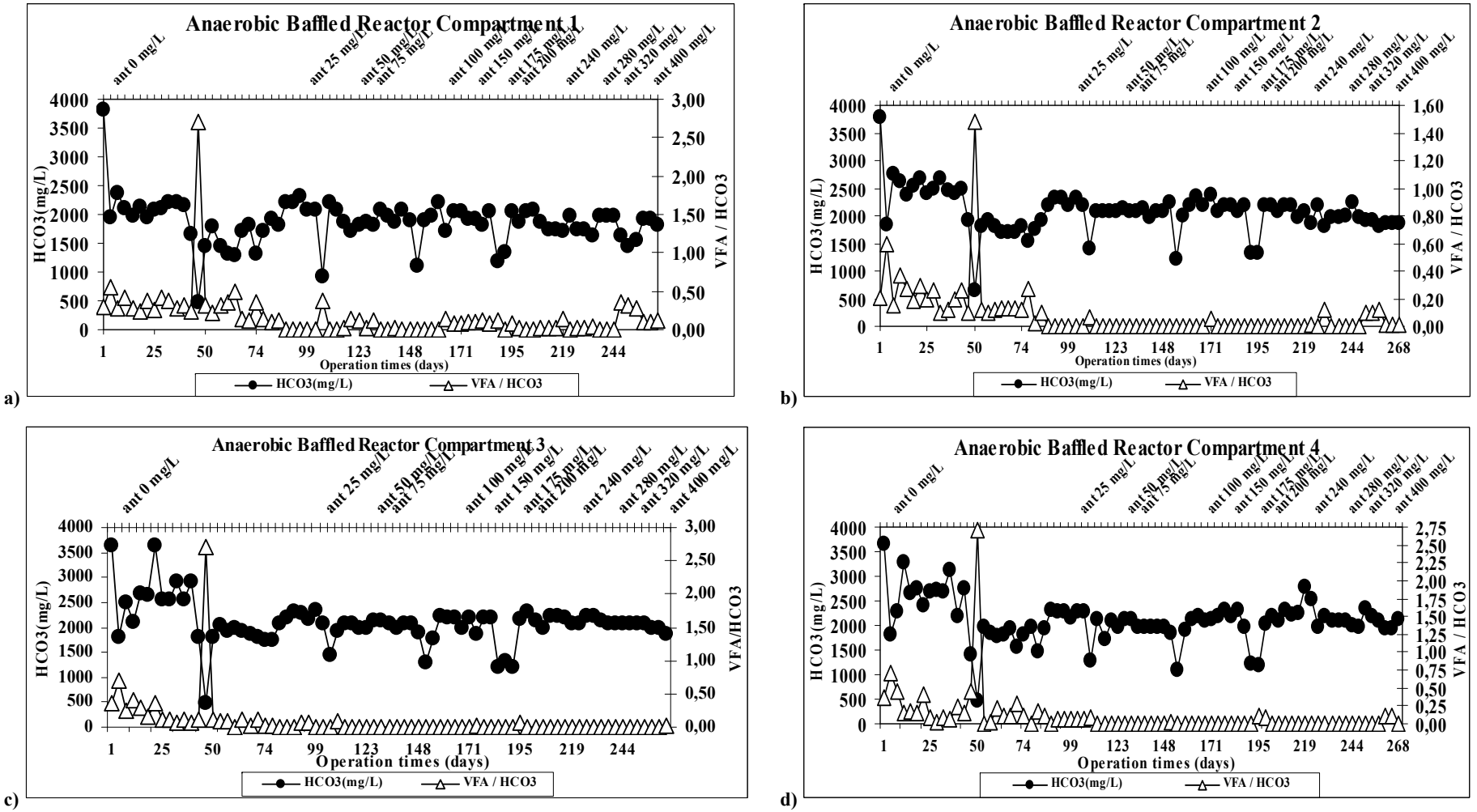


Figure 4.11 The variations of HCO_3 in ABR at increasing streptomycin concentrations in the all compartments. (a- compartment 1, b-compartment 2, c-compartment 3 d- compartment 4)

4.2.1.6 Effect of Increasing Antibiotic Dose on Gas Production and Methane Percentage in Anaerobic ABR Reactor.

Biogas production was monitored through the operation of the ABR reactor, particularly for detection the methanogenic activity. From Figure 4.12 it can be seen that the methane gas production rates increased from 0 L/day to 144 L/day at a streptomycin concentration of zero. Then methane gas production rates increased from 144 L/day to 259,2 L/day , respectively. As the streptomycin concentration was increased from 0 mg/L to 280 mg/L, the methane gas production decreased from 259 L/day to 172,8 L/day . The methane percentages of biogas increased from 0% up to 53% until a streptomycin concentration of 200 mg/L. The methane percentages of biogas were decreased to 48%, when the streptomycin concentration increased from 200 mg/L to 400 mg/L. In a study performed by Liu at all (2009) methane gas production was found as 12 L/day (OLR=1.04 kg COD/m^3*day), 30 L/day (OLR=2.01 kg COD/m^3*day) and 66 L/day (OLR=6.17 kg COD/m^3*day) for firth, second and third compartments, in ABR reactor respectively. In this study the methane percentages are comparable higher than that aforementioned study.

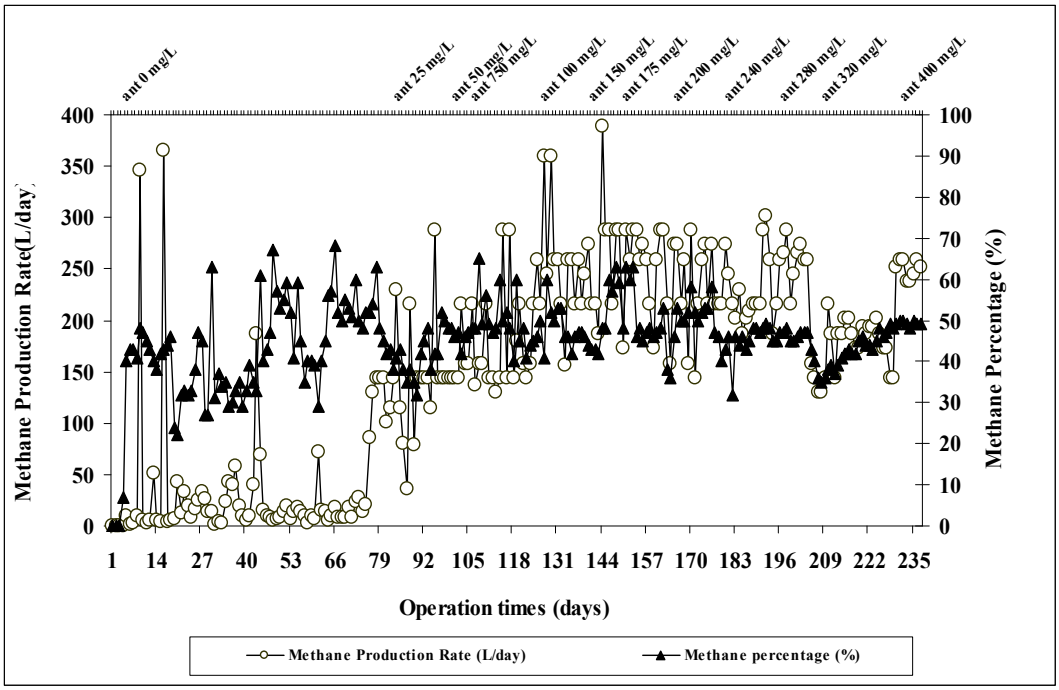


Figure 4.12 The variations of methane gas production and methane percentage in ABR at increasing streptomycin concentrations.

From Figure 4.13 it can be seen that the total gas production rates increased from 0 L/day to 259,2 L/day in the operation of ABR without streptomycin. After that the total gas production rates increased from 259,2 L/day to 504 L/day , respectively as the streptomycin concentration increased from 0 mg/L to 175 mg/L. The total gas production also, decreased from 504 mg/L to 208,8 mg/L . The methane percentages of biogas were increased from 0% up to 53% until a streptomycin concentration at 200 mg/L then the methane percentages of biogas decreased to 48%, when the streptomycin concentration increased from 200 mg/L to 400 mg/L.

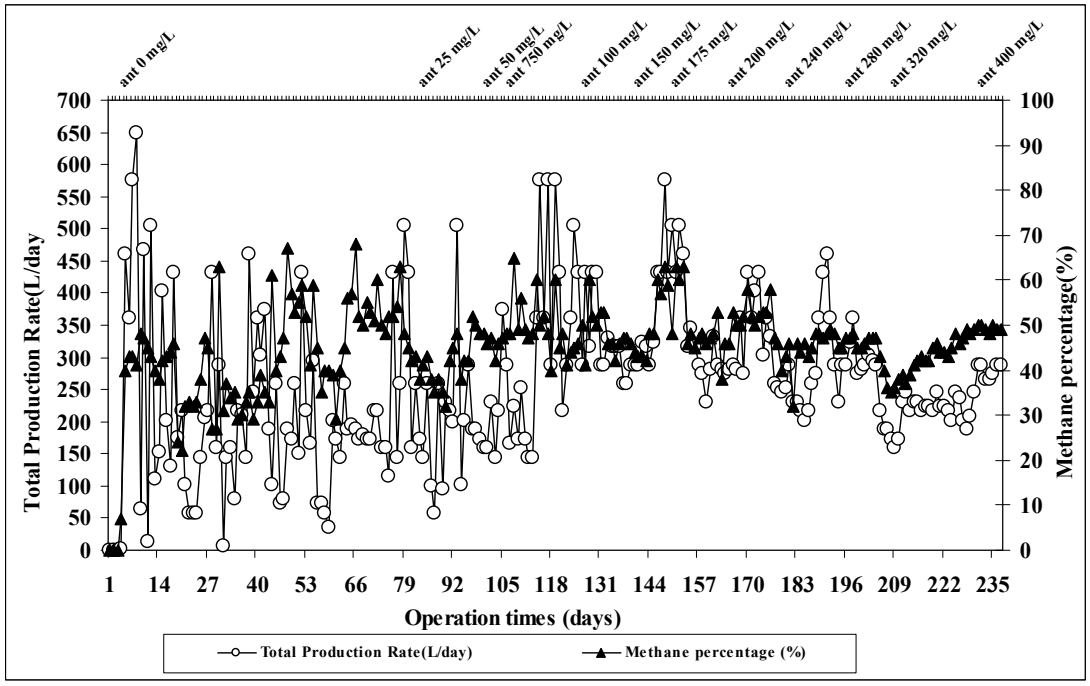


Figure 4.13 The variations of total gas production and methane percentage in ABR at increasing streptomycin concentrations.

4.2.1.7 Effect of Hydraulic Retention Time (HRT) on The Performance of ABR Reactor

4.2.1.7.1 Effect of HRTs on THE COD Removal Efficiency in ABR Reactor. The effect of hydraulic retention times (HRTs) on the COD removal efficiency was shown in Figure 4.14.The influent streptomycin concentration was kept constant as 200 mg/L. As shown in Figure 4.14, the influent COD concentration was

approximately 3600-2900 mg/L since 200 mg/L streptomycin gives an additional COD concentration to total COD thought continuous operation. 200 mg/L of streptomycin gave approximately a COD of 131,38 mg/L. 90% COD removal efficiency was obtained at a HRT of 19,2 days in ABR reactor. When the HRT was decreased from 12,8 days to 7,68 days, the COD removal efficiency decreased from 89% to 76%, respectively. Akunna & Clark, (2000) investigated the performance of an anaerobic baffled reactor treated a whisky distillery wastewater at different four HRTs (10, 7, 4 and 2 days). The maximum COD removal efficiency was observed at a HRT of 4 days (E=93%). Oktem, Ince, Sallis, Donnelly & Kasapgil, (2007) investigated the performance of anaerobic sludge blanket reactor treated a chemical synthesis – based pharmaceutical wastewater at two HRTs (1and 3 days). COD removal efficiency increased from 58% to 78% with the HRT was increased from 1 to 3 days. Kuscu & Sponza, (2009) found that as the HRT decreased from 10,38 days to 2,5 days the COD removal efficiencies in the anaerobic and anaerobic/aerobic reactor effluents decreased from 94% to 92% and from 98% to 97%, respectively.

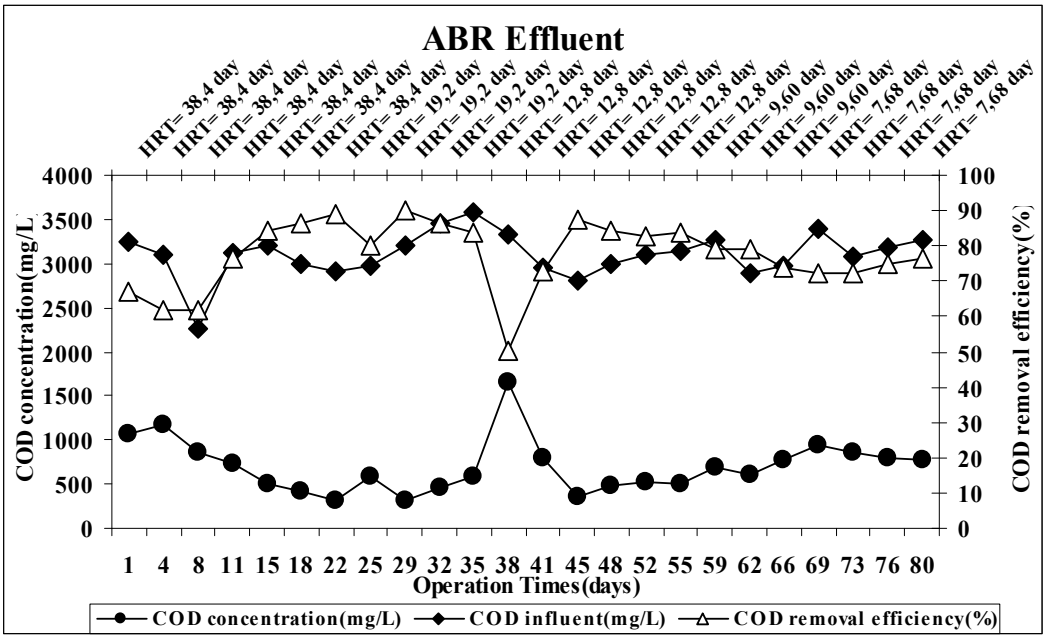


Figure 4.14 The effect of HRTs on COD removal efficiencies in ABR

4.2.1.7.2 Effect of HRTs on Total Volatile Fatty Acid (TVFA), Bicarbonate Alkalinity (Bic. Alk.) and TVFA/Bic.Alk. Ratio Variations in ABR Reactor. Figure 4.15 shows the TVFA in the effluent of ABR at decreased HRTs. The highest VFA concentration (191 mg/L) was found at a HRT of 38,4 days. After this HRT, TVFA concentration in the effluent decreased and was measured as 9 mg/L at a HRT of 7,68 days. From Fig. 4.15, it can be seen that Bic.Alk. concentrations in effluent decreased from 191 to 9 mg/l since the HRT were decreased.

HuaJun Feng, LıFang Hu, Dan Shan, ChengRan Fang and DongSheng Shen (2008) investigated the performance of an anaerobic baffled reactor treated dilute wastewater the HRT decreased from 18 h to 9 h, and the final concentration of effluent VFAs increased from 8 mg/L to 22 mg/L, respectively.

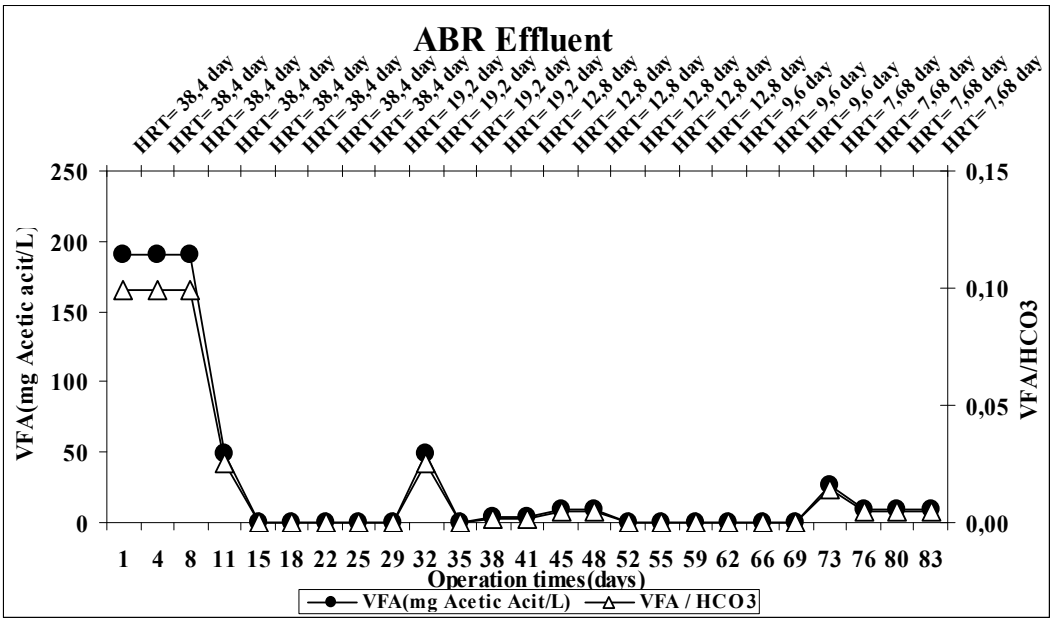


Figure 4.15 The variations of VFA and VFA/Bic.Alk. ratio in the effluent of ABR at decreased HRTs.

From Figure 4.16, it can be seen that Bic.Alk. concentrations increased from 1931 mg/L up to 2199 mg/L when HRT decreased from 38,4 to 9,60 days. After that Bic. Alk. concentrations decreased to 1972 mg/L a HRT of 7,68 days.

In anaerobic reactor system TVFA/Bic.Alk. ratio gives necessary information to determine the stability of 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). As shown in Fig. 4.15. The TVFA/Bic.Alk. ratio varied between 0.099 and 0.005 in effluent as the HRTs were decreased from 38,4 days to 7,68 days. ABR reactor was stable as reported by Behling et al., (1997) since the TVFA/Bic.Alk. ratios in the effluent were lower than 0.4.

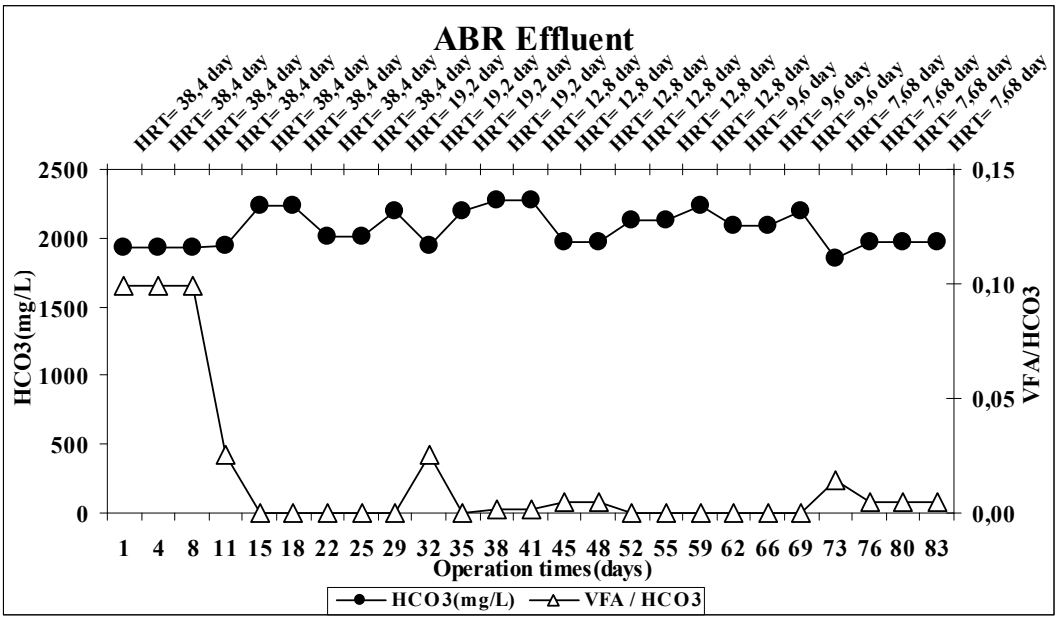


Figure 4.16 The variations of Bic.Alk. and VFA/Bic.Alk. ratio in the effluent of ABR at decreased HRTs

4.2.1.7.3 Effect of HRTs on Gas Productions and Methane Percentage in Anaerobic ABR Reactor. From figure 4.17 it can be seen that the methane gas production increased from 144 L/day up to 446,4 L/day as the HRT decreased from 38,4 to 9,60 days as the streptomycin concentration constant at a 200 mg/L. the maximum methane percentages (58%) has obtained at a HRT of 19,2 days. However, the methane gas production decreased from 446,4 L/day to 288 L/day at a HRT of 7,68 days. Maximum methane gas production (446,4 L/day) was obtained at 9,6 days of HRT.

From figure 4.18 it can be seen that the total gas production increased from 259,2 L/day up to 504 L/day as the HRT decreased from 38,4 to 9,60 days as the streptomycin concentration constant at a 200 mg/L . But total gas production decreased from 504 L/day to 432 L/day at a HRT of 7,68 days. Maximum total gas production (504 L/day) was obtained at 9,60 days of HRT.

The methane percentages of the biogas were approximately 38-40% at a HRT of 38,4 days. After that methane percentages increased from 36% up to 53% at a HRT of 19,2 days. However the methane percentages increased from 35% up to 46% as the HRT decreased from 19,2 to 7,68 days.

When the ABR system reached to a stabilized state under the OLR of 6.0 kg COD/m^3 d and HRT of 39,5 days, the total amounts of biogas in the four compartments were 73.2 L/d, 30.8 L/d, 8.6 L/d, and 1.3 L/d, respectively(Ge-Fu Zhu, Jian-Zheng Li ,Peng Wu, Hui-Zheng Jin , Zheng Wang , (2008)).

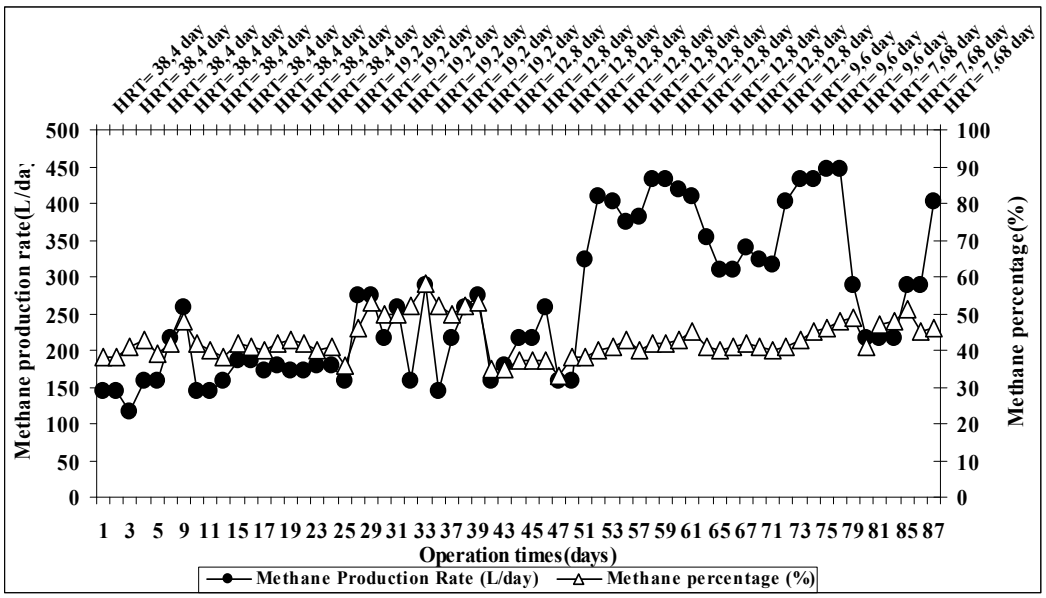


Figure 4.17 Methane gas production and methane percentage in ABR at decreased HRTs

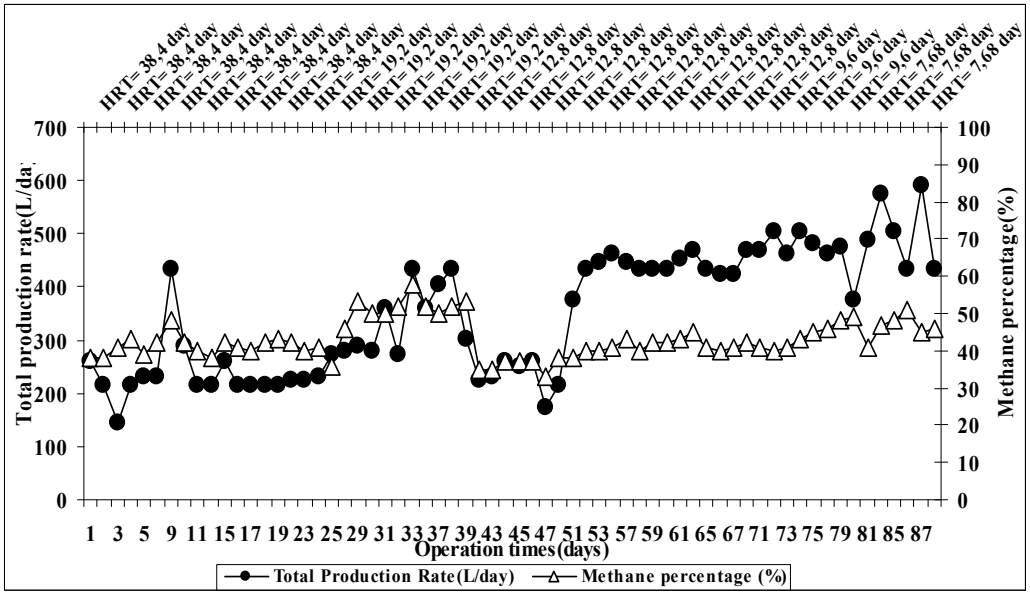


Figure 4.18 Total gas production and methane percentage in ABR at decreased HRTs

4.2.1.7.4 Effect of Compartments of ABR on COD Removal Efficiencies at Different HRTs. In this study, the effect of decreases in HRTs on COD removal efficiencies was investigated in four compartments of the ABR reactor. Figure 4.19 shows the effect of compartmentalization on COD removal efficiencies at different HRTs. As shown in the figure 4.19 (a), in compartment I the COD removal efficiencies were approximately 77% at a HRT of 19,2 days. The COD removal efficiency at a HRT of 19,2 days is smaller than the others HRTs. The COD removal efficiency was low at a HRT of 7,68 in the compartment I. Figure 4.19. (b),(c), shows the COD removal efficiencies in the compartment II (varied between 64,23% and 78,08%)and compartment III (varied between 71,61% and 86,02%) (see figure 4.19.(b),(c)). COD removal efficiency increased from 64,23% to 87,19% in the second compartment when HRTs decreased from 38,4 days to 19,2 days. However the COD removal efficiency decreased from 87,19% to 62,50% in compartment II when the HRT decreased from 19,2 to 7,68 days (see figure 4.19.(b)). COD removal efficiency increased from 71,61% to 88,10% in the initial compartment when the HRT decreased from 38,4 days to 19,2 days. However COD removal efficiency decreased from 88,10% to 70,44% in compartment III when the HRT decreased from 19,2 to 7,68 days (see figure 4.19.(c)).In compartment IV the COD removal efficiencies were high (89,89%) at a HRT of 19,2 days compared to

the other HRTs. The COD removal efficiency increased from 62,49 % to 89,89% as the HRT decreased from 38,4 days to 19,2 days . Then the COD removal efficiency decreased from 89,89 % to 78,59 % when the HRT decreased from 19,2 days to 7,68 days in compartment IV (see figure 4.19.(d)). Therefore for the maximum COD removal efficiency the optimum HRT was found to be 19,2 days.

In a study at soybean protein processing wastewater of Ge-Fu Zhu, Jian-Zheng Li ,Peng Wu, Hui-Zheng Jin , Zheng Wang , (2008) at a HRT of 39,5 days. After the acclimatization of the anaerobic activated sludge in 24 days, the ABR was subjected to a steady-state operation and the removal of total COD from the wastewater was remarkable (above 92%); At the second stage, the COD removal increased continually when the volume loading rate enhancing, basically about 94%. But at the third stage, when an influent 8000 mg COD/L was applied to the ABR, acidification phenomenon happened during the initial period (65–67 days) because of increasing volume loading rate that resulted in the declination of COD removal to 80%. Four days later, the COD removal was improved to 94% without adopting any measurement (influent COD concentration 8000 mg/L). The COD removal in the last stage was similar to the third stage, when increased volume loading rate further, the total COD removal efficiencies in the ABR system remained as 97% and the effluent COD concentration was under 300 mg/L.

The carrier anaerobic baffled reactor (CABR) was initially fed with domestic sewage from Zhejiang University during start-up at HRT of 48 h, derived mainly from restaurants and dormitories. The reactors were acclimatized for over a period of 21 days at ideal temperature under which digesters were known to perform in an optimal way. The HRT was gradually decreased from 48 h to18 h by increasing the flow rate for 3 months. The total COD and SS removal efficiency was 69% and 82% at HRT of 18 h, respectively. The average COD removal efficiency was 77.73% at a HRT of 18 h, 74.91% at a HRT of 12 h, and 58.51% at a HRT of 9 h, respectively. The difference in removal efficiencies was not significant between 18 h and 12 h of the HRT. However, drastic drop in removal efficiency was observed when the HRT decreased to 9 h, indicating that the HRT greatly affected the performance of CABR. However, the COD removal load at a short HRT was still higher than that at a long

HRT in this study, which was 0.31 kg/m^3d at a HRT of 18 h, 0.45 kg/m^3d at a HRT of 12 h, and 0.47 kg/m^3d at a HRT of 9 h, respectively, (HuaJun Feng, LiFang Hu, Dan Shan, ChengRan Fang, And DongSheng Shen, 2008).

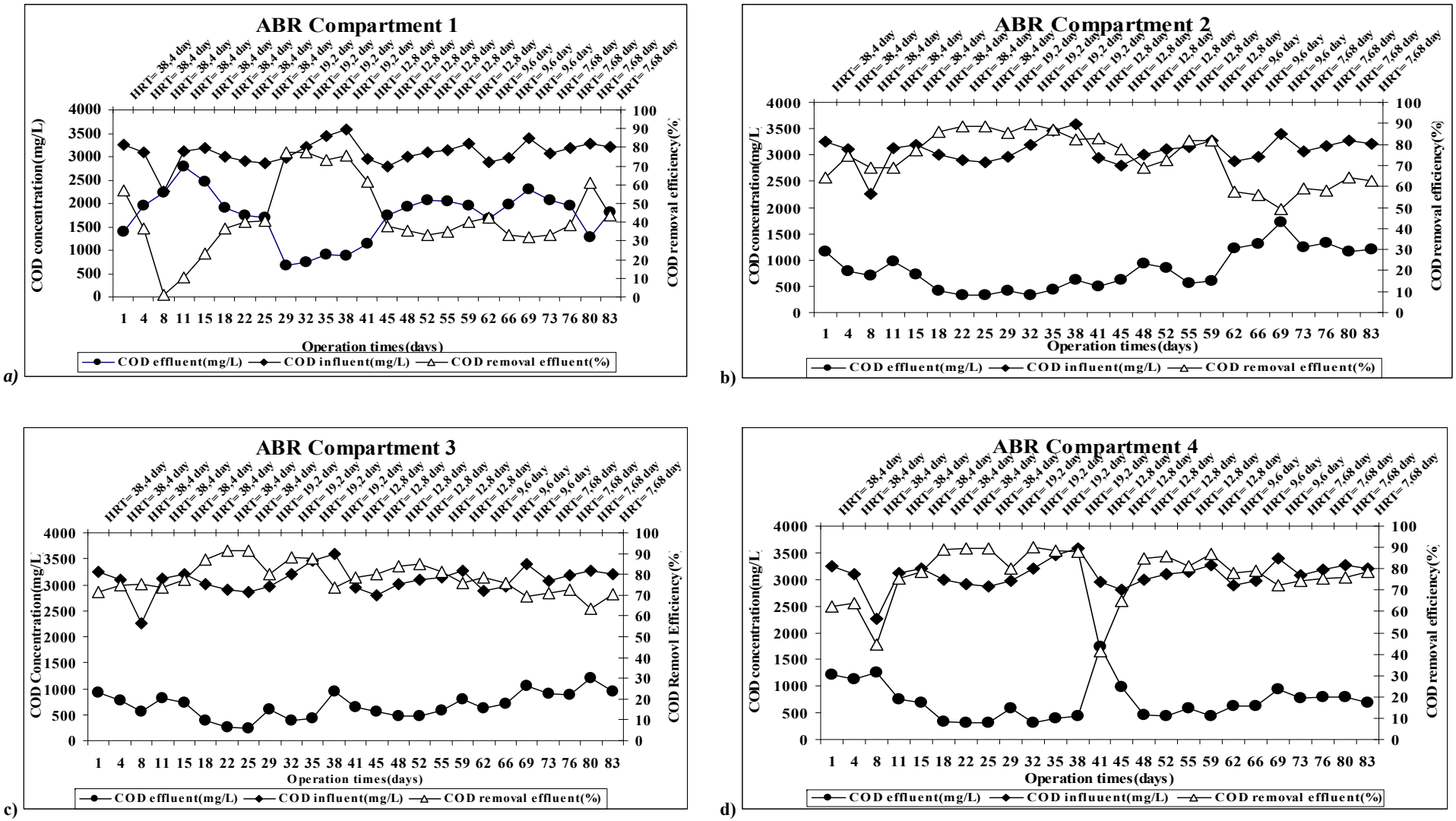


Figure 4.19 The variations of COD in ABR at decreased HRTs in the all compartments. (a- compartment 1, b-compartment 2, c-compartment 3 d- compartment 4)

4.2.1.7.5 Effect of Compartments of ABR on VFA, Bic. Alk. and VFA/Bic. Alk. ratio at Different HRTs

Figure 4.20 shows the TVFA and TVFA/Bic.Alk. ratio variations in all compartments on decreased HRTs. In the compartment I at the VFA decreased from 608 mg Acetic acid /L to 26 mg Acetic acid /L when HRT decreased from 38,4 days to 19,2 days. But VFA increased from 26 mg Acetic acid/L to 425 mg Acetic acid /L when the HRT decreased from 12,8 to 7,68 days(see figure 4.20.(a)). In the compartments II and III at the VFA nearly zero mg/L firths four HRTs (38,4 - 19,2 - 12,8 - 9,60 days), but VFA was found 26 mg/L at a HRT of 7,68 days (see figure 4.20.(b,c)).Figure 4.20(d) shows the VFA in the compartment IV , the VFA almost 9 mg/l at all HRTs. S. Ghaniyari-Benis, R. Borja,S. Ali Monemian, V. Goodarzi, (2009), who studied synthetic medium-strength wastewater, found that the VFA concentration of 913 mg/L, 1154 mg/L and 1258 mg/L were achieved at HRTs of 24h, 16h and 8h, respectively, in compartment I . In compartment II the VFA concentrations were found as 371 mg/L, 458 mg/L and 959 mg/L at HRTs of 24h, 16h and 8h, respectively. VFA concentration of 223 and 228 mg/L were achieved at HRTs of 24 and 16 h. Decreasing of HRT to 8 h gave a VFA concentration of 458 mg/L in compartment III of multistage anaerobic biofilm reactor. For all HRTs the VFA production in the first compartment was significantly greater than that in other compartments and it decreased from input to output.

Figure 4.21 shows the Bic.Alk. and TVFA/Bic.Alk. ratio variations in all compartments on decreased HRTs. In compartment I, when the HRT decreased from 38,4 days to 19,2 days, the HCO_3 concentrations increased from 1509 mg/L up to 1972 mg/L. When the HRT decreased from 38,4 days to 19,2 days, the HCO_3 concentrations decreased. When the HRT decreased from 19,2 days to 7,68 days the HCO_3 concentrations decreased from 1972 mg/L to 1550 mg/L (see 4.21(a)). Similar results were found for Compartments II, III and VI. The HCO_3 concentrations increased from 1972 mg/L up to 2045 mg/L when the HRT decreased from 38,4 days to 9,60 days in compartments II and IV. The HCO_3 concentrations increased from 1972 mg/L up to 2126 mg/L when the HRT decreased 38,4 days to 9,60 days in

compartment III. However the VFA concentrations decreased to 1858 mg/L and to 1972 mg/L in compartments II - III and at a HRT of 7,68, respectively(see figure 4.21(b,c,d)).

In anaerobic reactor system TVFA/Bic.Alk. ratio gives necessary information to determine the stability of 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). As shown in Fig. 4.20 and 4.21. The TVFA/Bic.Alk. ratio varied between 0.403 and 0.274 in compartment I, as the HRTs were decreased from 38,4 days to 7,68 days. The TVFA/Bic.Alk. ratio varied between 0.005 and 0.014 in compartments II, III and IV as the HRTs were decreased from 38,4 days to 7,68 days.ABR reactor was stable as reported by Behling et al., (1997) since the TVFA/Bic.Alk. ratios in the all compartments were lower than 0.4.

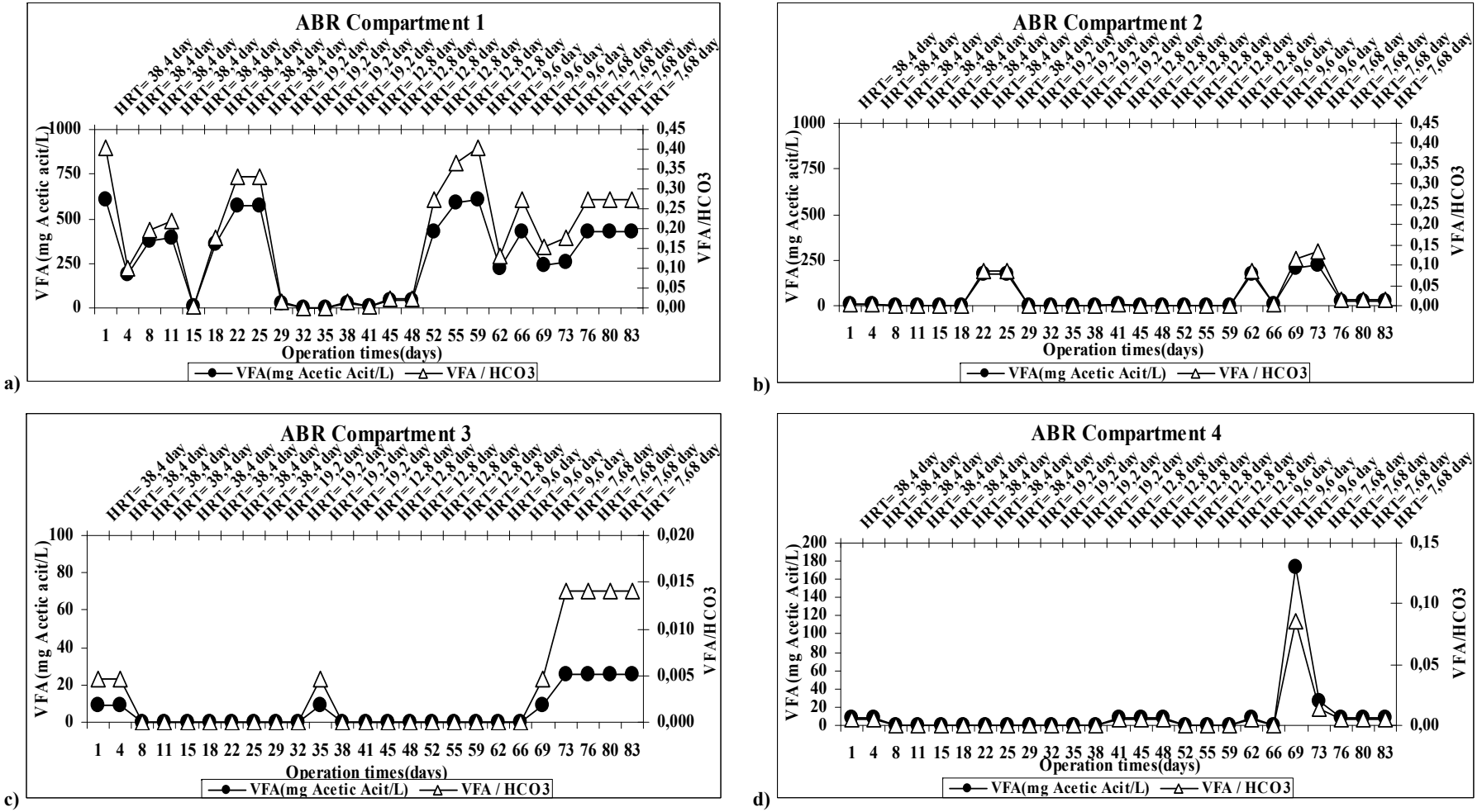


Figure 4.20 The variations of VFA in ABR at decreased HRTs in the all compartments. (a- compartment 1, b-compartment 2, c-compartment 3 d- compartment 4)

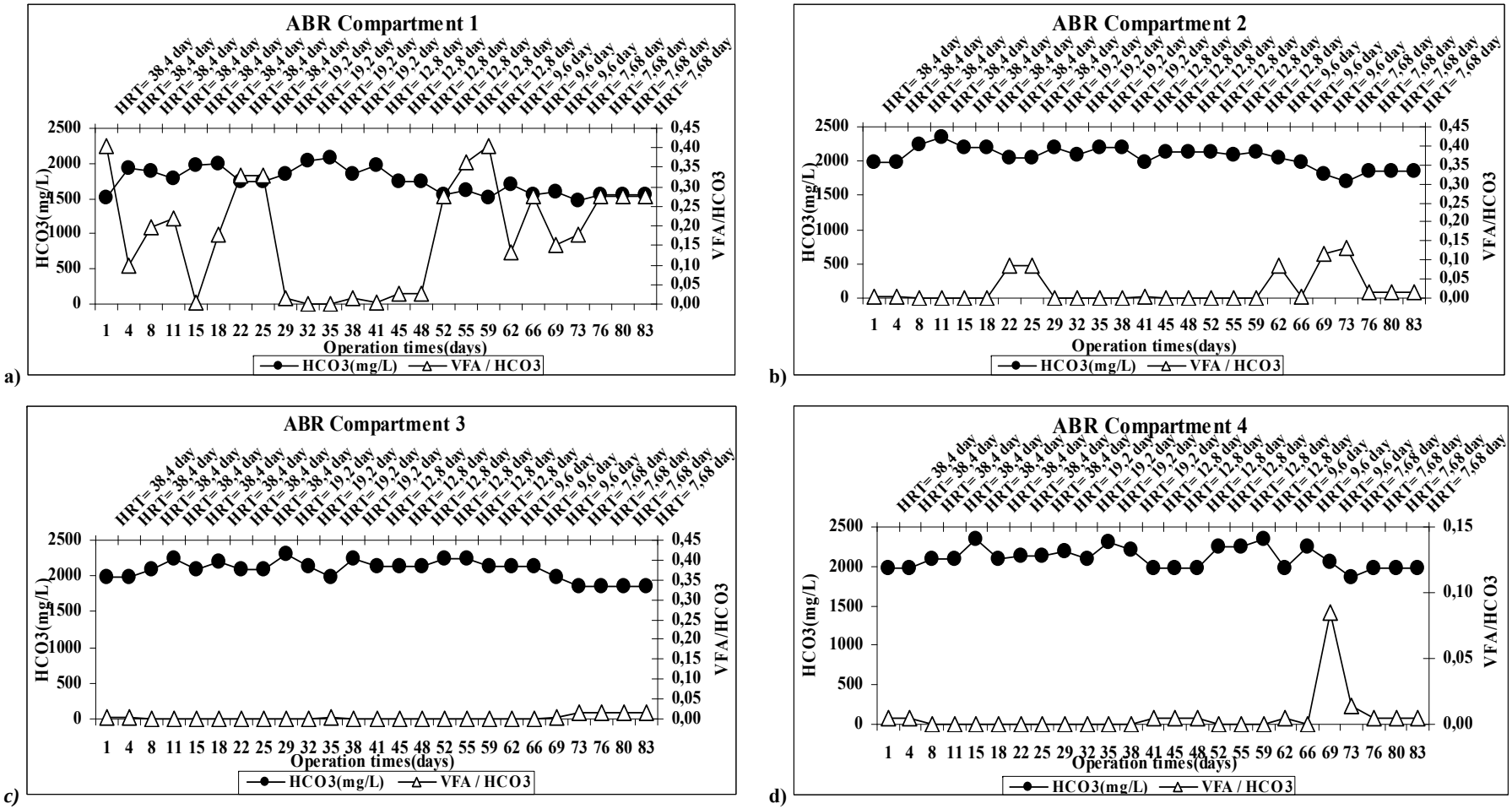


Figure 4.21 The variations of HCO_3 in ABR at decreased HRTs in the all compartments. (a- compartment 1, b-compartment 2, c-compartment 3 d- compartment 4)

4.2.1.7.6 Removal Efficiencies in Aerobic CSTR Reactor System. Figure 4.22 shows the COD removal efficiencies of aerobic CSTR reactor. The COD removal efficiency in this reactor system were up to 94,52% until a HRT of 19,2 days. After that COD removal efficiency of the reactor decreased from 94,52% to 85,70% when the HRT were decreased from 19,2 days to 7,68 days in. For maximum COD removal efficiency (E=94,52%) the optimum HRT was found as 19,2 days.

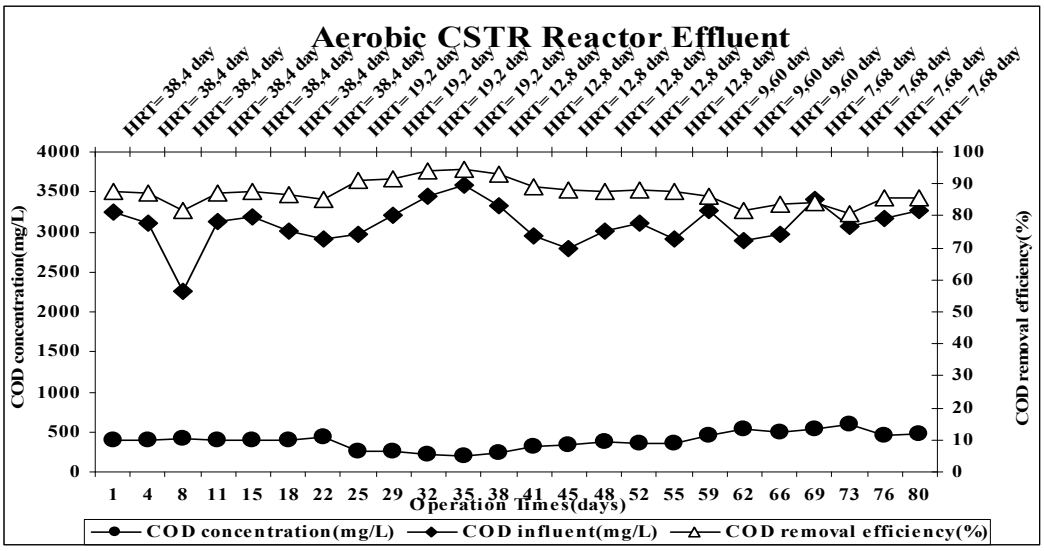


Figure 4.22 The overall COD removal efficiency in aerobic (CSTR) reactor system

Figure 4.23 shows the overall VFA and VFA/Bic.Alk. ratio of aerobic reactor. Although the VFA and HCO_3 alkalinity were the key parameters in the anaerobic reactors in this study it was aimed to monitor their concentrations in the aerobic CSTR reactor since the effluent of anaerobic ABR reactor it was used as the feed of the aerobic CSTR reactor. In aerobic CSTR reactor the VFA concentrations increased from 0 mg /L to 258 mg Acetic acid /L when the HRT were decreased from 38,4 days to 9,60 days. However the VFA concentrations decreased from 258 mg/L to 0 mg/L when the HRT decreased from 9,60 days to 7,68 days. For the lowest VFA concentrations (258 mg/L) the optimum HRT was found as 9,60 days.

Figure 4.24 shows the Bic.Alk. and VFA/Bic.Alk. ratios of aerobic reactor system. In aerobic CSTR reactor system the HCO_3 concentrations decreased from

123 mg /L to 81 mg /L when the HRT decreased from 38,4 days to 19,2 days. However, the HCO_3 concentrations increased from 81 mg /L to 1087 mg /L when the HRT decreased from 19,2 days to 7,68 days. The best HCO_3 concentrations was found to be 1087 mg/L, for the maximum growth of methanogen in the anaerobic conditions at a HRT of 7,68 days.

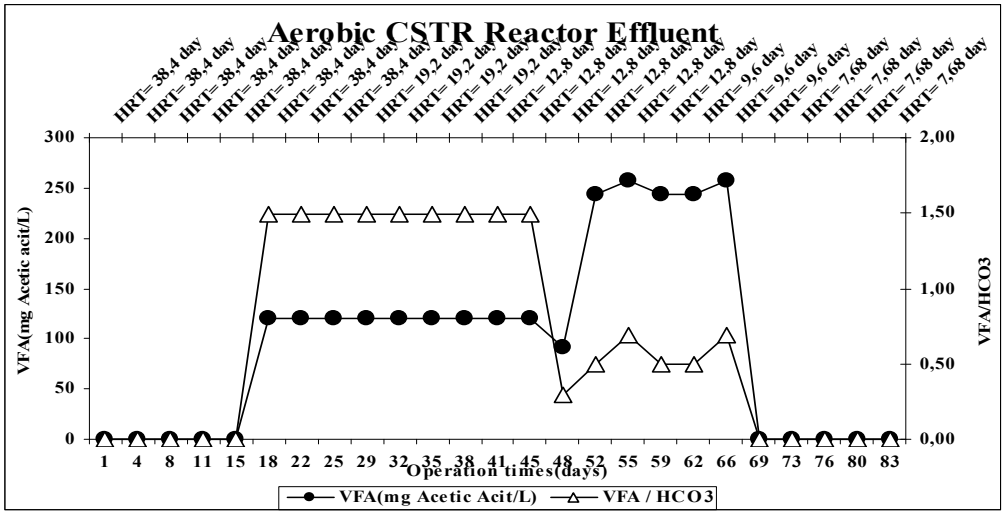


Figure 4.23 The overall VFA and VFA/Bic.Alk. ratio in aerobic (CSTR) reactor

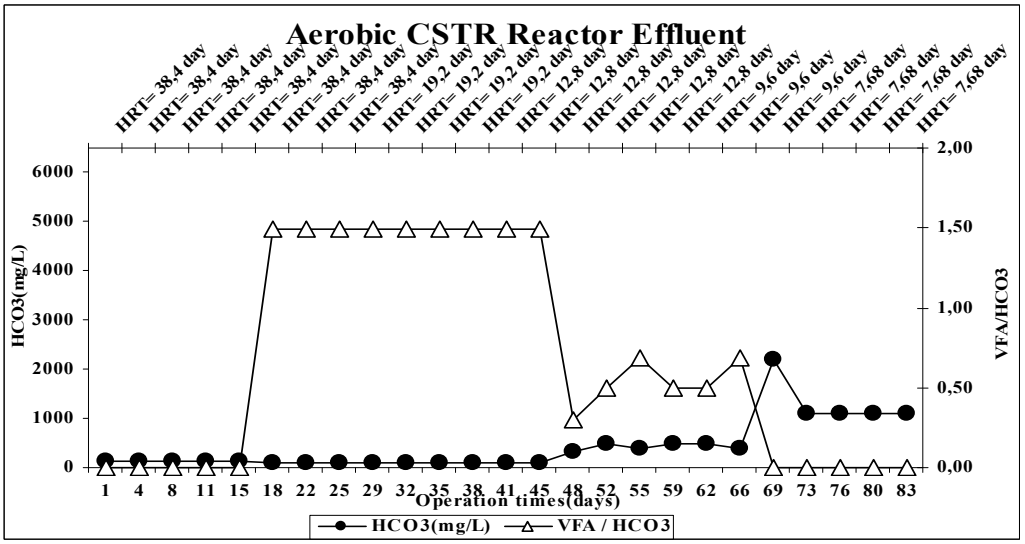


Figure 4.23 The overall HCO_3 and VFA/Bic.Alk. ratio in aerobic (CSTR) reactor

4.2.1.7.7 Specific Methanogenic Activity (SMA) in ABR at Different HRTs. Figure 4.24 shows the SMA values of mixed sludge taken from the all

compartments of ABR during continuous operation of ABR at different HRTs. The SMA is an indicator of methanogenic activity in anaerobic systems. As shown in Figure 4.24, the SMA values increased from 0.111 to 0.218 g COD-CH_4/ gVSS when the HRT decreased from 38.4 days to 7.68 days. In other words the maximum SMA was found to be 0.218 g COD-CH_4/ gVSS day for HRTs between 7.68 and 9.60days. This could be explained by the high flow rates in the ABR reactor resulting in increases in the activity of the methanogenes.

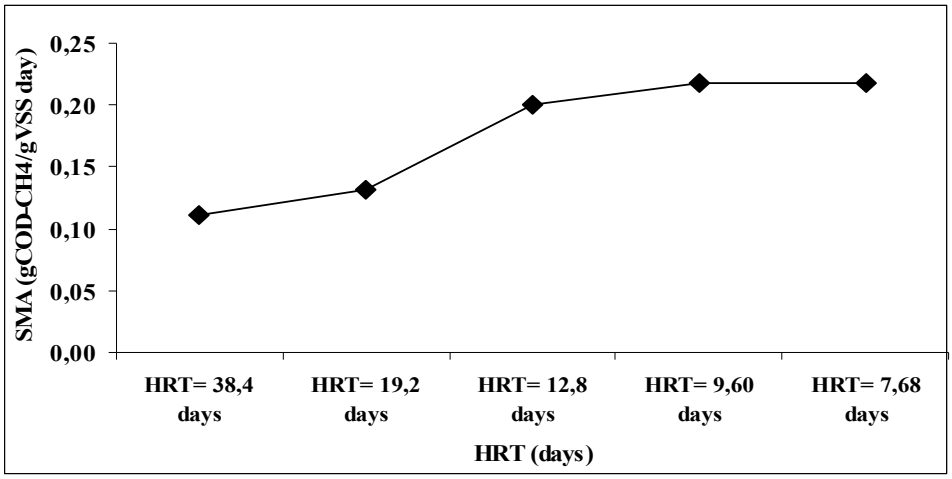


Figure 4.24 SMA values in ABR at different HRTs.

4.2.1.7.8 Assessment of Toxicity of Sequential Anaerobic ABR/Aerobic CSTR Reactor System. Daphnia magna test is accepted as acute toxicity test. Results were expressed as mortality percentage of the Daphnids. After the test samples contining streptomycin was diluted, the experiments were carried out using 10 Daphnids. The Daphnids was added to into every one test vessel at the beginning time (t=0).After 24 h of incubation time, EC_{50} value (the concentration inhibited 50% of Daphnia magna) was found.

Table 4.1 shows the *Daphnia magna* toxicity test results for samples taken from the compartments II, III and IV of the anaerobic ABR reactor, from the effluent of the anaerobic ABR reactor and from the e effluent of the aerobic CSTR system at a HRT of 38,4 days.

Table 4.1 Toxicity values in the compartments II, III, IV, effluent of ABR reactor and effluent of CSTR system (Streptomycin = 200 mg/L, HRT = 38,4 days)

200 mg/L HRT=38,4 days	ANAEROBIC				AEROBİC	
	Compartment 2	Compartment 3	Compartment 4	Effluent	Effluent	
Dilution ratio	Daphnia magna number First start=10				Dilution ratio	Daphnia magna number First start =10
%ww	24 hours				%ww	24 hours
%0	0	0	0	0	%0	7
%30	0	0	0	0	%30	8
%50	0	0	0	3	%50	10
%75	0	0	0	4	%75	10
%95	0	0	2	10	%95	10

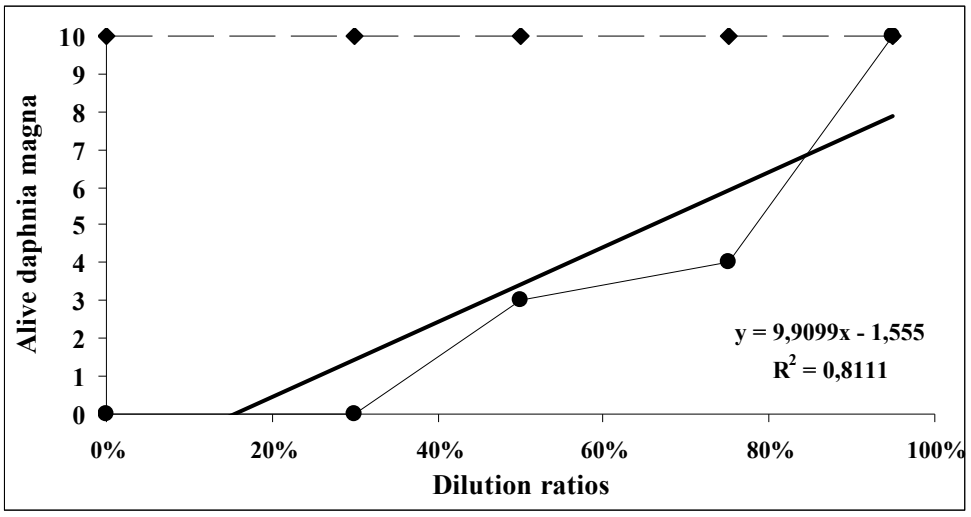


Figure 4.25 Toxicity values in the effluent of ABR reactor at a HRT of 38,4 days

If the effluent wastewater is diluted 66% times, 50% of *Daphnids* was dead at a HRT of 38,4 days. In Figure 4.25, the EC_{50} value was found to be 132 mg/L in the effluent of ABR reactor in the sample diluted at a ratio of 66%.The other dilution ratios did not show any mortality effect to *Daphnids*.

In Figure 4.26, the EC_{10} value was 100 mg/L (EC_{50}= 20 mg/L) in the effluent of CSTR system in the sample diluted at a ratio of 50%. The other dilution ratios did not show any mortality effect to *Daphnids*.

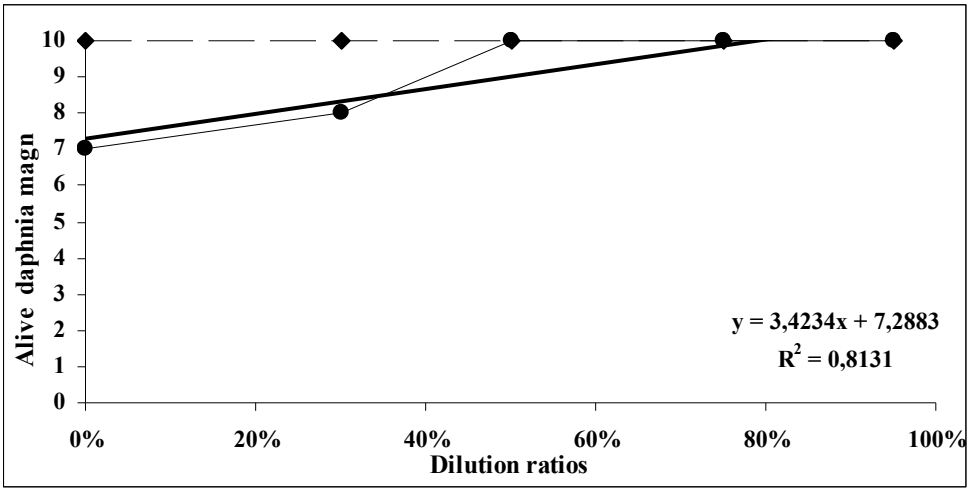


Figure 4.26 Toxicity values in the effluent of CSTR system at a HRT of 38,4 days

Table 4.2 shows the *Daphnia magna* toxicity test results for samples taken from the compartments II, III and IV of anaerobic ABR reactor, the effluent of anaerobic ABR reactor and the effluent of aerobic CSTR system at HRTs of 19,2 days.

Table 4.2 Toxicity values in the compartments II, III, IV, effluent of ABR reactor and effluent of CSTR system (Streptomycin = 200 mg/L, HRT = 19,2 days)

200 mg/L HRTs=19, 2 days	ANAEROBIC				AEROBİC	
	Compartment 2	Compartment 3	Compartment 4	Effluent	Effluent	
Dilution ratio	Daphnia magna number First start=10				Dilution ratio	Daphnia magna number First start =10
%ww	24 hours				%ww	24 hours
%0	0	0	0	0	%0	8
%30	0	0	0	1	%30	9
%50	0	0	0	3	%50	10
%75	0	0	1	8	%75	10
%95	0	8	8	8	%95	10

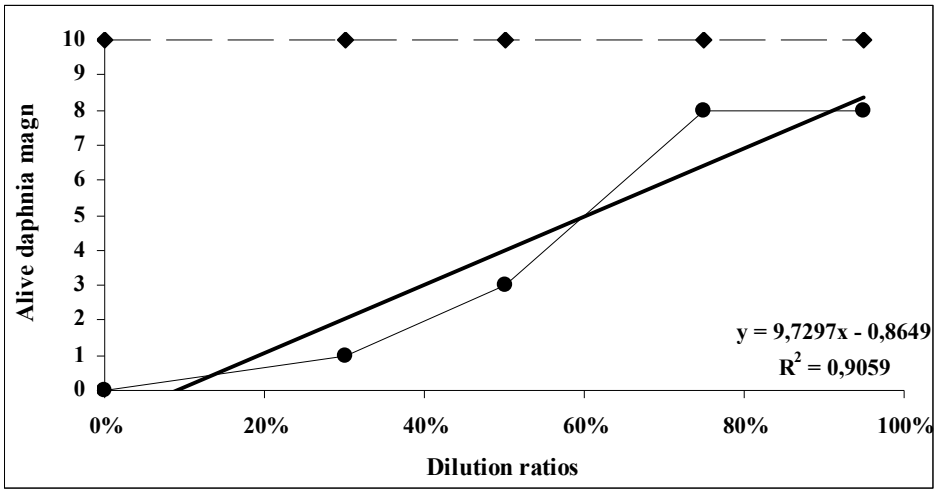


Figure 4.27 Toxicity values in the effluent of ABR reactor at a HRT of 19,2 days

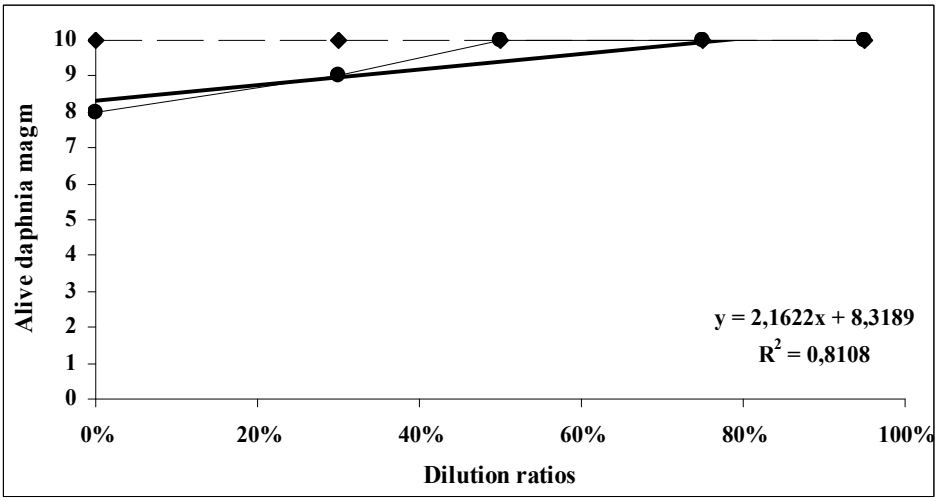


Figure 4.28 Toxicity values in the effluent of CSTR system at a HRT of 19,2 days

The EC_{50} value was 120 mg/L in the effluent of ABR reactor at a HRT of 19,2 days in the sample diluted at a ratio of 60%.The other dilution ratios did not show any mortality effect to *Daphnids* (see in figure 4.27).

In Figure 4.28, EC_{10} value was 306 mg/L (EC_{50}=61,2 mg/L) in the effluent of CSTR at a HRT of 63 days in the system sample diluted at a ratio of 31,5%. The other dilution ratios did not show any mortality effect to *Daphnids*.

Table 4.3 shows the *Daphnia magna* toxicity test results for samples taken from the compartments II, III and IV of anaerobic ABR reactor, the effluent of anaerobic ABR reactor and the effluent of aerobic CSTR system at HRTs of 12,8 days.

Table 4.3 Toxicity values in the compartments II, III, IV, effluent of ABR reactor and effluent of CSTR system (Streptomycin = 200 mg/L, HRT = 12,8 days)

200 mg/L HRTs=12, 8 days	ANAEROBIC				AEROBİC	
	Compartment 2	Compartment 3	Compartment 4	Effluent	Effluent	
Dilution ratio	Daphnia magna number First start=10				Dilution ratio	Daphnia magna number First start =10
%ww	24 hours				%ww	24 hours
%0	0	0	0	0	%0	3
%30	0	0	2	5	%30	5
%50	0	0	6	5	%50	8
%75	7	7	10	10	%75	10
%95	8	9	10	10	%95	10

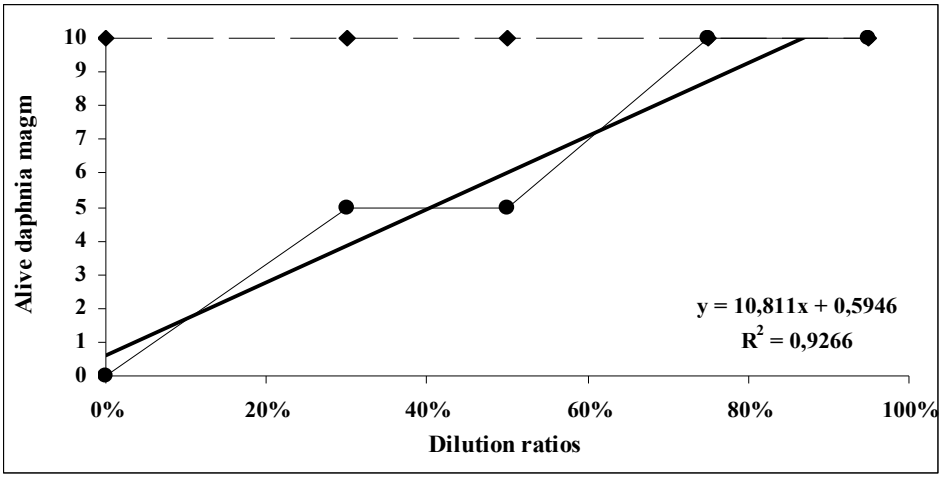


Figure 4.28 Toxicity values in the effluent of ABR reactor at a HRT of 12,8 days

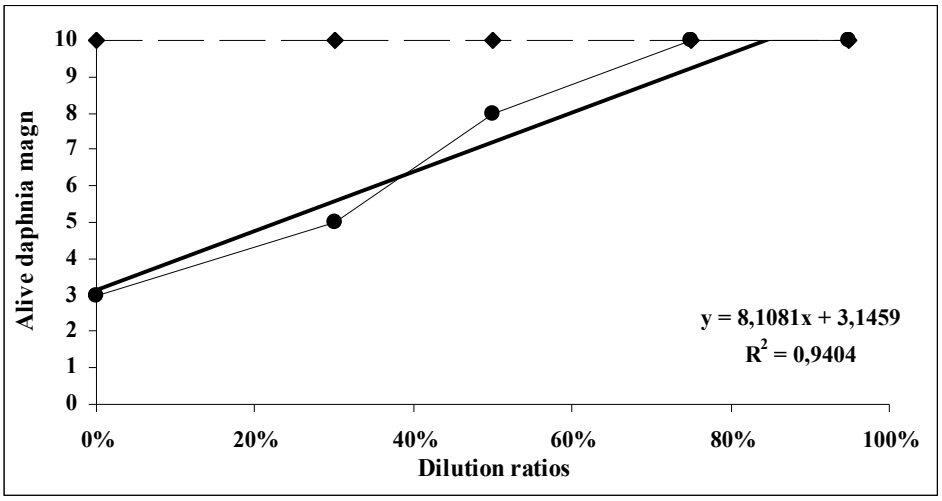


Figure 4.29 Toxicity values in the effluent of CSTR system at a HRT of 12,8 days

The EC_{50} value was 82 mg/L in the effluent of ABR reactor at a HRT of 12,8 days sample diluted at a ratio of 41%.The other dilution ratios did not show any mortality effect of *Daphnid* (see figure 4.28).

In figure 4.29, The EC_{50} value was 46 mg/L in the effluent of CSTR at a HRT of 12,8 days system sample diluted at a ratio of 23%. The other dilution ratios did not show any mortality effect of *Daphnid*.

Table 4.4 shows the *Daphnia magna* toxicity test results for samples taken from the compartments II, III and IV of anaerobic ABR reactor, the effluent of anaerobic ABR reactor and the effluent of aerobic CSTR system at HRTs of 9,60 days.

Table 4.4 Toxicity values in the compartments II, III, IV, effluent of ABR reactor and effluent of CSTR system (Streptomycin = 200 mg/L, HRT = 9,6 days)

200 mg/L HRTs=9,6 days	ANAEROBIC				AEROBİC	
	Compartment 2	Compartment 3	Compartment 4	Effluent	Effluent	
Dilution ratio	Daphnia magna number First start=10				Dilution ratio	Daphnia magna number First start =10
%ww	24 hours				%ww	24 hours
%0	0	0	0	0	%0	2
%30	0	0	1	3	%30	7
%50	4	6	7	7	%50	8
%75	8	8	10	10	%75	10
%95	10	10	10	10	%95	10

The EC_{50} value was 82 mg/L in the effluent of ABR reactor at a HRT of 9,60 days in samples diluted at a ratio of 41%.The other dilution ratios did not show any mortality effect to *Daphnids* (see figure 4.30).

In Figure 4.31, the EC_{50} value was 42 mg/L in the effluent of CSTR at a HRT of 9,60 days system sample diluted at a ratio of 21%. The other dilution ratios did not show any mortality effect of *Daphnids*.

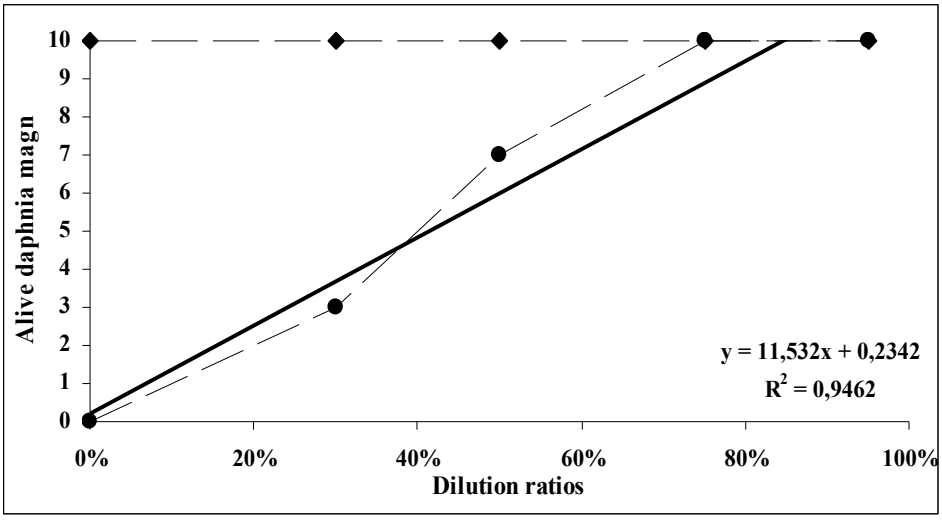


Figure 4.30 Toxicity values in the effluent of ABR reactor at a HRT of 9,60 days

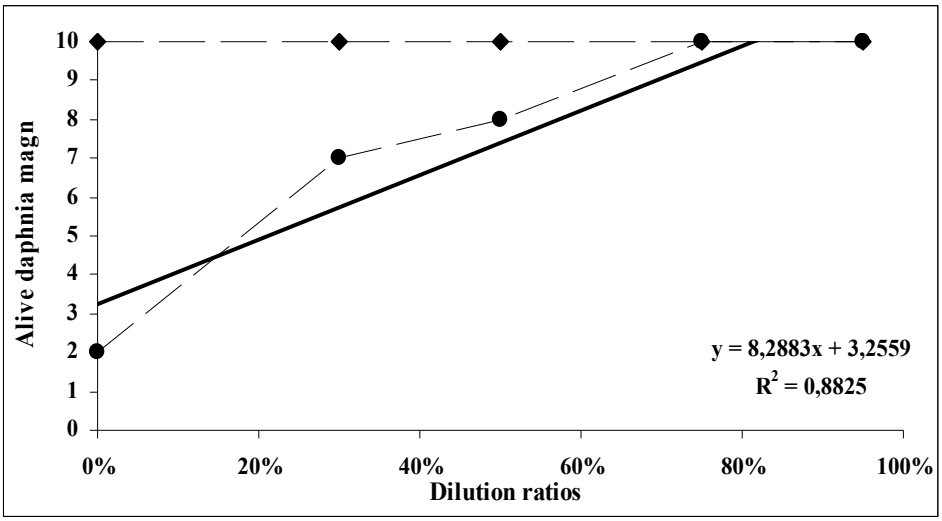


Figure 4.31 Toxicity values in the effluent of CSTR system at a HRT of 9,60 days

Table 4.5 shows the *Daphnia magna* toxicity test results for samples taken from the compartments II, III and IV of anaerobic ABR reactor, the effluent of anaerobic ABR reactor and the effluent of aerobic CSTR system at HRTs of 7,68 days.

Table 4.5 Toxicity values in the compartments II, III, IV, effluent of ABR reactor and effluent of CSTR system (Streptomycin = 200 mg/L, HRT = 7,68 days)

200 mg/L HRTs=7,68 days	ANAEROBIC				AEROBİC	
	Compartment 2	Compartment 3	Compartment 4	Effluent	Effluent	
Dilution ratio	Daphnia magna number First start=10				Dilution ratio	Daphnia magna number First start =10
%ww	24 hours				%ww	24 hours
%0	0	0	0	0	%0	0
%30	0	0	0	0	%30	5
%50	0	0	2	4	%50	6
%75	6	4	6	7	%75	10
%95	10	10	10	10	%95	10

The EC_{50} value was 114 mg/L in the effluent of ABR reactor at a HRT of 7,68 days in samples diluted at a ratio of 57%.The other dilution ratios did not show any mortality effect to *Daphnids* (see figure 4.32).

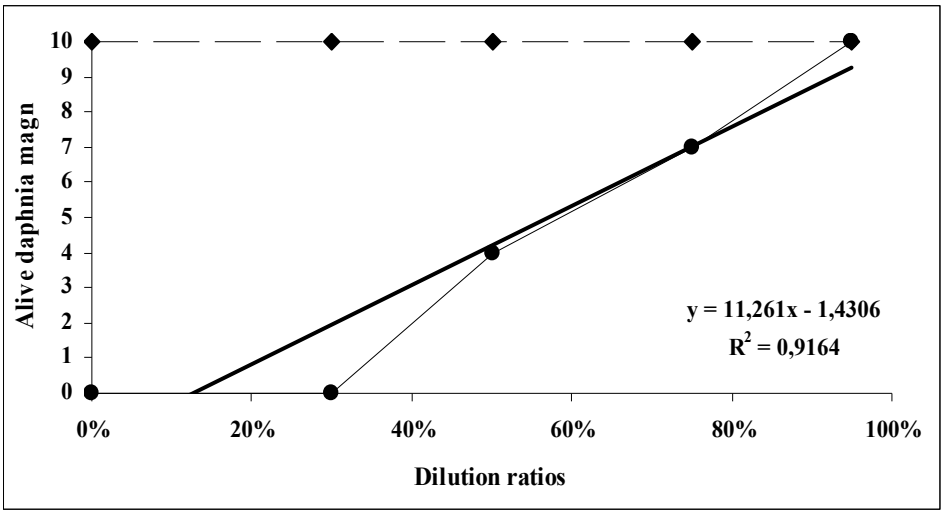


Figure 4.32 Toxicity values in the effluent of ABR reactor at a HRT of 7,68 days

In Figure 4.33, the EC_{50} value was 78 mg/L in the effluent of CSTR at a HRT of 7,68 days system in the sample diluted at a ratio of 39%. The other dilution ratios did not show any mortality effect of *Daphnid*.

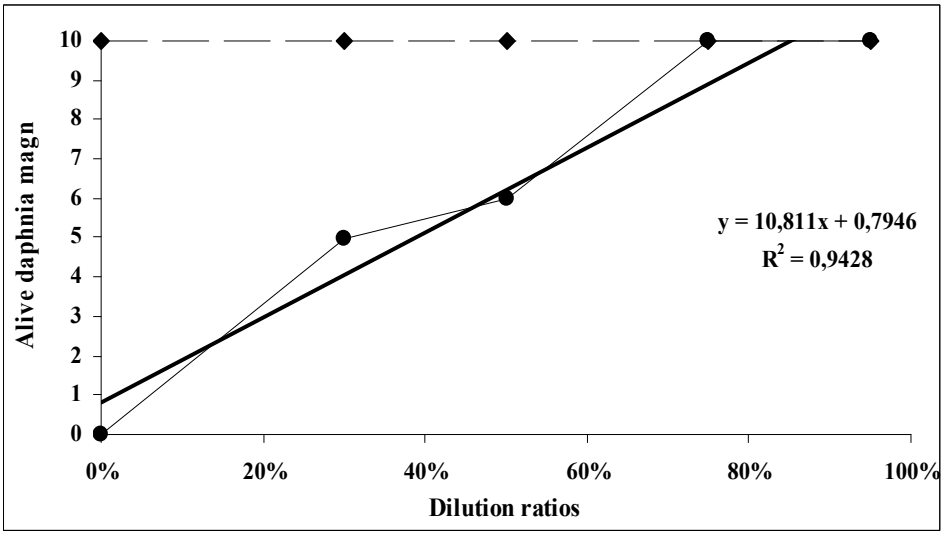


Figure 4.33 Toxicity values in the effluent of CSTR system at a HRT of 7,68 days

Table 4.6 Variations of acute toxicity values (EC_{50} for Daphnia magna) through influent, ABR, CSTR reactor effluents and total sequential reactor system

HRT Days	EC_{50} ABR influent (mg/L)	Dilution (%)	EC_{50} ABR Effluent (mg/L)	Toxicity Removal (%)	EC_{50} Aerobic Effluent (mg/L)	Toxicity Removal (%)	EC_{50} Removal in Sequential Total System Effluent (%)
38,4	400	66	132	%67	20	%85	%95
19,2	400	60	120	%70	61,2	%49	%85
12,8	400	41	82	%80	46	%43	%89
9,60	400	41	82	%80	42	%49	%90
7,68	400	57	114	%72	78	%32	%80

The acute toxicity test results performed with *Daphnia magna* showed that the EC_{50} values decreased from influent 400 mg/L to 132 mg/L , and to 20 mg/L in the effluents of ABR , in aerobic reactor effluent at a HRT of 38.4 days. (see in a table 4.6) . The total acute toxicity reduction in sequential ABR CSTR reactor effluent was 95%. At a HRT of 19,2 days EC_{50} values decreased from influent 400 mg/L to 120 mg/l , and to 61,2 mg/L in the effluents of ABR , in aerobic reactor effluent. The total acute toxicity reduction in sequential ABR CSTR reactor effluent was 85%. At a HRT of 12,8 days EC_{50} values decreased from influent 400 mg/L to 82 mg/L , and to 46 mg/l in the effluents of ABR , in aerobic reactor effluent. The total acute toxicity reduction in sequential ABR CSTR reactor effluent was 89%. At a HRT of 9,60 days EC_{50} values decreased from influent 400 mg/L to 82 mg/L , and to 42 mg/L in the effluents of ABR , in aerobic reactor effluent. The total acute toxicity reduction in sequential ABR CSTR reactor effluent was 90%. After that the EC_{50} values decreased from influent 400 mg/L to 114 mg/L, and to 78 mg/L in the effluents of ABR , in aerobic reactor effluent at a HRT of 7,68 days. The total acute toxicity reduction in sequential ABR CSTR reactor effluent was 80%. The maximum acute toxicity removal was found at the maximum HRT of 38.4 days studied during the operation of anaerobic ABR reactor. This could be attributed to high HRT which is enough for toxicity removals of 200 mg/L streptomycin antibiotic. During this HRT the microorganisms have enough time to contact and to acclimate to 200mg/L streptomycin antibiotic in ABR.

4.2.1.7.9 Variations of Streptomycin Removal Efficiency in the ABR Reactor at Increasing HRTs. Figures 4.34, 4.35 and 4.36 shows the HPLC chromatogram of the samples taken from the anaerobic reactor influent, effluent and aerobic CSTR effluent at a constant influent streptomycin concentration of 200 mg/L at a HRT of 12,8 days. A Streptomycin peak was 59,79 mg/L in HPLC chromatogram of the effluent of ABR reactor samples. This showed that streptomycin was biodegraded with removal efficiencies of 66%- 74% in ABR and CSTR reactors at a HRTs of 12,8 days. (See table 4.7). In figure 4.34 the chromatogram of streptomycin showed that the peak was appeared after 2.319 min, in HPLC analysis. This corresponded to a streptomycin concentration of 179,57 mg/L. As seen in figure 4.35, the streptomycin concentration was measured as 59,79 mg/L in the effluent of the ABR. The streptomycin removal efficiency was 66% at a HRT of 12,8 days. The streptomycin concentration was measured as 47,54 mg/L in the aerobic CSTR effluent corresponding a removal efficiency 74%. In this study it was found that the "streptomycin" antibiotic was mainly degraded (179,57 mg/L) in anaerobic ABR reactor while the remaining small part of this antibiotic (47,54 mg/L) was removed in the aerobic CSTR reactor.

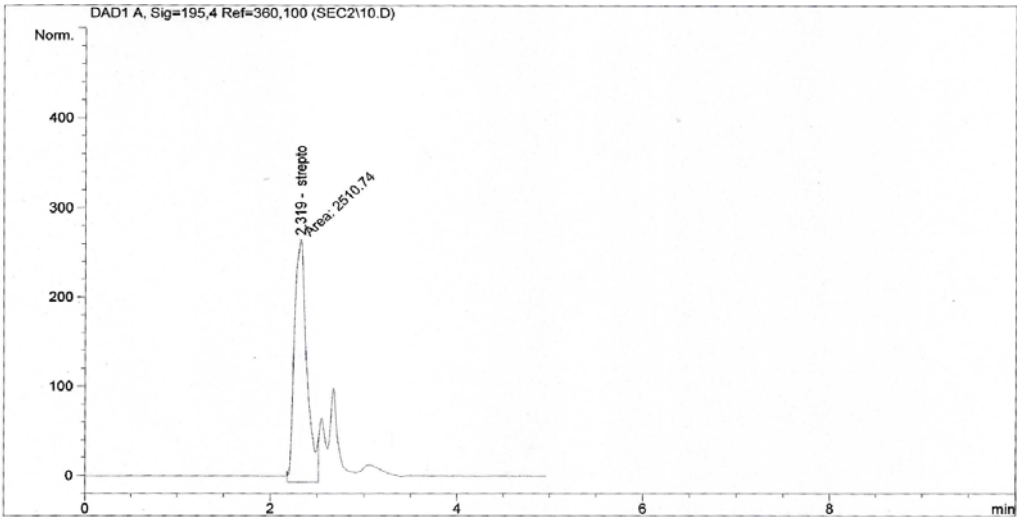


Figure 4.34 HPLC chromatogram in the influent of ABR at 12,8 days of HRT

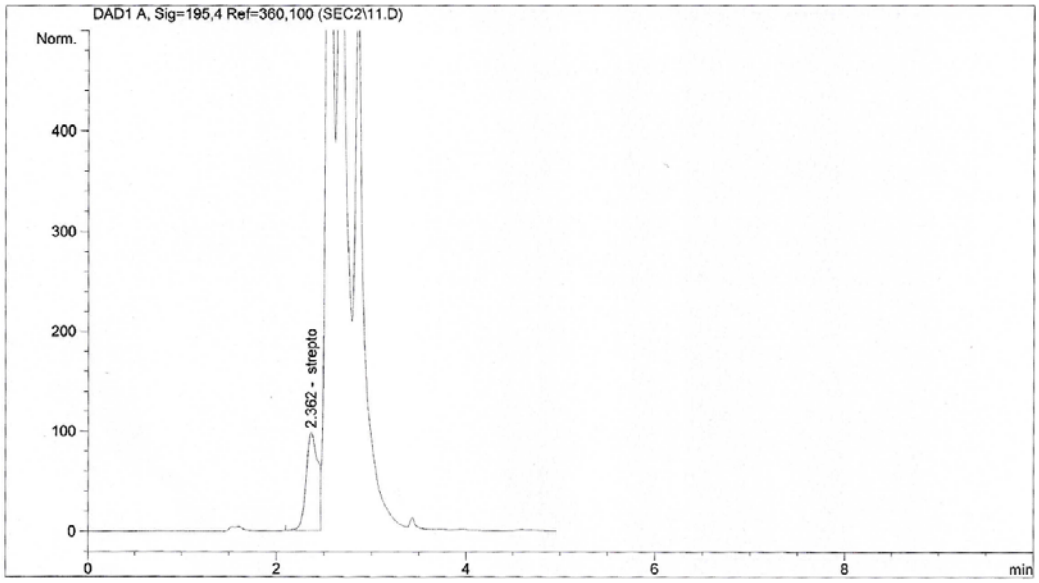


Figure 4.35 HPLC chromatogram in the effluent of ABR at 12,8 days of HRT

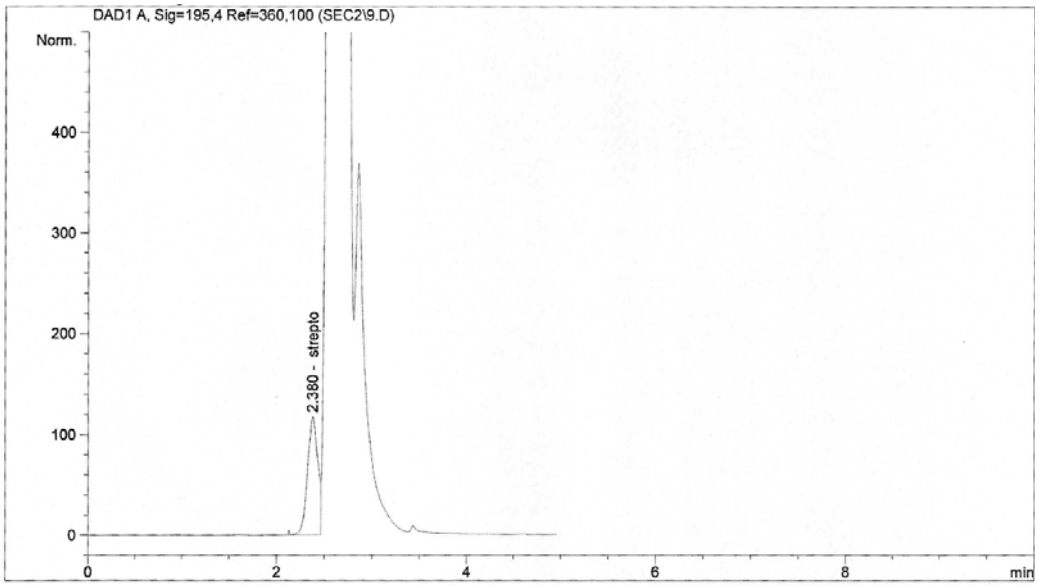


Figure 4.36 HPLC chromatogram in the effluent of CSTR at 12,8 days of HRT

Table 4.7 Variations of Streptomycin concentrations in the influent, effluent of the ABR Reactor in the effluent of the Aerobic CSTR and in total reactor system versus decreasing HRTs at an initial Streptomycin concentration of 200 mg/L

HRT Days	Antibiotic ABR Influent (mg/L)	Antibiotic ABR Effluent (mg/L)	Antibiotic ABR Effluent Removal (%)	Antibiotic Aerobic Effluent (mg/L)	Antibiotic Removal in Sequential Total System Effluent (%)
38,4	180,71	83,74	54%	48,54	73%
19,2	178,41	82,12	53,4%	49,90	72%
12,8	179,57	59,79	66%	47,54	74%
9,60	181,48	75,38	58%	54,26	71%
7,68	180,48	86,43	52%	72,04	60%

4.2.2 The Removal of Chloramphenicole in Anaerobic Baffled Reactor (ABR) and Sequential ABR/CSTR Reactor System

4.2.2.1 Start-up of Anaerobic Baffled Reactor (ABR)

The ABR reactor was operated through 12 days without chloramphenicole under steady-state conditions to acclimate the granular sludge to ABR reactor. Figure 4.37 shows the COD removal efficiencies in the ABR during the start-up period. The COD removal efficiency was 84% at the operation time of 4 days. The COD removal efficiencies remained stable around 82% after an operation period of 12 days. Figure 4.38 shows the methane gas percentages in the ABR during the start-up period. The daily methane gas production and methane percentage remained stable at 439,2 L/day and 48%, respectively, after 12 days of the start-up period. Figure 4.36 shows the total gas percentages in the ABR during the start-up period. The daily total gas production and methane percentage remained stable at 475,2 L/day and 48%, respectively, after 12 days of the start-up period.

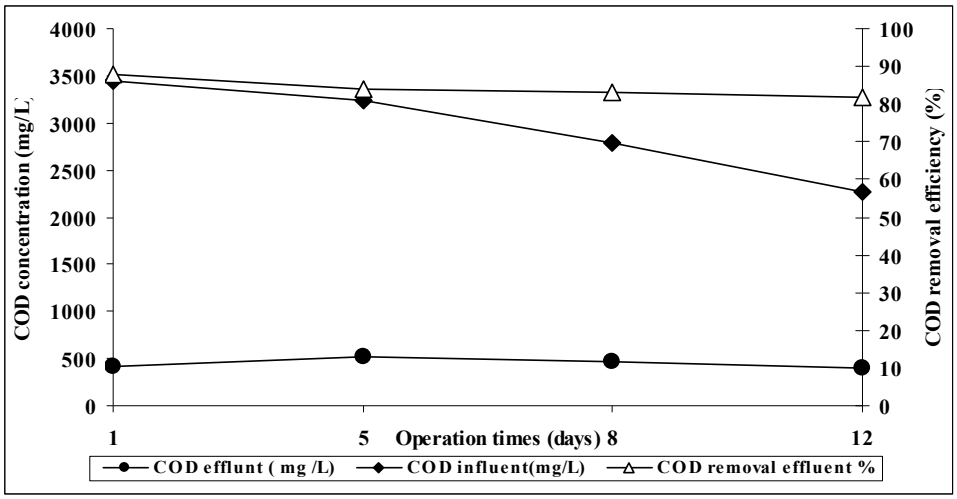


Figure 4.37 COD removal efficiencies in the ABR during the start-up period in ABR

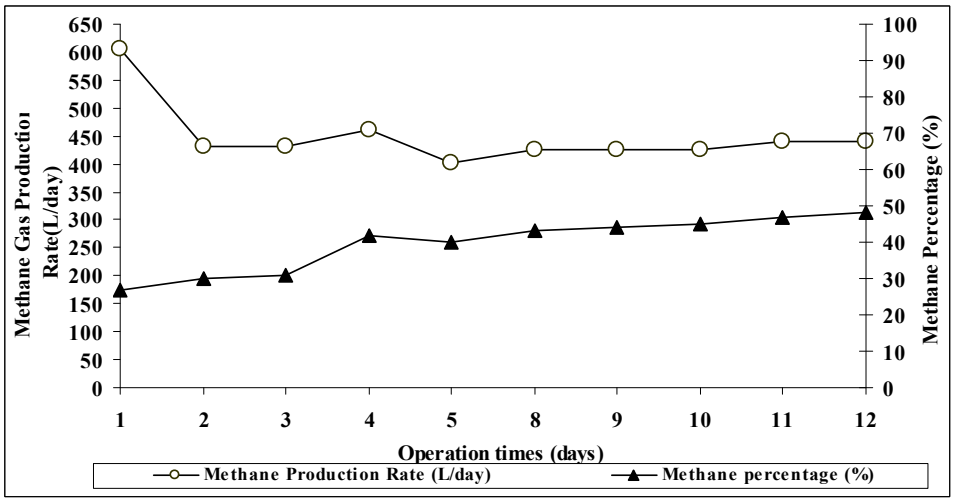


Figure 4.38 Methane gas production and methane percentages in the ABR during the start-up period in ABR

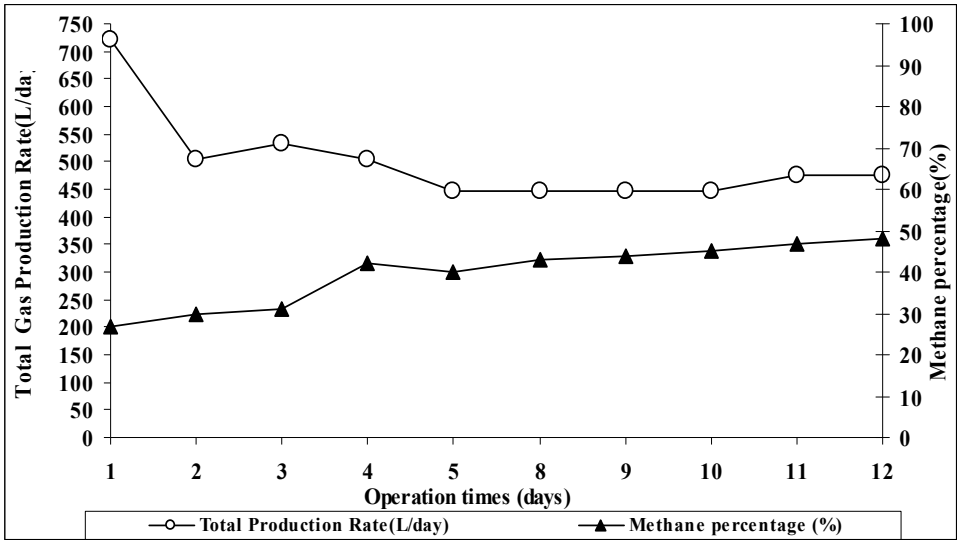


Figure 4.39 Total gas production and methane percentages in the ABR during the start-up period in ABR

4.2.2.2 Effect of Increasing Chloramphenicole Concentration on the COD Removal Efficiencies in ABR Reactor

In this study, the effect of increasing chloramphenicole concentrations on COD removal efficiencies was investigated in ABR. The operation of the ABR with chloramphenicole was started at an influent chloramphenicole concentration of 50 mg/L, and then chloramphenicole concentration was subsequently increased from 50, 75, 100, 130, 180, 230, 280, to 340 mg/L (at OLRs from 0,167 to 0,178 kg COD/m^3 day). The effect of chloramphenicole concentration on the COD removal efficiencies in ABR was shown in Figure 4.40. Although the influent COD concentration was kept constant at 3000 mg/L with glucose, the influent COD concentrations increased with increasing chloramphenicole concentration since chloramphenicole give additional COD to synthetic wastewater. The influent COD concentration was 3206 mg/L at a chloramphenicole concentration of 50 mg/L while it was measured as 3418 mg/L at a chloramphenicole concentration of 340 mg/L. The COD removal efficiency was 96,95% at an initial chloramphenicole concentration of 50 mg/L introduced to ABR. In a study performed by Liu at al.,(2009) the COD removal efficiency was found as 82.47% at a organic loading rate of (ORL) 2 kg COD/m^3*day in a periodic anaerobic baffled reactor (PABR) treating traditional medicine industrial wastewater. The COD removal efficiency found in this study is

comparable higher than that aforementioned study. The maximum COD removal efficiencies were around 94-96 % for a chloramphenicole concentration of 130 mg/L and around 99% at a chloramphenicole concentration of 50 mg/L. When the chloramphenicole concentration was increased to 340 mg/L the COD removal efficiency was measured as 65,29 % (Figure 4.40).

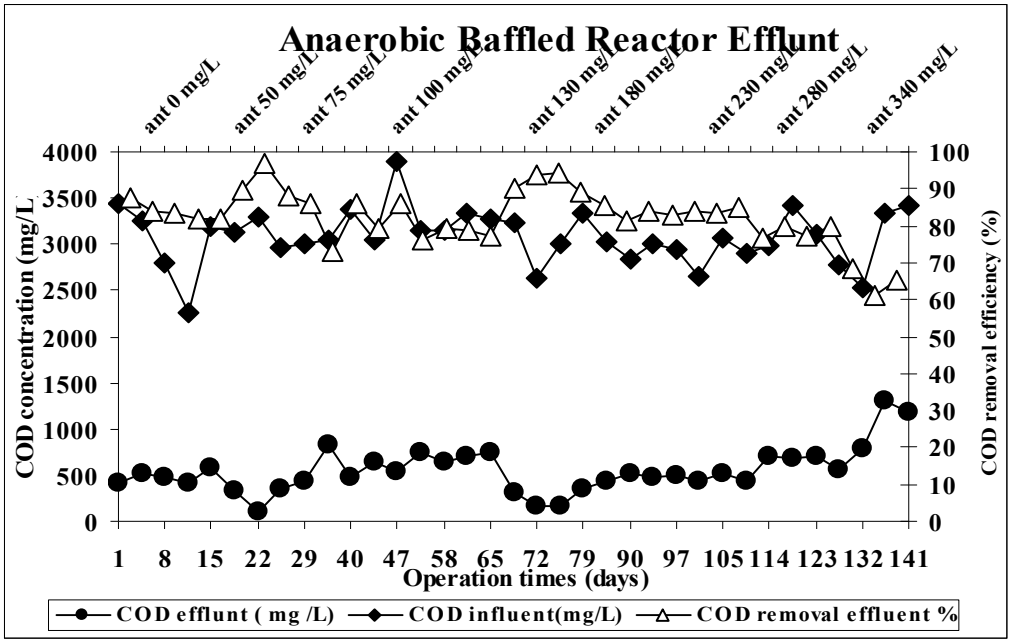


Figure 4.40 Effect of chloramphenicole concentration on COD removal efficiencies in ABR reactor

4.2.2.3 Effect of Increasing Chloramphenicole Concentration on the VFA, Bicarbonate Alkalinity (Bic.Alk.) concentrations and VFA/Bic.Alk. ratio in ABR Reactor

Figure 4.41 shows the variations in VFA concentrations and VFA/Bic.Alk. ratios in the ABR reactor at increasing chloramphenicole concentrations. As the chloramphenicole concentrations increased from 0 mg/l to 340 mg/l the VFA concentration increased from 0 mg/l to 26 mg/l. Figure 4.38 shows the variations of Bic.Alk. concentrations through 141 days of operation period. The Bic.Alk. concentrations decreased in the effluent, step by step. As the chloramphenicole concentration increased from 0 to 340 mg/L the VFA concentrations decreased from 2822 to 1858 mg/L (see figure 4.42). VFA/Bic.Alk. ratios varied between 0,005 and

0,014 in the effluent of ABR reactor at increasing chloramphenicole concentration (from 0 mg/L up to 340 mg/L).This showed that the ABR reactor operated under steady-state conditions since the VFA/ BİC.Alk. ratios were lower than 0.5.The HCO_3 alkalinity also remained between 1250 and 2500 mg/L indicating the buffer capacity of the ABR reactor for methanogenesis (Speece, 1996).

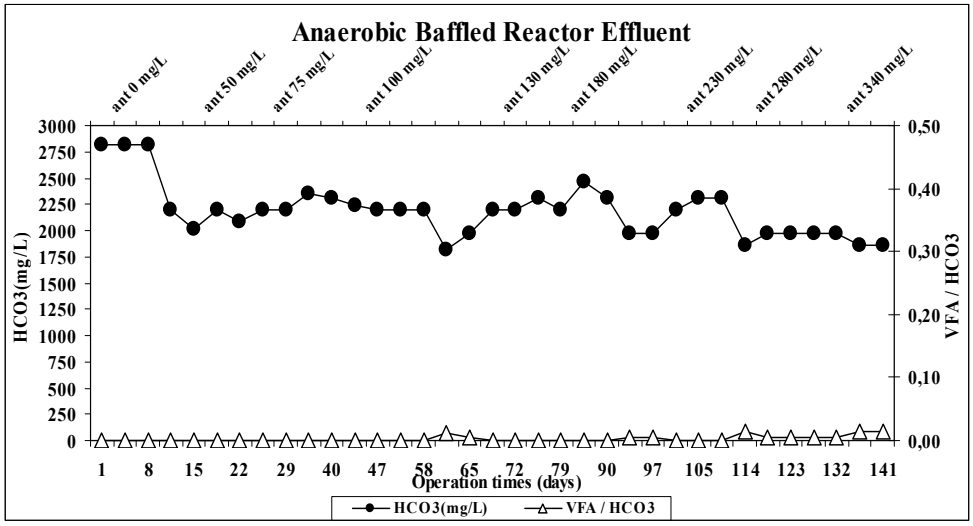


Figure 4.41 The variations of HCO_3 in ABR at increasing chloramphenicole concentrations

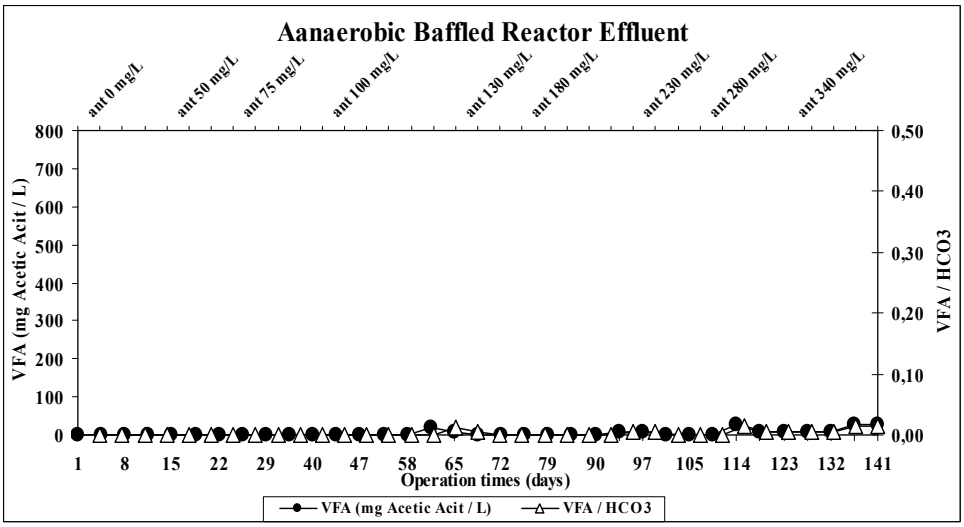


Figure 4.42 The variations of VFA in ABR at increasing chloramphenicole concentrations

4.2.2.4 The Variations of COD Removal Efficiency in Compartments of the ABR Reactor at Increasing Chloramphenicole Concentrations

In this study, the effect of increasing chloramphenicole concentrations on COD removal efficiencies was investigated in four compartments of the ABR reactor. As shown in fig.4.43 (a), the COD removal efficiency values in the compartment I was lower than the other compartments. The COD removal efficiency values in the first compartment varied between 23,78% and 54,33% at all chloramphenicole concentrations. The COD removal efficiency values increased to 78,67% and to 91,14% in compartments II and III at a chloramphenicole concentration of 130 mg/L, respectively. Figure 4.43. (b),(c), shows the COD removal efficiencies in the compartment II(varied between 75,67% and 71,72%)and compartment III (varied between 77,52% and 74,45%). COD removal efficiencies were high (97%) in compartment IV compared to the other compartments. The COD removal efficiency increased from 77,37% to 97% until a chloramphenicole concentration of 130 mg/L in compartment IV. Then the COD removal efficiency decreased from 97% to 62% after 130 mg/L at compartment IV (see 4.43.(d)).

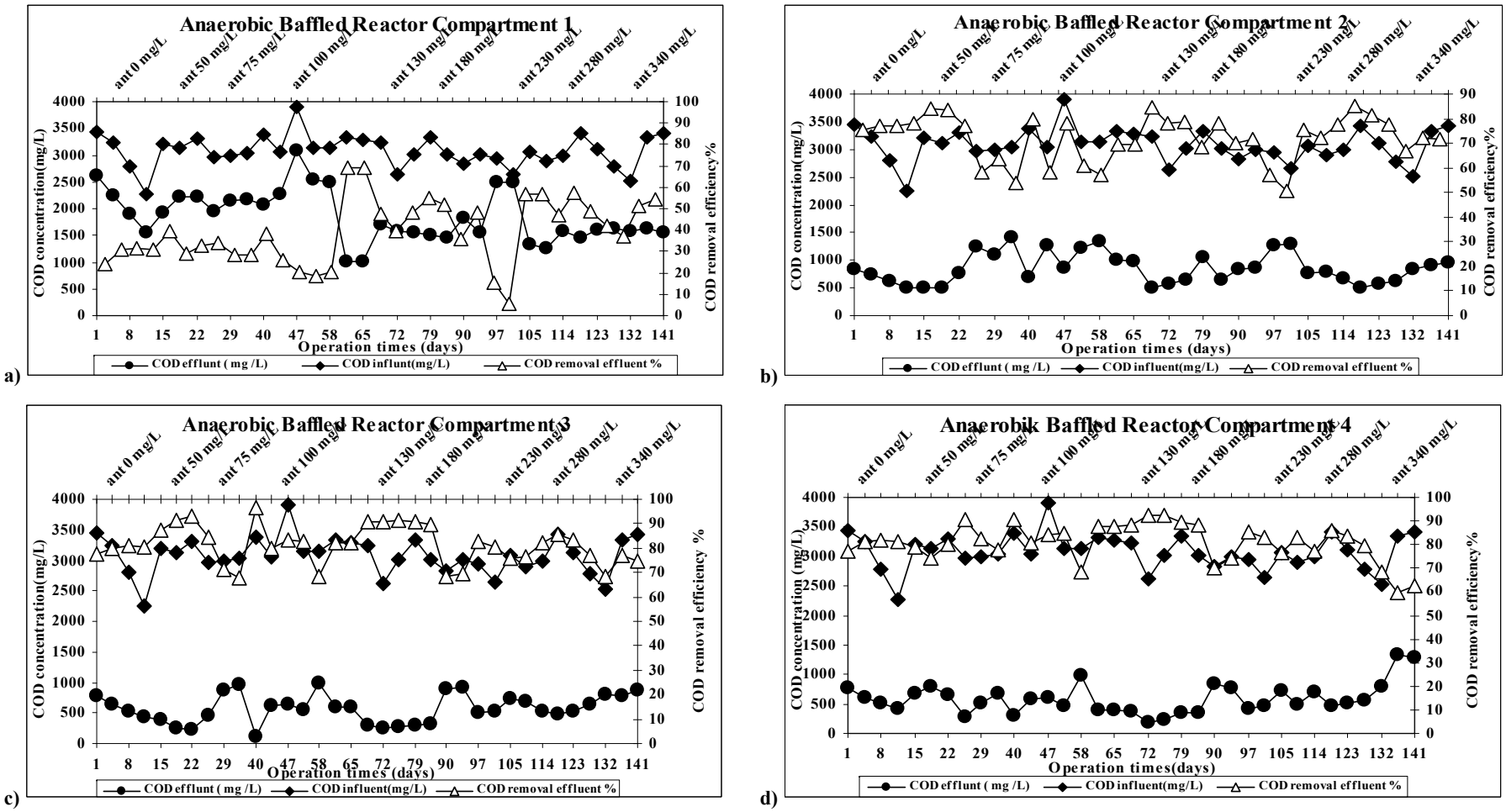


Figure 4.43 Effect of chloramphenicole concentration on COD removal efficiencies in all compartments. (a- compartment 1, b-compartment 2, c-compartment 3, d-compartment 4)

4.2.2.5 The Variations of VFA, Bicarbonate Alkalinity (Bic.Alk.) and VFA/Bic.Alk. ratio in Compartments of the ABR Reactor at Increasing Chloramphenicole Concentrations

Figure 4.44 shows the VFA, VFA/Bic.Alk. ratio variations in the ABR reactor at increasing chloramphenicole concentrations from 0 mg/L up to 340 mg/L. VFA concentrations were high in the compartment I compared to the other compartments, because in compartment I the activity of acidogens was a maximum rate (See 4.44. (a)). VFA concentrations decreased from 590 mg/L to 0 mg/L as the chloramphenicole concentrations increased from 50 mg/L up to 340 mg/L in first compartment. VFA concentrations decreased in compartments II, III and IV. VFA concentrations decreased from 623 mg/L to 0 mg/L until a chloramphenicole concentration of 100 mg/L while the VFA concentration was zero until a chloramphenicole concentration of 340 mg/L in compartment IV(See 4.44.(d)). The VFA concentration was zero at a chloramphenicole concentration of 340 mg/L in compartment III (See 4.44. (c)).The VFA concentrations decreased from 157 mg/L to 0 mg/L until a chloramphenicole concentration of 340 mg/L in compartment II (See 4.44. (b)).

The Bicarbonate Alkalinity (HCO_3) and VFA/Bic.Alk. ratio variations in all compartments of the ABR reactor at increasing chloramphenicole concentrations (from 0 mg/L up to 340 mg/L) were shown in Figure 4.42. Figure (4.45.(a)) indicates a low decreases in HCO_3 concentrations (from 1622 mg/L to 1104 mg/L) was present in the compartment I when the ABR reactor was operated at chloramphenicole concentrations in the range of 0 mg/L - 340 mg/L. similarly no significant decreases in HCO_3 concentrations was observed in compartment IV. the hco_3 concentrations were between 2126 mg/l and 2240 mg/l in the compartment IV when the ABR reactor was operated at chloramphenicole concentrations varying between 0 mg/L and 340 mg/L (see figure 4.45 (d)).The HCO_3 concentrations decreased from 2085 mg/L to 1218 mg/L at a chloramphenicole concentration of 340 mg/L in compartment II. These HCO_3 concentrations decreased from 2240 mg/L to 1285 mg/L at a chloramphenicole concentration of 340 mg/L in compartment III.

The HCO_3 concentrations in the compartment IV is higher than the others compartments in the ABR reactor.

Generally it was found that the acidogenesis is the major mechanism of the anaerobic treatment in the first compartment of the ABR reactor. The third and fourth compartments are the major removal steps for methanogenesis. Therefore the VFA concentrations were high in the first compartment while the HCO_3 alkalinity concentrations were low in the same compartment of the ABR.

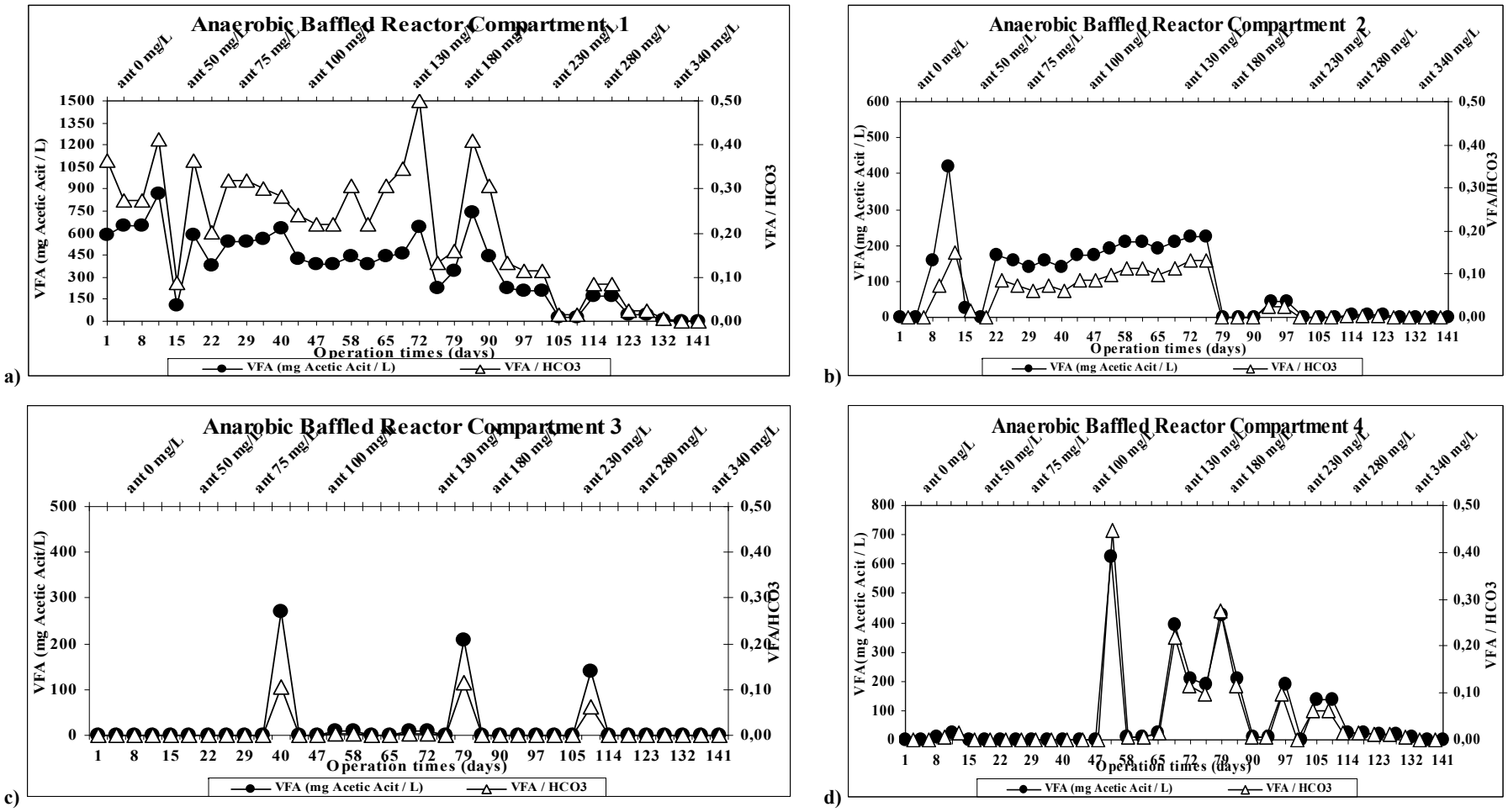


Figure 4.44 The variations of VFA in ABR at increasing chloramphenicole concentrations in the all compartments. (a- compartment 1, b-compartment 2, c-compartment 3, d- compartment 4)

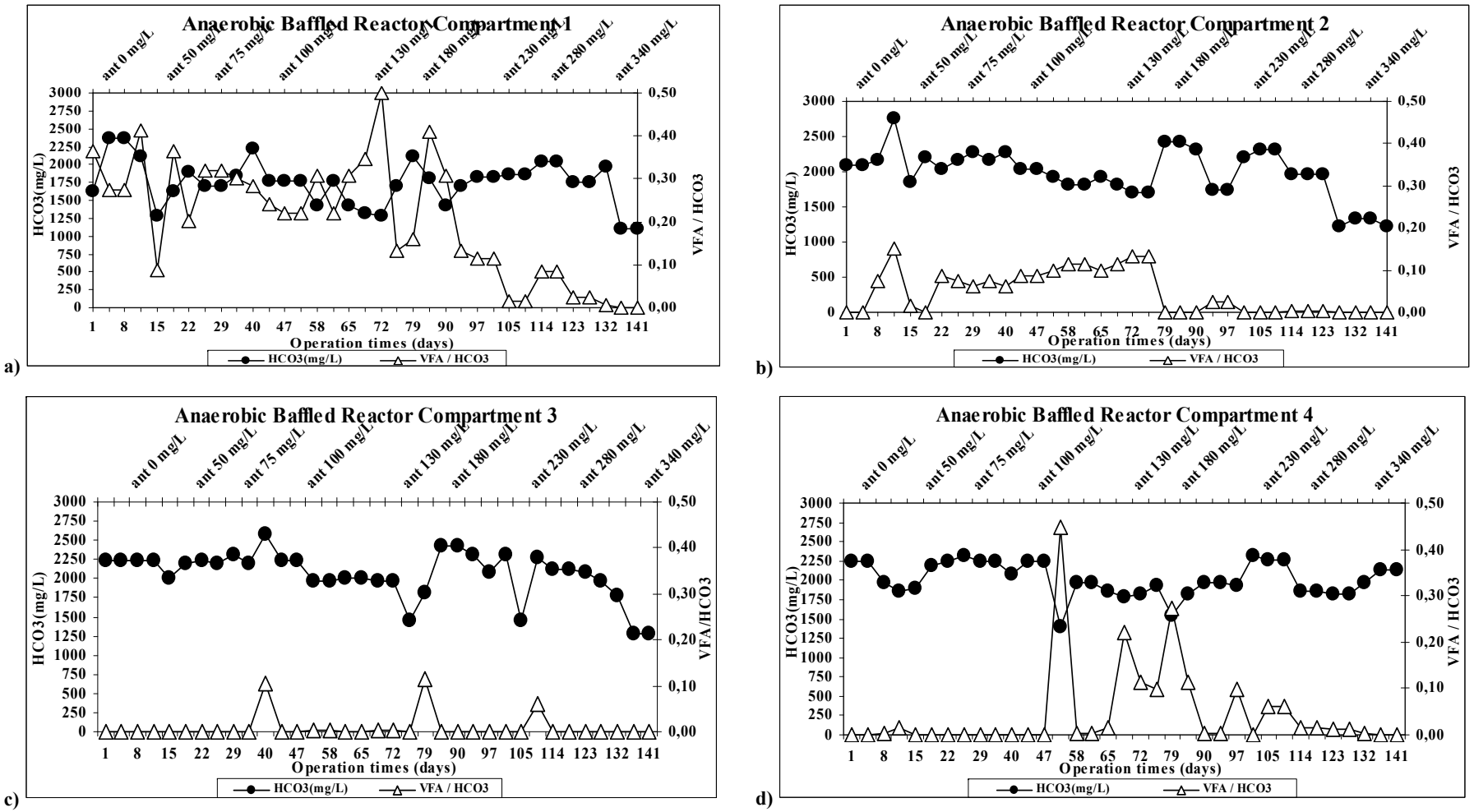


Figure 4.45 The variations of HCO_3 in ABR at increasing chloramphenicole concentrations in the all compartments. (a- compartment 1, b-compartment 2, c-compartment 3 d- compartment 4)

4.2.2.6 Effect of Increasing Antibiotic Dose on Gas Production and Methane Percentage in Anaerobic ABR Reactor.

Biogas production was monitored through the operation of the ABR reactor, particularly for detection the methanogenic activity. From Figure 4.46 it can be seen that the methane gas production rates decreased from 432 L/day to 403,2 L/day at chloramphenicole of zero. Then methane gas production rates increased from 403,2 L/day to 504 L/day , as the chloramphenicole concentration increased from 0 mg/L up to 130 mg/L . As the chloramphenicole concentration was increased from 130 mg/L to 340 mg/L, the methane gas production decreased from 504 L/day to 273,6 L/day . The methane percentages of biogas increased from 27% up to 58% until a chloramphenicole concentration of 130 mg/L. The methane percentages of biogas were decreased to 44%, when the chloramphenicole concentration increased from 130 mg/L to 340 mg/L. In a study performed by Liu at all (2009) methane gas production was found as 12 L/day (OLR=1.04 kg COD/m^3*day), 30 L/day (OLR=2.01 kg COD/m^3*day) and 66 L/day (OLR=6.17 kg COD/m^3*day) for firth, second and third compartments, in ABR reactor respectively. In this study the methane percentages are comparable higher than that aforementioned study.

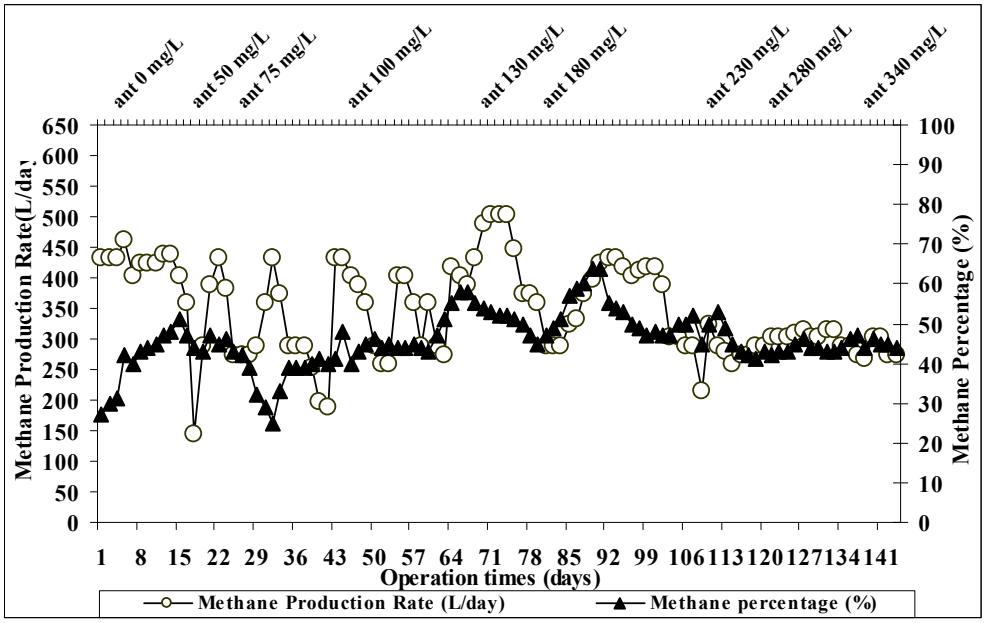


Figure 4.46 The variations of methane gas production and methane percentage in ABR at increasing chloramphenicole concentrations

From Figure 4.47 it can be seen that the total gas production rates decreased from 720 L/day to 432 L/day in the operation of ABR without chloramphenicole. After that the total gas production rates increased from 432 L/day up to 547,2 L/day , respectively as the chloramphenicole concentration increased from 50 mg/L to 130 mg/L. The total gas production also, decreased from 547,2 mg/L to 302,4 mg/L when chloramphenicole concentration increased from 130 mg/L up to 340 mg/L. The methane percentages of biogas increased from 27% up to 58% until a chloramphenicole concentration of 130 mg/L. The methane percentages of biogas were decreased to 44%, when the chloramphenicole concentration increased from 130 mg/L to 340 mg/L.

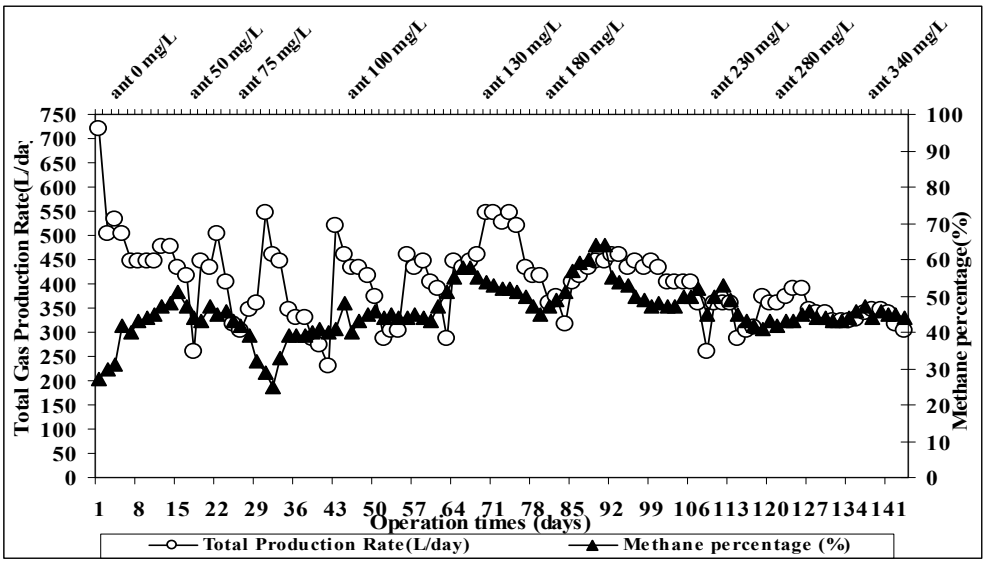


Figure 4.47 The variations of total gas production and methane percentage in ABR at increasing chloramphenicole concentrations

4.2.2.7 Effect of Hydraulic Retention Time (HRT) on the Performance of ABR Reactor

4.2.2.7.1 Effect of HRTs on the COD Removal Efficiency in ABR Reactor. The effect of hydraulic retention times (HRTs) on the COD removal efficiency was shown in Figure 4.48.The influent chloramphenicole concentration was kept constant at 130 mg/L. As shown in Figure 4.48, the influent COD concentration was

approximately 3450-3000 mg/L since 130 mg/L chloramphenicole gives an additional COD concentration to the total COD thought continuous operation. 130 mg/L of chloramphenicole gave approximately a COD of 161,85 mg/L**.** 77,94% COD removal efficiency was obtained at a HRT of 38,4 days in ABR reactor. 95,13% COD removal efficiency was obtained at a HRT of 19,2 days in ABR reactor at a chloramphenicole concentration of 130 mg/L. When the HRT were decreased from 12,8 days to 7,68 days, the effluent COD removal efficiency decreased from 84,23% to 83,82%, respectively. To the maximum COD removal efficiency (E= 98,12%)was reached at a HRT of 19,2 days. Akunna & Clark, (2000) investigated the performance of an anaerobic baffled reactor treated a whisky distillery wastewater at different four HRTs (10, 7, 4 and 2 days). The maximum COD removal efficiency was observed at a HRT of 4 days (E=93%). Oktem, Ince, Sallis, Donnelly & Kasapgil, (2007) investigated the performance of anaerobic sludge blanket reactor treated a chemical synthesis – based pharmaceutical wastewater at two HRTs (1and 3 days). COD removal efficiency increased from 58% to 78% with the HRT was increased from 1 to 3 days. Kuscu & Sponza, (2009) found that as the HRT decreased from 10,38 days to 2,5 days the COD removal efficiencies in the anaerobic ABR and anaerobic/aerobic reactor effluents decreased from 94% to 92% and from 98% to 97%, respectively. The results obtained in this study exhibited beter 98,12% COD removal efficiencies than that aforementioned literature studies at a HRT of 19,2 days.

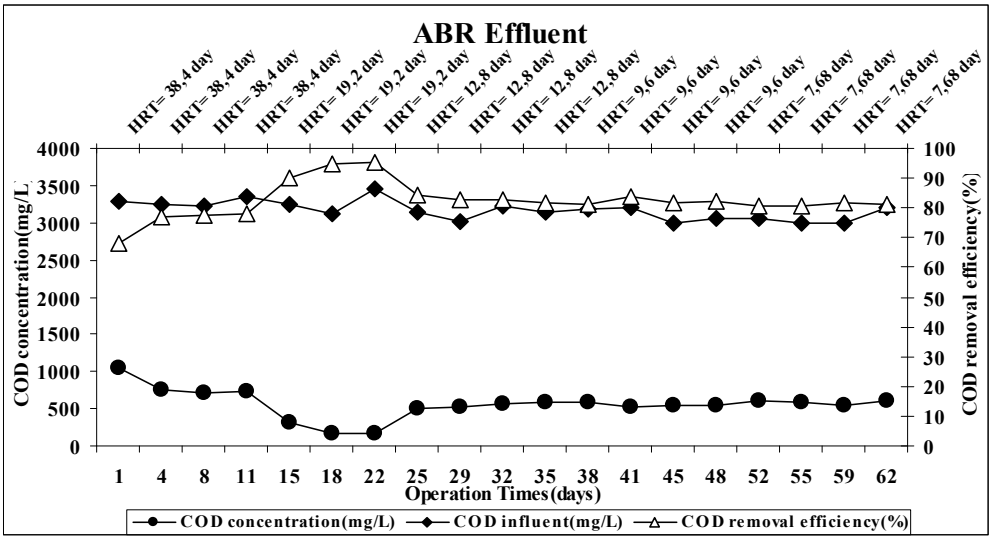


Figure 4.48 The effect of HRTs on COD removal efficiencies in ABR

4.2.2.7.2 Effect of HRTs on Total Volatile Fatty Acid (TVFA), Bicarbonate Alkalinity (Bic. Alk.) and TVFA/Bic.Alk. Ratio Variations in ABR Reactor. Figure 4.49 shows the VFA in the effluent of ABR at decreasing HRTs. The VFA concentration was found as 0 mg/L at all HRTs. In other words, the VFA concentrations were zero as the HRTs were decreased from 38.4 days from 7.68 days throughout continuous operation of ABR reactor. From Fig. 4.50, it can be seen that Bic.Alk. concentrations in the effluent were at between 2085 mg/L to 2226 mg/L although the HTRs was decreased from 38.4 days from 7.68 days. This shows the stability of ABR reactor.

HuaJun Feng, LıFang Hu, Dan Shan, ChengRan Fang and DongSheng Shen (2008) investigated the performance of an anaerobic baffled reactor treating the dilute wastewater. In this study as the HRT decreased from 18 h to 9 h, the final concentration of effluent VFAs increased from 8 mg/L to 22 mg/L, respectively. However, in our study decreasing of HRT did not increase the VFA concentrations in the effluent indicating the steady-state conditions in the ABR reactor.

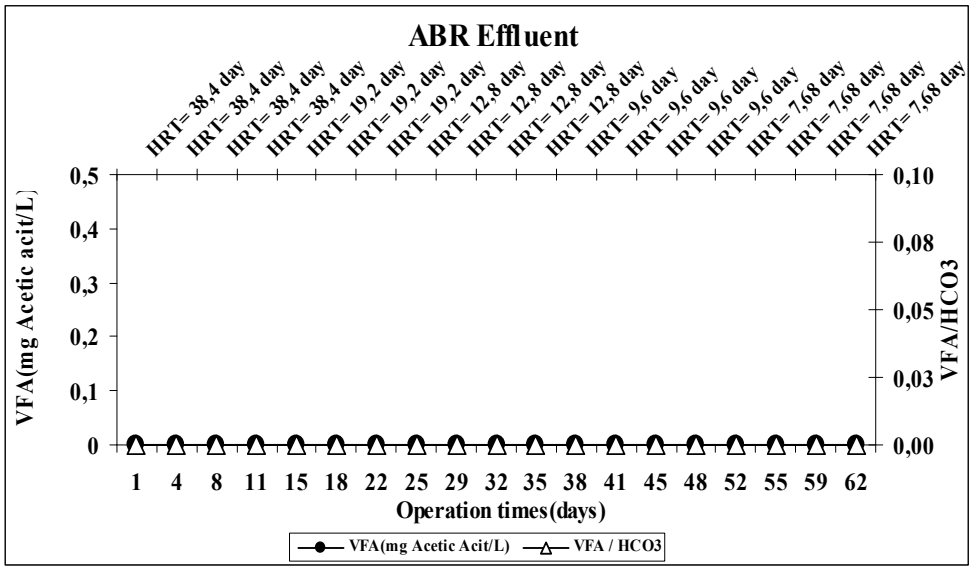


Figure 4.49 The variations of VFA and VFA/Bic.Alk. ratio in the effluent of ABR at decreased HRTs.

In anaerobic reactor system TVFA/Bic.Alk. ratio gives necessary information to determine the stability of 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). As shown in Figs. 4.49.and 4.50, the TVFA/Bic.Alk. ratio was zero in the effluent at all HRTs. This showed that the ABR reactor was stable as reported by Behling et al., (1997) since the TVFA/Bic.Alk. ratios in the effluent were lower than 0.4.

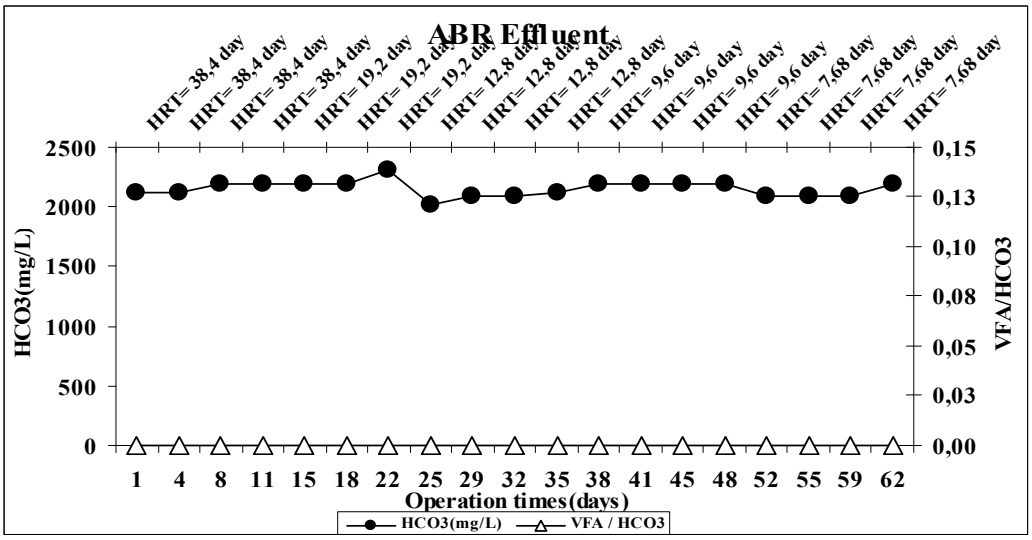


Figure 4.50 The variations of Bic.Alk. and VFA/Bic.Alk. ratio in the effluent of ABR at decreased HRTs

4.2.2.7.3 Effect of HRTs on Gas Productions and Methane Percentage in Anaerobic ABR Reactor: From figure 4.51 it can be seen that the methane gas production increased from 216 L/day up to 504 L/day as the HRT decreased from 38,4 to 19,2 days at a constant chloramphenicole concentration of 130 mg/L. However, the methane gas production decreased from 504 L/day to 324 L/day until at a HRT of 12.8 days. Then the methane gas production increased from 324 L/day up to 482,4 L/day, as the HRT decreased from 12.8 days to 7.68 days. The maximum methane gas production rate (504 L/day) was obtained at 19,2 days of HRT.

From figure 4.52 it can be seen that the total gas production increased from 259,2 L/day up to 547 L/day as the HRT decreased from 38,4 to 19.2 days at a constant

chloramphenicole concentration of 130 mg/L. The total gas production decreased from 547 L/day to 432 L/day when the HRT decreased to 12.8 days. The total gas production increased from 432 L/day up to 547,2 L/day As the HRT decreased from 12.8 days to 7,68 days. To the maximum total gas production (547,2 L/day) was reached at a HRT of 19,2 days.The maximum methane percentages varied between 50-58% at a HRT of 19,2 days

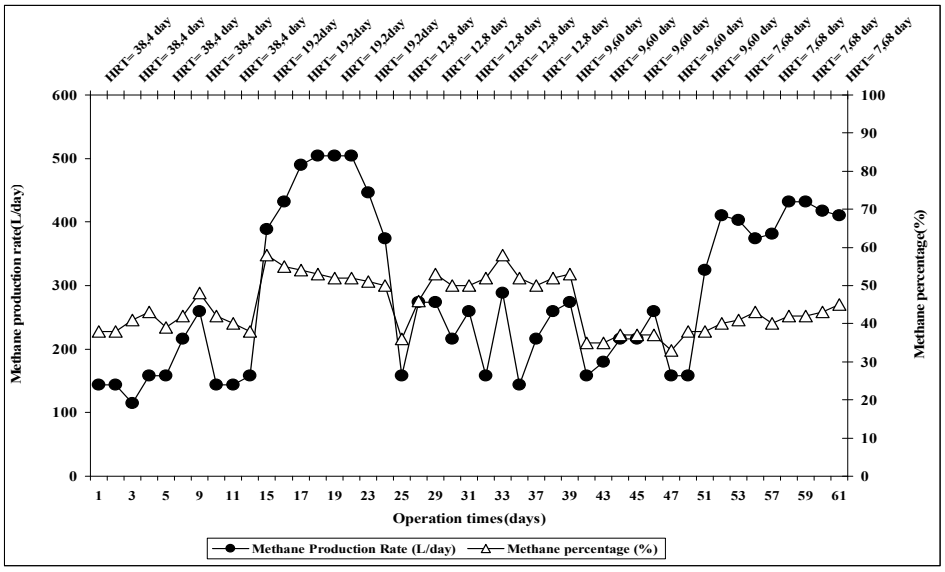


Figure 4.51 Methane gas production and methane percentage in ABR at decreased HRTs

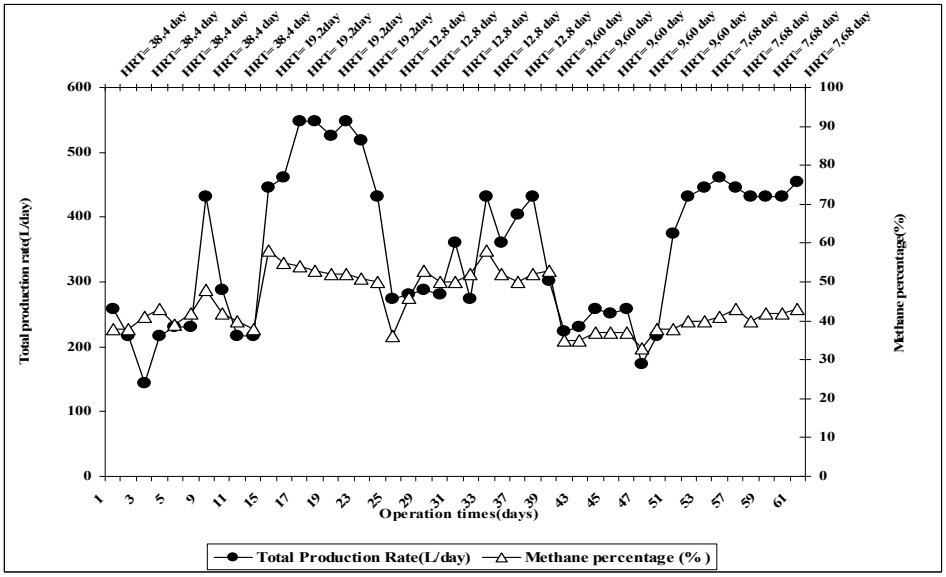


Figure 4.52 Total gas production and methane percentage in ABR at decreased HRTs

As illustrated in Figures 4.51 and 4.52, the methane percentages of the biogas varied between 42% and 55% indicating the stability of the ABR reactor. Decreasing of HRTs from 38.4 days to 7.68 days in the continuous operation of the ABR reactor did not affect significantly the methane percentage. The maximum methane percentages varied between 50-58% at a HRT of 19,2 days.

When the ABR system reached to a stabilized state under the OLR of 6.0 kg COD/m^3 d and HRT of 39,5 days, the total amounts of biogas in the four compartments were 73.2 L/d, 30.8 L/d, 8.6 L/d, and 1.3 L/d, respectively.(Ge-Fu Zhu, Jian-Zheng Li ,Peng Wu, Hui-Zheng Jin , Zheng Wang , (2008)). These results are lower 518,2 L/day than that finding of our findings at a HRT of 19,2 days and a methane percentage of 55%.

4.2.2.7.4 Effect of Compartments of ABR on COD Removal Efficiencies at Different HRTs. In this study, the effect of decreases in HRTs on COD removal efficiencies was investigated in four compartments of the ABR reactor. Figure 4.50 shows the effect of compartmentalisation on COD removal efficiencies at different HRTs. As shown in the figure 4.53(a), in compartment I the COD removal efficiencies were approximately 68,14% at a HRT of 38,4 days. It was found that the COD removal efficiency obtained in this SRT for compartment I was the highest than that obtained in the other SRTs applied to the ABR. The lowest COD removal efficiency (E=39,05%) was found at a HRT of 12,8 days in the compartment I. Figure 4.53. (b),(c), shows the COD removal efficiencies in the compartment II (varied between 64,60% and 84,49%)and compartment III (varied between 71,98% and 92,29%) at HRTs 38,4 and 19,2 respectively. COD removal efficiency increased from 64,60% to 84,49% in the second compartment when the HRT decreased from 38,4 days to 19,2 days. The COD removal efficiency decreased from 84,49% to 70,20% in compartment II when the HRT decreased from 19,2 to 7,68 days (see figure 4.53.(b)). The COD removal efficiency decreased from 92,29% to 76,42% in compartment III when the HRT decreased from 19,2 to 7,68 days (see figure 4.53.(c)).In compartment IV the COD removal efficiencies were high (E=97,68%) at a HRT of 19,2 days compared to the other HRTs. The COD removal efficiency

increased from 62,98 % to 97,68% as the HRT decreased from 38,4 days to 19,2 days . Then the COD removal efficiency decreased from 98,11 % to 76,92 % when the HRT decreased from 19,2 days to 7,68 days in compartment IV (see figure 4.53.(d)). It can be concluded that for maximum COD removal efficiency the optimum HRT was found to be 19,2 days.

In a study performed by Ge-Fu Zhu, Jian-Zheng Li, Peng Wu, Hui-Zheng Jin, Zheng Wang, (2008) after the acclimatization of the anaerobic activated sludge in 24 days, the removal of total COD from the wastewater was above 92% in an ABR reactor treating soybean protein processing wastewater at a HRT of 39,5 days. In the second stage of the ABR reactor, the COD removal increased to 94% continually when the volumetric loading rate increased, However, in the third stage of the ABR reactor, when an influent 8000 mg COD/L was applied to the ABR, acidification phenomenon happened during the initial period (65–67 days) because of increasing volume loading rate that resulted in the declination of COD removal to 80%. Four days later, the COD removal was improved to 94% .The COD removal in the last stage was similar to the third stage. When the volumetric loading rate increased further, the total COD removal efficiencies in the ABR system remained as 97% and the effluent COD concentration was under 300 mg/L. My result is better than the findings obtained in this study, because my result is 98,11% in compartment IV.

The carrier anaerobic baffled reactor (CABR) was initially fed with domestic sewage derived mainly from restaurants and dormitories from Zhejiang University during start-up at a HRT of 48 h. The CABR reactors were acclimatized for over a period of 21 days at 25^{O}C. The HRT was gradually decreased from 48 h to18 h by increasing the flow rate for 3 months. The total COD and SS removal efficiency was 69% and 82% at a HRT of 18 h, respectively. The average COD removal efficiency was 77,73% at a HRT of 18 h; 74.91% at a HRT of 12 h, and 58.51% at a HRT of 9 h, respectively. The difference in removal efficiencies was not significant between 18 h and 12 h of the HRT. However, a drastic drop in removal efficiency was observed when the HRT decreased to 9 h, indicating that the HRT greatly affected the performance of CABR. However, the COD removal load at short HRTs was still

higher than that at a long HRT in this study, which was 0.31 kg/m^3d at a HRT of 18 h, 0.45 kg/m^3d at a HRT of 12 h, and 0.47 kg/m^3d at a HRT of 9 h, respectively, (HuaJun Feng, LiFang Hu, Dan Shan, ChengRan Fang, And DongSheng Shen, 2008). The COD performance found in this study were lower than that my study.

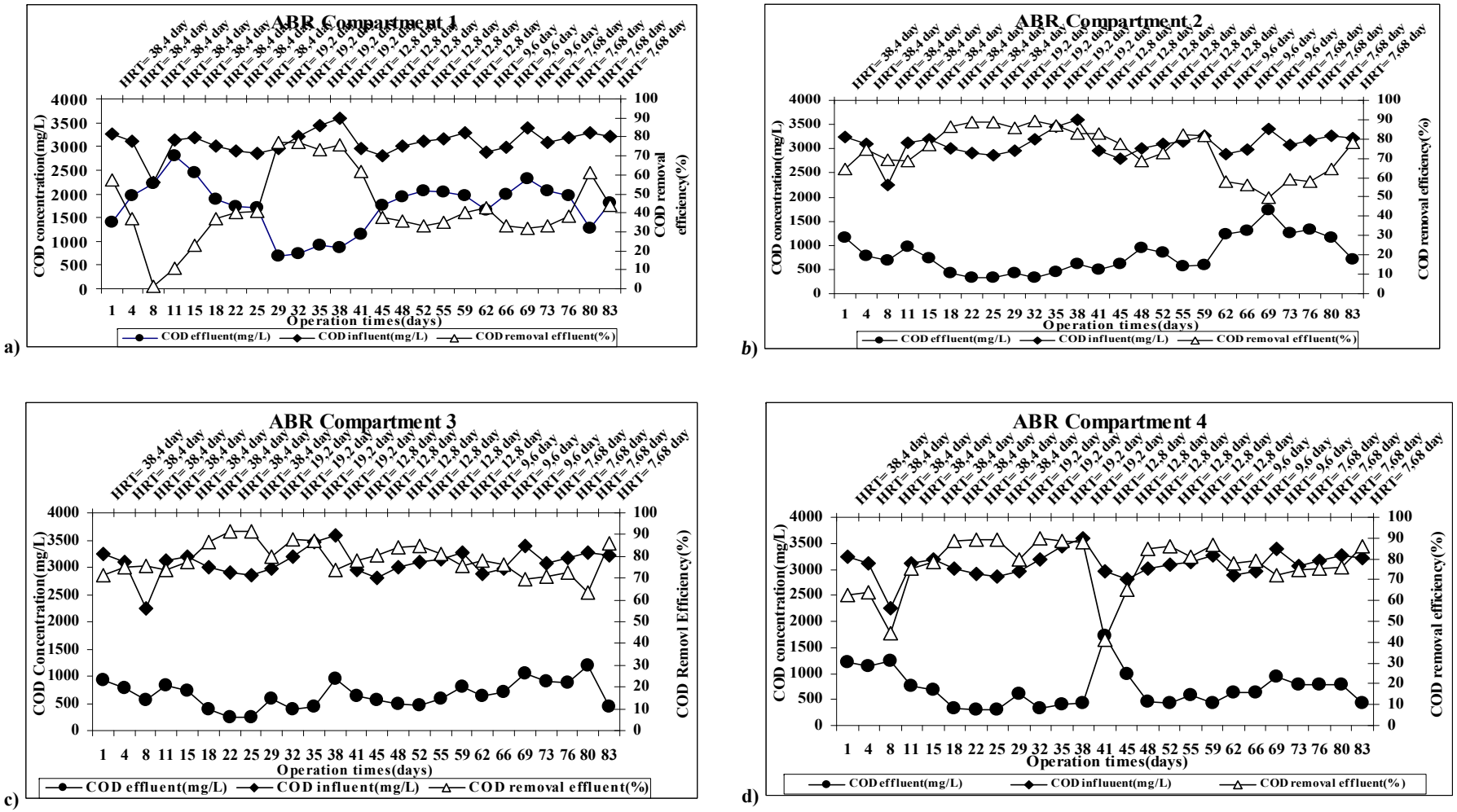


Figure 4.53 The variations of COD in ABR at decreased HRTs in the all compartments. (a- compartment 1, b-compartment 2, c-compartment 3 d- compartment 4)

*4.2.2.7.5 Effect of Compartments of ABR on VFA, Bic. Alk. and VFA/Bic. Alk. ratio at Different HRTs :*Figure 4.54 shows the TVFA concentrations and the TVFA/Bic.Alk. ratio variations in all compartments at decreasing HRTs. In the compartment I, the VFA concentration decreased from 642 mg Acetic acid /L to 0 mg Acetic acid /L when the HRT decreased from 38,4 days to 9,60 days. However, the VFA concentration increased from 0 mg Acetic acid/L to 174 mg Acetic acid /L when the HRT decreased from 9,60 to 7,68 days(see figure 4.54.(a)). In the compartments II the VFA increased from 9 mg/L to 225 mg/L when the HRT decreased from 38,4 to 19,2 days. The VFA concentration decreased from 225 mg/L to zero when the HRT decreased from 19,2 to 7,68 days (see figure 4.54.(b)). In the compartment III the VFA concentration was nearly zero mg/L through first four HRTs (38,4 - 12,8 - 9,60 - 7,68 days). The VFA concentration was found to be 9 mg/L at a HRT of 19,2 days (see figure 4.54.(c)). Figure 4.54(d) shows the VFA concentrations in the compartment IV. The VFA concentration was almost zero at all HRTs. For minimum VFA production the optimum HRTs were found to be 19,2-12,8-9,60-7,68 days. Ghaniyari-Benis, R. Borja,S. Ali Monemian, V. Goodarzi, (2009), who studied the synthetic medium-strength wastewater, found that the VFA concentration of 913 mg/L, 1154 mg/L and 1258 mg/L were achieved at HRTs of 24h, 16h and 8h, respectively, in compartment I in a multistage anaerobic biofilm reactor. In compartment II, the VFA concentrations were found as 371 mg/L, 458 mg/L and 959 mg/L at HRTs of 24h, 16h and 8h, respectively. The VFA concentration of 223 and 228 mg/L were achieved at HRTs of 24 and 16 h. Decreasing of HRT to 8 h gave a VFA concentration of 458 mg/L in compartment III of multistage anaerobic biofilm reactor. For all HRTs the VFA production in the first compartment was significantly greater than that in other compartments and it decreased from input to output. Similar results was found in our study, compartment I greater than that in other compartments.

Figure 4.55 shows the Bic.Alk. and TVFA/Bic.Alk. ratio variations in all compartments versus decreasing HRTs. In compartment I, when the HRT was decreased from 38,4 days to 12,8 days, the HCO_3 concentrations increased from 1282 mg/L up to 2199 mg/L. When the HRT decreased from 12,8 days to 7,68 days,

the HCO_3 concentrations decreased. (see 4.55(a)). Figure 4.52 (b) shows the HCO_3 concentrations measured in compartment II. The HCO_3 concentrations increased from 1972 to 2353 mg/L, when the HRT decreased from 38,4 days to 7,68 days. Similar results were found for Compartment III, at all HRTs (see 4.55(c)). The HCO_3 concentrations increased from 2085 mg/L up to 2312 mg/L when the HRT decreased from 38,4 days to 9,60 days in compartment IV. After the HCO_3 concentrations decreased from 2312 mg/L to 2199 mg/L when the HRT decreased from 9,60 days to 7,68 days in compartment IV(see 4.55(d)).

In anaerobic reactor system TVFA/Bic.Alk. ratio gives necessary information to determine the stability of 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). As shown in Fig. 4.54 and 4.55. The TVFA/Bic.Alk. ratio varied between 0.501 and 0.085 in compartment I, as the HRT was decreased from 38,4 days to 7,68 days. The TVFA/Bic.Alk. ratio varied between 0.005 and 0.132 in compartments II as the HRT was decreased from 38,4 days to 7,68 days. After that the TVFA/Bic.Alk. ratio was only 0.005 in compartment III, at a HRT of the 19,2 days. The TVFA/Bic.Alk. ratio varied between 0.501 and 0.085 in compartment I, as the HRT was decreased from 38,4 days to 7,68 days. In compartment IV the TVFA/Bic.Alk. ratio varied between 0,220 and 0,099, at a HRT of 19,2 days. ABR reactor was stable as reported by Behling et al., (1997) since the TVFA/Bic.Alk. ratios were lower than 0.4 except compartment I among all compartments. The ABR reactor was not stable only at a HRT of 38,4 days.

For maximum HCO_3 alkalinity (2353mg/L) and available TVFA/Bic.Alk. ratio (0) the optimum HRT was 7,68 days.

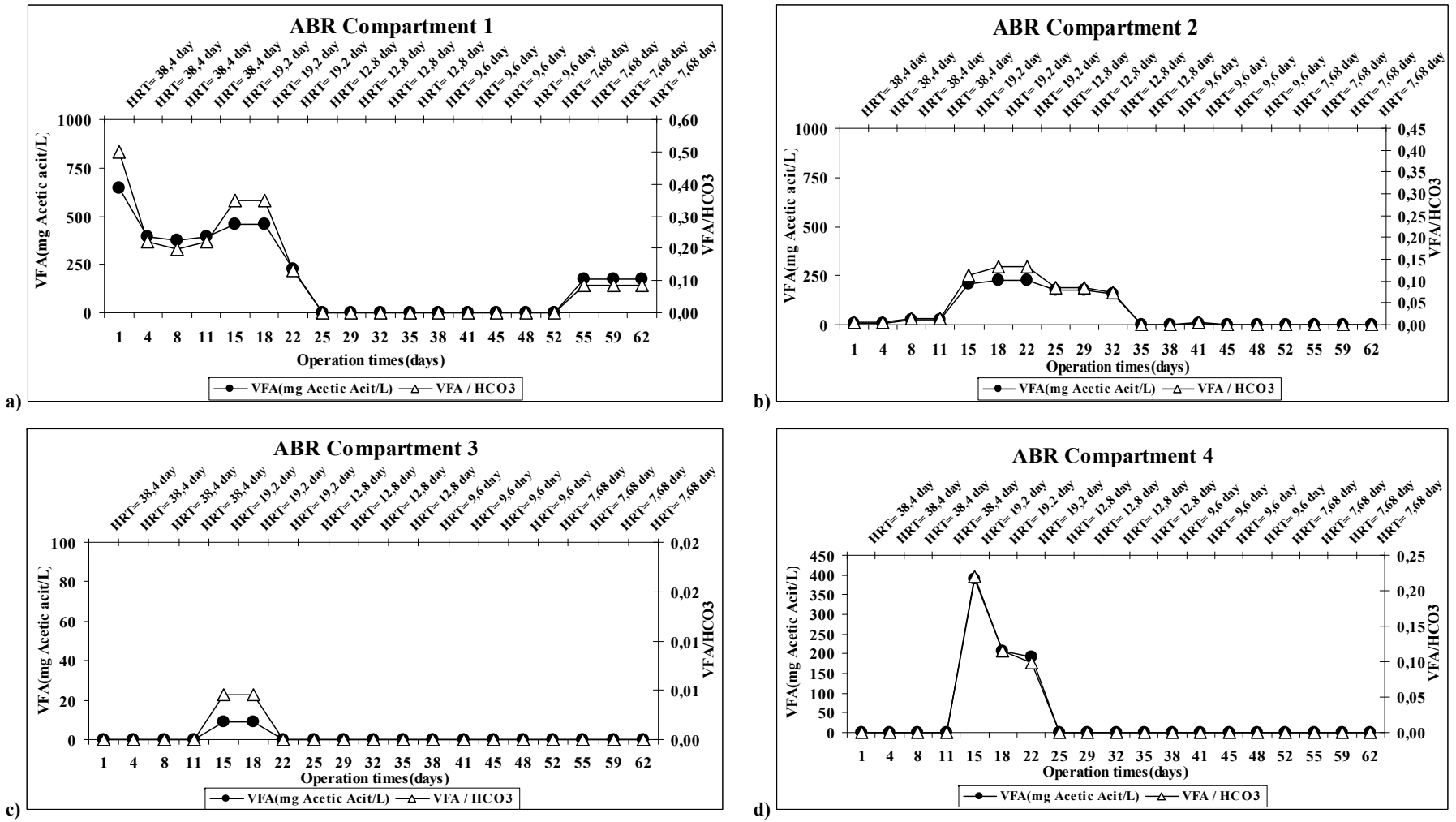


Figure 4.54 The variations of VFA in ABR at decreased HRTs in the all compartments. (a- compartment 1, b-compartment 2, c-compartment 3 d- compartment 4)

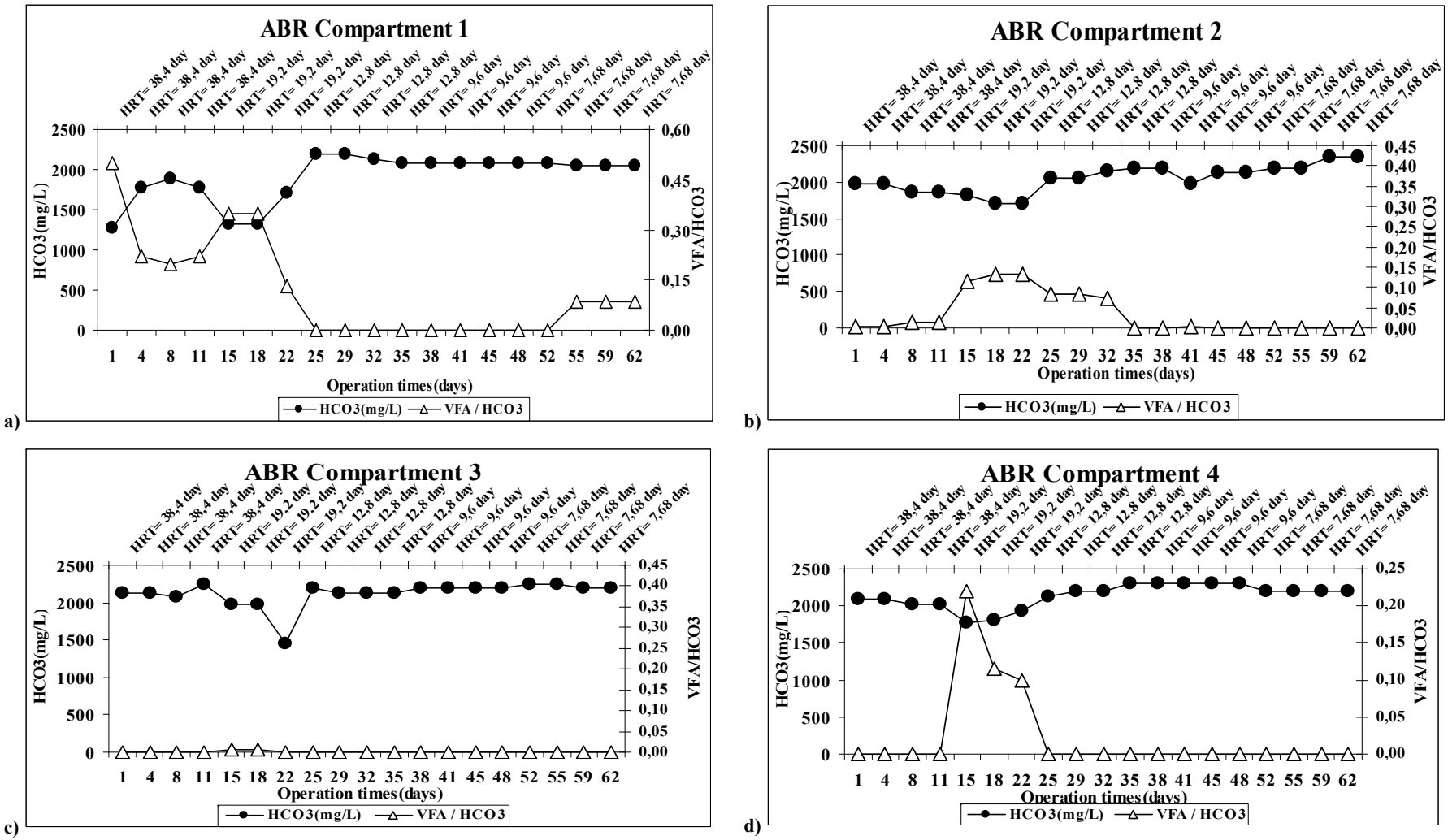


Figure 4.55 The variations of HCO_3 in ABR at decreased HRTs in the all compartments. (a- compartment 1, b-compartment 2, c-compartment 3 d- compartment 4)

4.2.2.7.6 Removal Efficiencies in Aerobic CSTR Reactor Effluent

Figure 4.56 shows the COD removal efficiencies in the aerobic CSTR reactor system. The COD removal efficiency in this reactor system were up to 98,12% until a HRT of 19,2 days. The COD removal efficiency of the reactor decreased from 98,12% to 89,37% when the HRT was decreased from 19,2 days to 7,68 days in aerobic CSTR reactor. For maximum COD removal efficiency (E=98,12%) the optimum HRT was found as 19,2 days.

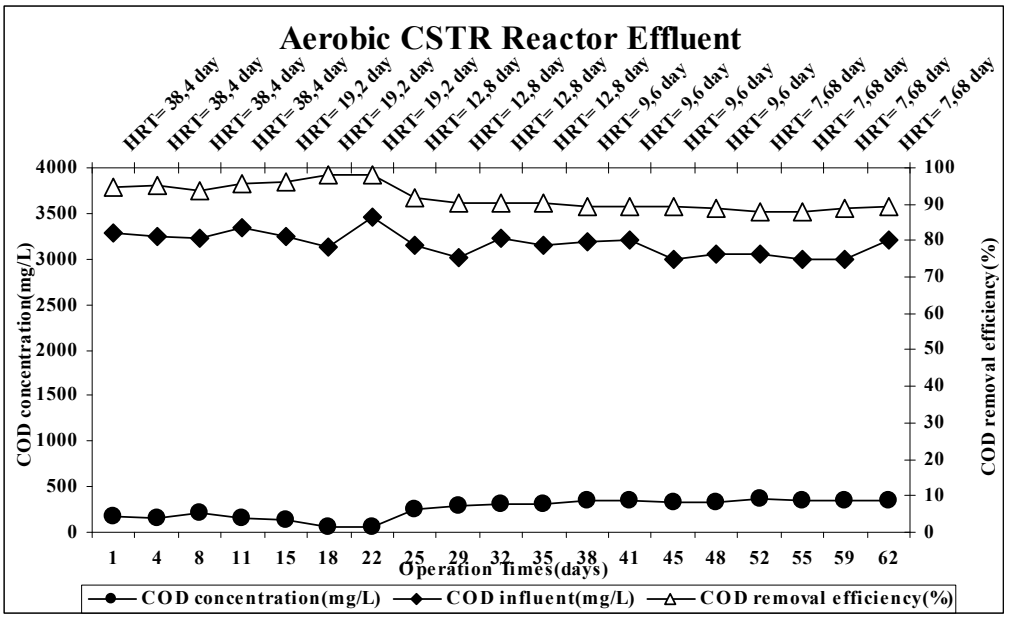


Figure 4.56 The COD removal efficiency in aerobic (CSTR) reactor system

Figure 4.57shows the VFA and VFA/Bic.Alk. ratio in the aerobic reactor system. Although the VFA and HCO_3 alkalinity were the key parameters in the anaerobic reactors in this study it was aimed to monitor their concentrations in the aerobic CSTR reactor since the effluent of anaerobic ABR reactor it was used as the feed of the aerobic CSTR reactor. In aerobic CSTR reactor the VFA concentrations increased from 0 mg /L to 123 mg Acetic acid/L when the HRT were decreased from 38,4 days to 7,68 days. For the lowest VFA concentrations (123 mg/L) the optimum HRT was found as 7,68 days.

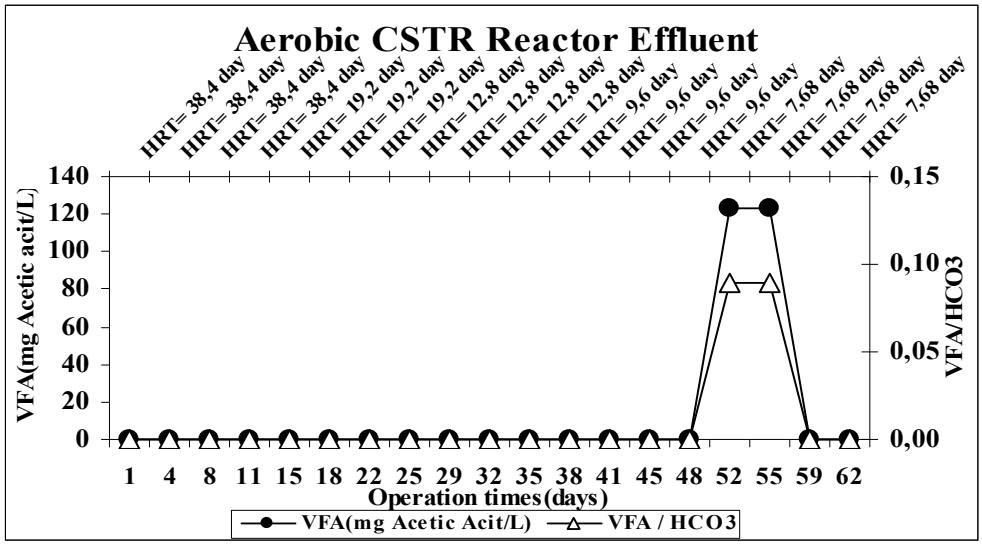


Figure 4.57 The overall VFA and VFA/Bic.Alk. ratio in aerobic (CSTR) reactor

Figure 4.58 shows the overall Bic.Alk. and VFA/Bic.Alk. ratios of aerobic reactor system. In aerobic CSTR reactor system the HCO_3 concentrations decreased from 1420 mg /L to 1328 mg /L when the HRT decreased from 38,4 days to 7,68 days.

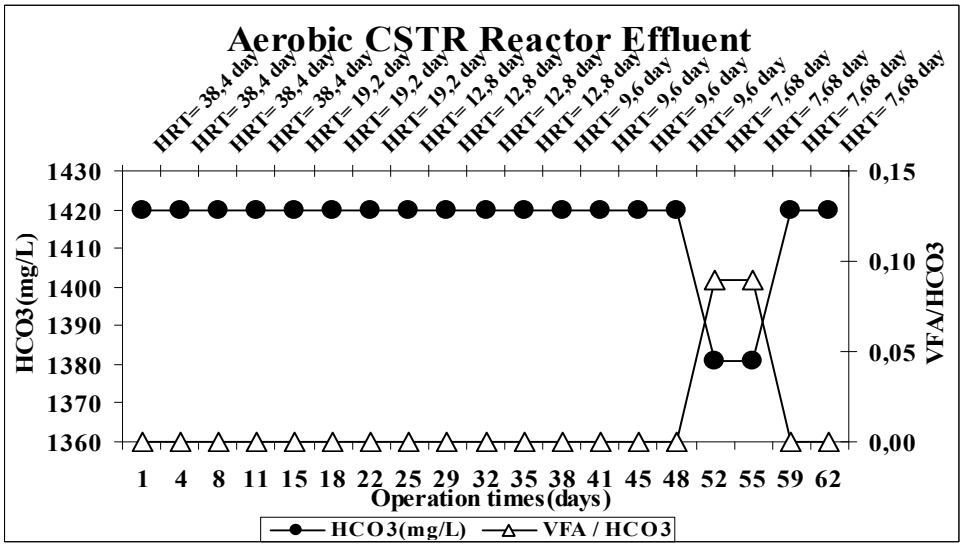


Figure 4.58 The overall HCO_3 and VFA/Bic.Alk. ratio in aerobic (CSTR) reactor

4.2.2.7.7 Specific Methanogenic Activity (SMA) in ABR at Different HRTs

Figure 4.59 shows the SMA values of mixed sludge taken from the all compartments of ABR during continuous operation of the ABR at different HRTs.

The SMA is an indicator of methanogenic activity in anaerobic systems. As shown in Figure 4.56, the SMA values increased from 0.035 to 0.048 g COD-CH_4/ gVSS when the HRT decreased from 38,4 days to 19,2 days. After that, the SMA values decreased from 0.048 to 0,033 g COD-CH_4/ gVSS when the HRT decreased from 19,2 days to 7,68 days. In other words the maximum SMA was found to be 0.048 g COD-CH_4/ gVSS day for HRT of 19,2 days.

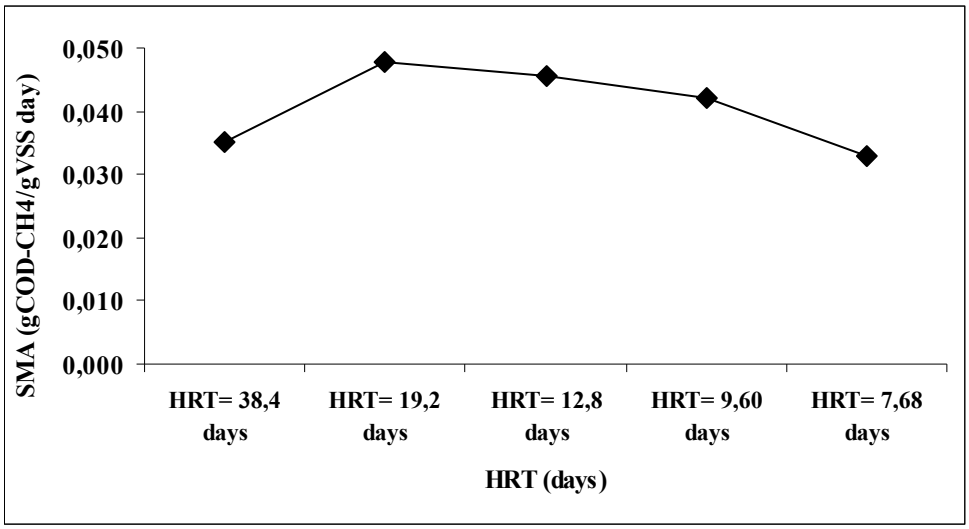


Figure 4.59 SMA values in ABR at different HRTs.

4.2.2.7.9 Variations of Chloramphenicole Removal Efficiency in the ABR Reactor at Increasing HRTs. Figures 4.60, 4.61 and 4.62 shows the HPLC chromatogram of the samples taken from the anaerobic reactor influent, effluent and aerobic CSTR effluent at a constant influent chloramphenicole concentration of 130 mg/L at a HRT of 19,2 days. A Chloramphenicole peak was almost zero in HPLC chromatogram of the effluent of ABR reactor samples. This showed that chloramphenicole was biodegraded with high removal efficiencies (almost completely) in ABR and CSTR reactors particularly at high HRTs (See table 4.8). In figure 4.60 the chromatogram of chloramphenicole showed that the peak was appeared after 4.214 min, in HPLC analysis. This corresponded to a chloramphenicole concentration of 128.42 mg/L. As seen in figure 4.61, the chloramphenicole concentration was measured as 1,90 mg/L in the effluent of the ABR. The chloramphenicole removal efficiency was 98,5% at a HRT of 19,2 days. In figure 4.62 the chloramphenicole concentration was

measured as 0.60 mg/L in the aerobic CSTR effluent corresponding a removal efficiency 99,5%. In this study it was found that the “chloramphenicole” antibiotic was mainly degraded (129.05 mg/L) in anaerobic ABR reactor while the remaining small part of this antibiotic (0.95 mg/L) was removed in the aerobic CSTR reactor.

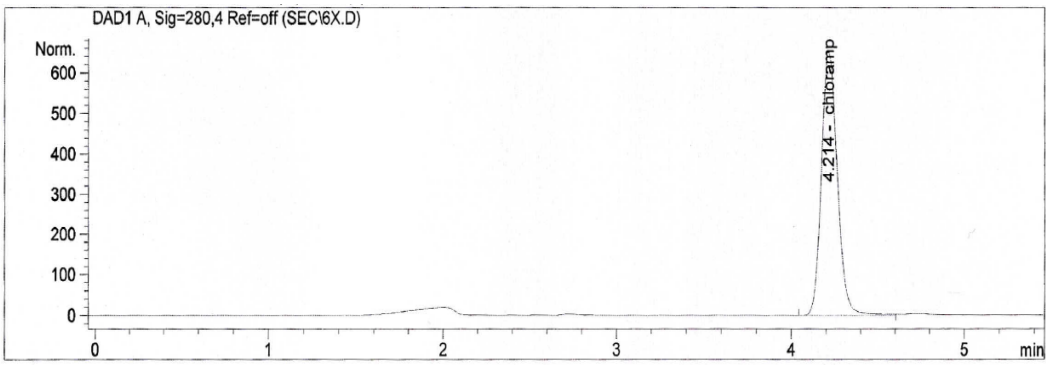


Figure 4.60 HPLC chromatogram in the influent of ABR at 19,2 days of HRT

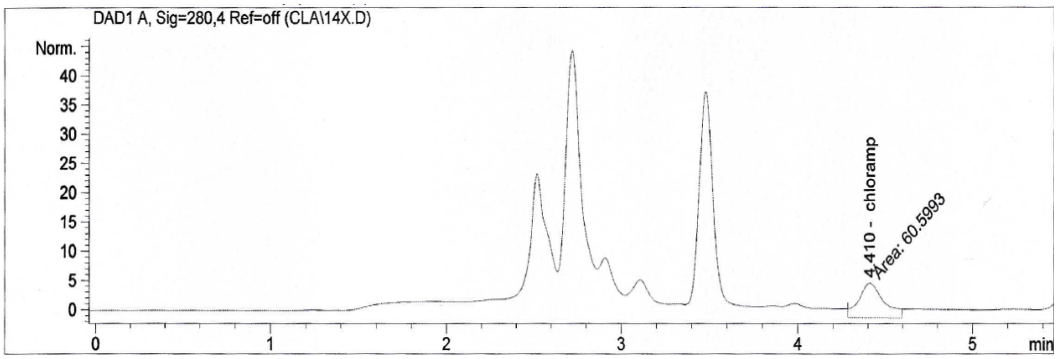


Figure 4.61 HPLC chromatogram in the effluent of ABR at 19,2 days of HRT

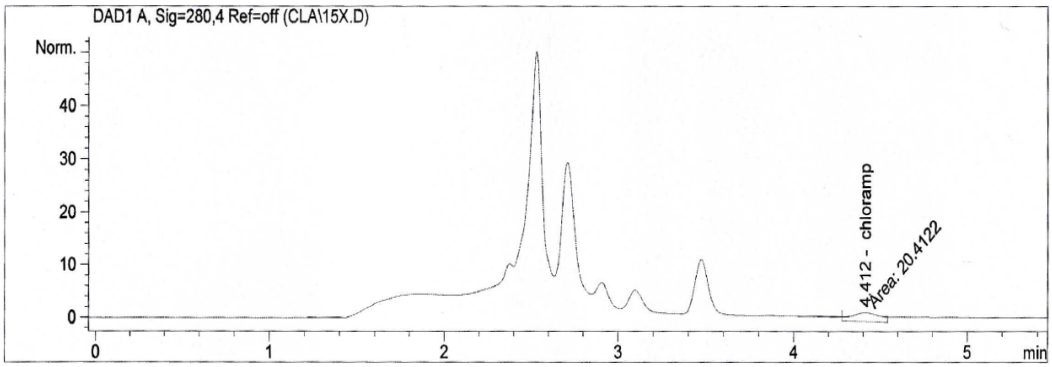


Figure 4.62 HPLC chromatogram in the effluent of CSTR at 19,2 days of HRT

As the HRT increased from 7.68 days to 38.4 days the chloramphenicole removal efficiencies increased from 99% to 100% in sequential total (anaerobic

ABR+aerobic CSTR reactor system) (Table 4.8). At all HRTs the big part of the chloramphenicole (around 96%-98.5%) was removed in anaerobic ABR reactor while the remaining small part of the antibiotic was removed in aerobic CSTR reactor.

Table 4.8 Variations of Chloramphenicole concentrations in the influent, effluent of the ABR Reactor in the effluent of the Aerobic CSTR and in total reactor system versus decreasing HRTs at an initial Chloramphenicole concentration of 130 mg/L

HRT Days	Antibiotic ABR Influent (mg/L)	Antibiotic ABR Effluent (mg/L)	Antibiotic ABR Effluent Removal (%)	Antibiotic Aerobic Effluent (mg/L)	Antibiotic Removal in Sequential Total System Effluent (%)
38,4	128,60	4,80	96%	0	100%
19,2	128,42	1,89	98,5%	0,63	99,5%
12,8	128,44	4,34	96%	0,72	99%
9,60	128,42	4,00	97%	0,80	99,5%
7,68	128,23	2,25	98%	1,15	99%

4.2.3Determination of Kinetic Constants

Determination of kinetic constant of an ABR reactor is a useful tool to be able to describe and predict the performance of the anaerobic system. Therefore in this study, the kinetic constant of the ABR treating streptomycin and chloramphenicole were evaluated according to the experimental data at five HRTs. In order to determine the most suitable biokinetic model in the ABR treating streptomycin and chloramphenicole, some kinetic models such as Monod, Grau second-order, contois kinetic and Modified Stover-Kincannon models were applied to the experimental results obtained from the continuous operation. The interpretions of the models and the kinetic constants were performed in this step.

4.2.3.1 Determination of Kinetics Constant through anaerobic degradation of Streptomycin in ABR at decreasing HRTs

In order to obtain the kinetic coefficient for different kinetic models the ABR reactor was operated with synthetic wastewater containing at a streptomycin concentration of 200 mg/L at five different HRTs**.**

4.2.3.1.1 Monod Kinetic Model. Five steady state sets datas were used to determine the kinetic constants for Monod Model. Figure 4.63 was plotted from the Eq 3.20 (See chapter 3.6.1.1.1) for determining the values of Y and k_d in this model. Growth yield coefficient (Y) (gVSS/gCOD) and endogenous decay coefficient (k_d) (day^{-1}) values calculated from the intercept and the slope of the straight line are illustrated in Figure 4.63 with regression coefficient of R^2=0.99, (y=0,4679x+0,0015) for COD. Y and k_d values was calculated as 2,137 g VSS /g COD and 0,00321 day^{-1}, respectively. The values of maximum specific substrate utilization rate (**μ_{max}**) (mgCOD/mgVSS.day) and half saturation concentration (K_S) (mg/L) for COD was determined from Figure 4.64 using Eq (3.15). (**μ_{max}**) and (K_S) for COD were calculated as 1,3033 day^{-1} and 0.029 mg/L, respectively with regression coefficient of R^2= 0.95, (y= 44,602x+ 34,22).

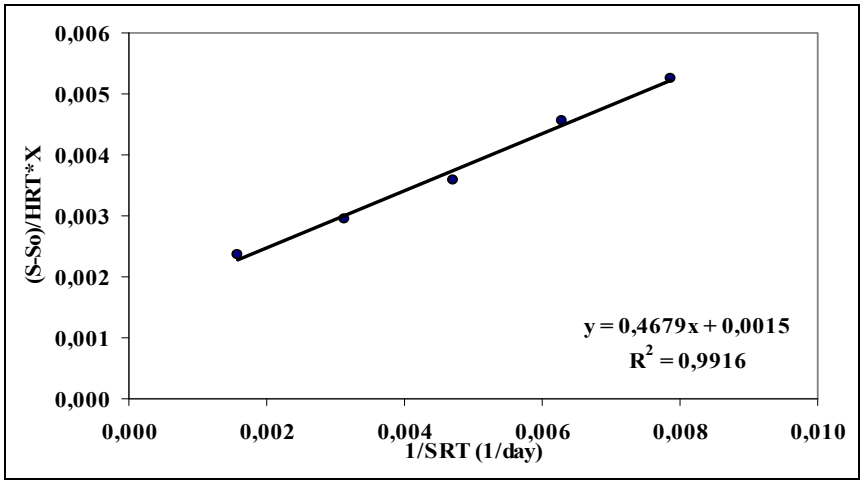


Figure 4.63Determination of yield coefficient (Y) and death rate constant (k_d) values for COD

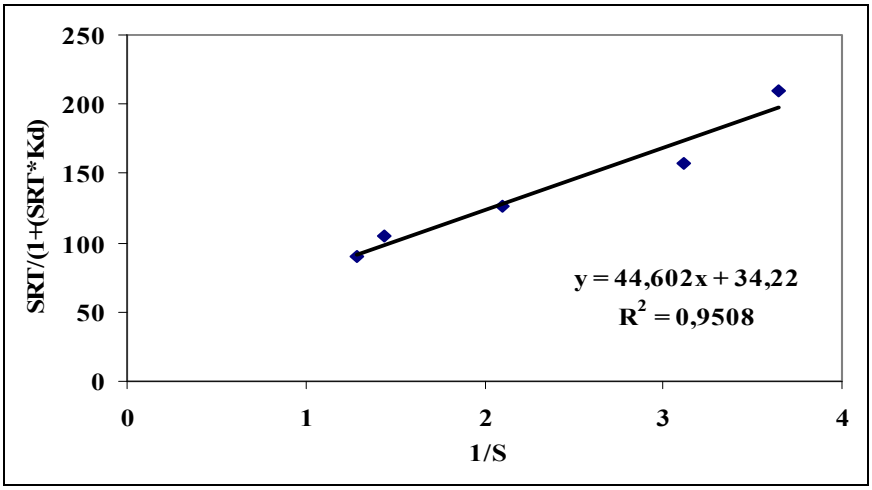


Figure 4.64 Determination of maximum specific substrate utilization rate (μ_{max}) and half saturation concentration (K_S) values for COD

4.2.3.1.2 Grau Second-Order Multicomponent Substrate Removal Model. In order to determine a (S_i/k_s*X) (day), b (dimensionless) and second order substrate removal rate constant (k_s) (day^{-1}), Equation 3.26 were plotted in Figure 4.65. The values of a and b were calculated from the intercept and slope of the straight line on graph. The values of a, and b were found to be 1,0461 day and 1,8645 (dimensionless) with a regression coefficient of R^2=0.99, (y= 1,0461x+ 1,8645) for COD. Second order multicomponent substrate removal rate constant (k_s) was calculated as 0.062 L/day from the equation $a=S_i/(k_s.X)$**,** indicating the substrate removal for each unit of microorganism depends on second order substrate removal rate constant (k_s).

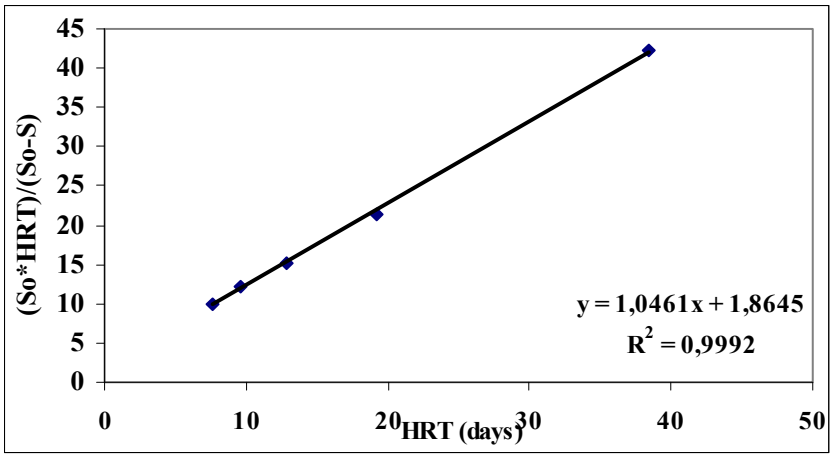


Figure 4.65 Determination of kinetic constants (a, b and k_s) for COD for Grau second order multicomponent substrate removal model.

4.2.3.1.3 Modified Stover-Kincannon Model. Figure 4.66 shows the graph plotted between reciprocal of total removed organic loading removal rate, [V/(Q*(Si-Se))], against to the reciprocal of total organic loading rate, V/(Q*Si) using Eq (3.29). Since the pilot of [V/(Q*(Si-Se))] versus V/(Q*Si) was found to be linear, linear regressions were used to determine the intercept ($1/R_{max}$) and the slope (K_B/R_{max}). Saturation value constant (K_B) (g/L*day) and maximum utilization rate (R_{max}) (g/L*day) for COD was calculated from the line plotted on graph given in Figure 4.66. K_B and R_{max} was found as 1,87 gCOD/L*day and 1,78 gCOD/L*day, respectively with high regression coefficient (R^2=99; y= 1.0514x+0.5626) for COD.

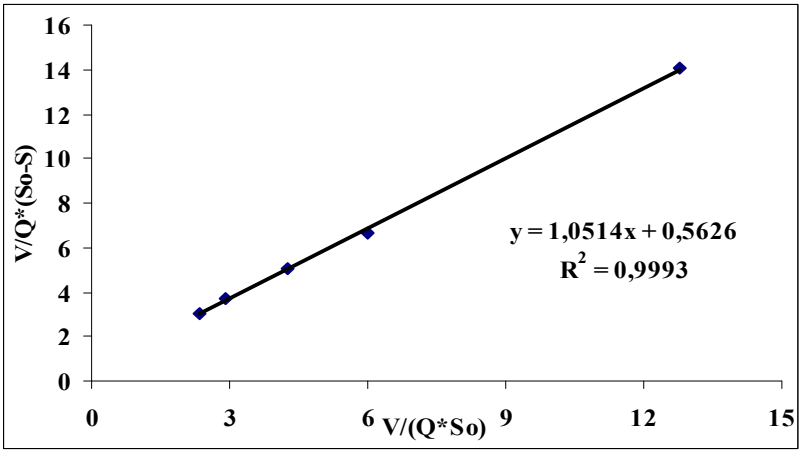


Figure 4.66 Determination of kinetic constants (R_{max} and K_B) in Stover-Kincannon model for COD

4.2.3.1.4 Contois Kinetic Model. Figure 4.67 was plotted from the Eq 3.23 for determining the values of μ_{max} and β in this model. Maximum specific grow rate (μ_{max}) (day^{-1}) and kinetic constant (β) (g COD/g biomass) values calculated from the intercept and the slope of the straight line illustrated in Figure 4.67 with regression coefficient of R^2=0.26, (y= 0,8557X+50,729) for COD. μ_{max} and β values was calculated as 0,0197 day^{-1} and 0,0169 (g COD/g biomass), respectively.

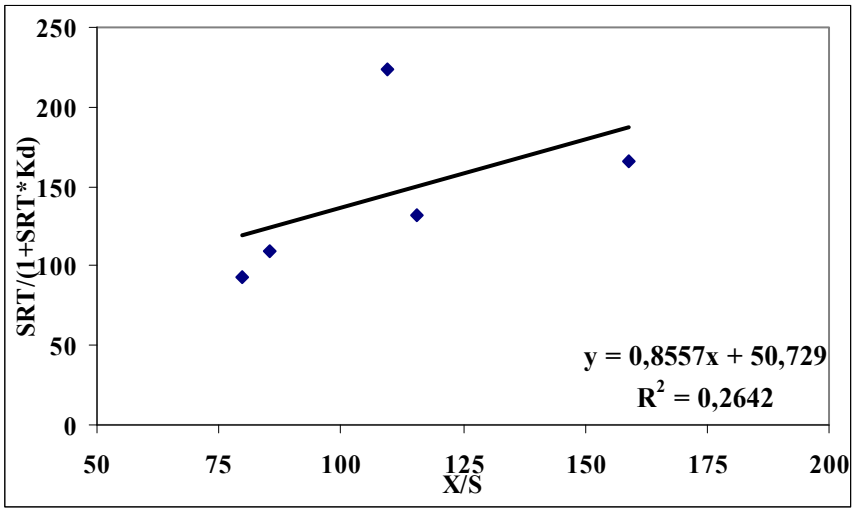


Figure 4.67 Determination of kinetic constants (μ_{max} and β) in Contois Kinetic Model for COD

4.2.3.1.5 Evaluation of the Kinetic Models through anaerobic degradation of Streptomycin in ABR Reactor. All kinetic coefficients calculated from the models are summarized in Table 4.9 with regration coefficients. The kinetic data showed that the Monod kinetic was more appropriate model than other models for predicting the performance of the lab scale ABR reactor when the regression coefficients and kinetic coefficients were compared with each other.

The yield coefficiency (Y) was higher compared to the death rate coefficient (k_d) in Monod kinetic model. Half saturation constant (K_s) was lower (K_s= 0.029 mg/L) compared to initial COD concentration of 3000 mg/L (Table 4.8). Maximum specific grow rate (μ_{max}) was higher compared to the death rate constant (k_d). This can be explained by the long HRT and sludge retention time. Low Ks value indicates a

higher affinity to COD treating anaerobic methanogens. In the study performed by Pavlostathis & Giraldo-Gomez, (1991) μ_{max}, Y, and K_S values were 0.77–6.67 mg COD (mgVSS/day), 0.04–0.11 mgVSS/mgCOD, and 105–3180 mgCOD/L, respectively, for anaerobic oxidation of long-chain fatty acids.

The regression coefficients for COD under five different HRTs were higher in Stover Kincannon kinetic model (R^2=0.9993), Monod kinetic model (R^2=0.9916) and Grau second order model (R^2=0.9992) compared to Contois kinetic model (R^2=0.2642). Contois kinetic model constatns was not significant (Table 4.8). Furthermore the kinetic constants determined in Stover Kincannon kinetic model, Monod kinetic model and Grau second order model are more meaningful than that observed in Contois kinetic model. Both maximum substrate utilization rate (R_{max}=1,78 gCOD/L*day) and saturation value constant (K_B=1,87 gCOD/L*day) are higher during COD degradation. High COD utilization rate (R_{max}) increase the reactor efficiency while high substrate saturation constant (K_B) indicates the un-utilization of COD by the methanogens in the ABR.

In this study, the saturation constant (K_B) (1,87 g/L*day) and maximum utilization rate (R_{max}) (1,78 g/L*day) values obtained from the Modified Stover-Kincannon model are lower than those obtained by Işık & Sponza (2005) (7.5 g/L*day and 8.2 g/L*day, respectively) in UASB reactor treating simulated textile wastewater and than those obtained by Kapdan, (2005) (12.9 g/L*day and 37.7 g/L*day, respectively) in packed colum treating textile dye stuff in modified Stover-Kincannon model. The multicomponent substrate rate constant (k_s) value obtained from the Grau second order model in this study (0,062 day^{-1}) was lower (than those obtained by Işık & Sponza (2005) in a UASB reactor (k_s=0.337 day^{-1}). Furthermore, the k_s value was 0.217 day^{-1} in a study performed by Ubay (1989) in a UASB reactor treating municipal wastewater using Grau second order kinetic model.

Although the regression coefficient obtained in the Stover- Kincannon model (R^2=99) is high the saturation value constant (K_B) found according this kinetic is extremely high. The kinetic constants found in the Monod and Grau kinetic models

were found to be meaningful. Therefore, it can be concluded that the streptomycin antibiotic is removed according to the Monod and Grau kinetic model with COD as substrate under anaerobic conditions in ABR reactor.

Table 4.9 Kinetic parameters of ABR reactor treating streptomycin

Kinetic models	Kinetic paramaters	Values	Regression coefficients(R^2)
Monod	Y(mgVSS/mgCOD)	2,137	0.9916
	k_d (day^{-1})	0,0321	0.9916
	μ_{max} (day^{-1})	1,3033	0.9508
	$k_{max}(\mu_{max}/Y)(day^{-1})$	0,61	0.9508
	K_s (mg/l)	0,029	0.9508
Grau second order	k_s (day^{-1})	0,062	0.9992
	a (day)	1,0461	0.9992
	b (dimensionless)	1,8645	0.9992
Modified Stover-Kincannon	K_B (g COD/L day)	1,87	0.9993
	R_{max} (g COD/L day)	1,78	0.9993
Contois	μ_{max} (day^{-1})	0,0197	0,2642
	β(dimensionless)	0,0169	0,2642

4.2.3.2 1 Determination of Kinetics Constant through anaerobic degradation of chloramphenicole in ABR at decreasing HRTs. In order to obtain the kinetic coefficient for different kinetic models the ABR reactor was operated with synthetic wastewater containing at a chloramphenicole concentration of 130 mg/L at five different HRTs**.**

4.2.3.2.1 Monod Kinetic Model. Five steady state sets datas were used to determine the kinetic constants for Monod Model. Figure 4.68 was plotted from the Eq 3.20 (See chapter 3.6.1.1.1) for determining the values of Y and k_d in this model. Growth yield coefficient (Y) (gVSS/gCOD) and endogenous decay coefficient (k_d) (day^{-1}) values calculated from the intercept and the slope of the straight line are illustrated in Figure 4.68 with regression coefficient of R^2=0.98, (y=0,0739x+0,0018) for COD. Y and k_d values was calculated as 1.35 mgVSS/mgCOD and 0,00244 day^{-1}, respectively. The values of maximum specific substrate utilization rate **(**μ_{max}) (mgCOD/mgVSSday) and half saturation concentration (K_S) (mg/L) for COD was determined from Figure 4.69 using Eq (3.15). **(**μ_{max}) and (K_S) for COD were

calculated as 0,3559 day^{-1} and 0.0071 mg/L, respectively with regression coefficient of R^2= 0.88, (y= 4,9655x+ 13,945).

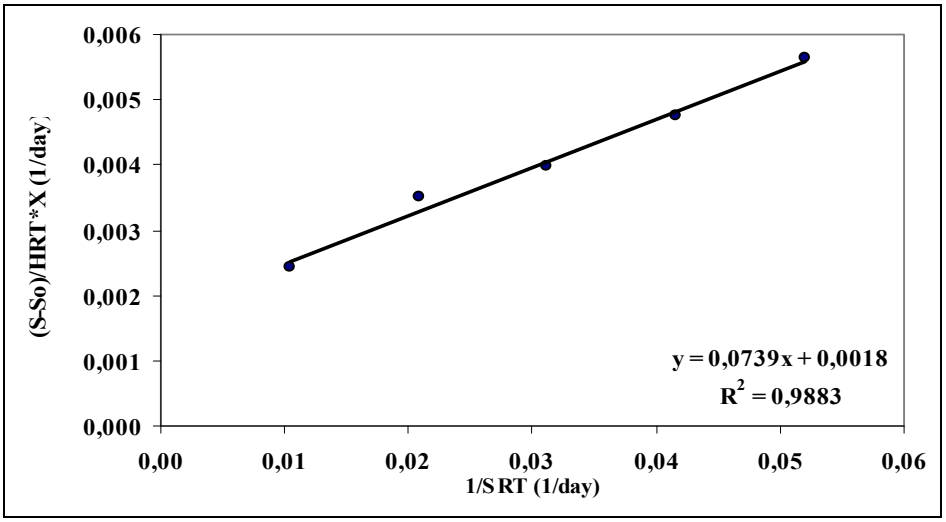


Figure 4.68 Determination of yield coefficient (Y) and death rate constant (k_d)values for COD

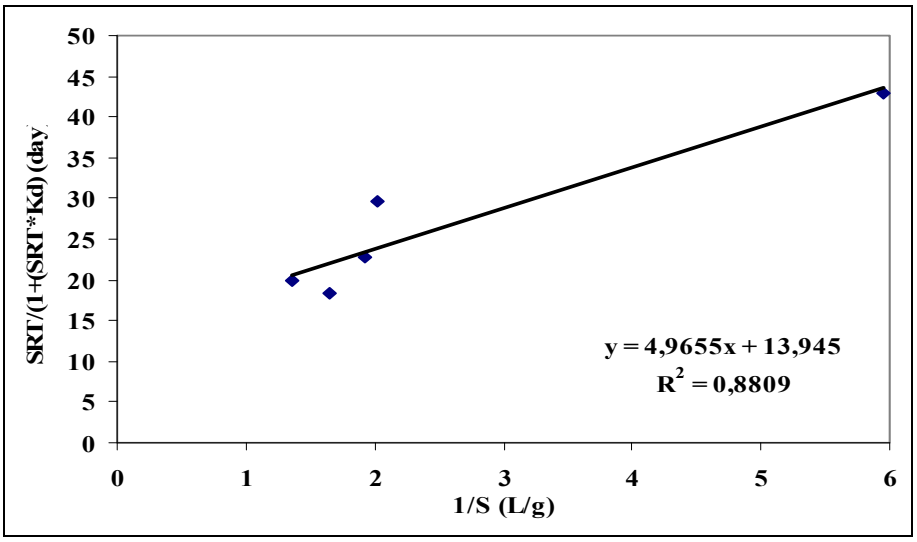


Figure 4.69 Determination of maximum specific substrate utilization rate (μ_{max}) and half saturation concentration (K_S) values for COD

4.2.3.2.2 Grau Second-Order Multicomponent Substrate Removal Model. In order to determine a (S_i/k_s*X) (day), b (dimensionless) and second order substrate removal rate constant (k_s) (day^{-1}) kinetic constants for COD, Equation 3.26 were plotted in Figure 4.70. The values of a and b were calculated from the intercept and slope of the

straight line on graph. The values of a, and b were found to be 1,2975 day and 1,6384 (dimensionless) with a regression coefficient of R^2=0.98, (y= 1.2975x–1,6384) for COD. Second order multicomponent substrate removal rate constant (k_s) was calculated as 0.055 day^{-1} from the equation a= $S_i/(k_s*X)$, indicating the substrate removal for each unit of microorganism depends on second order substrate removal rate constant (k_s).

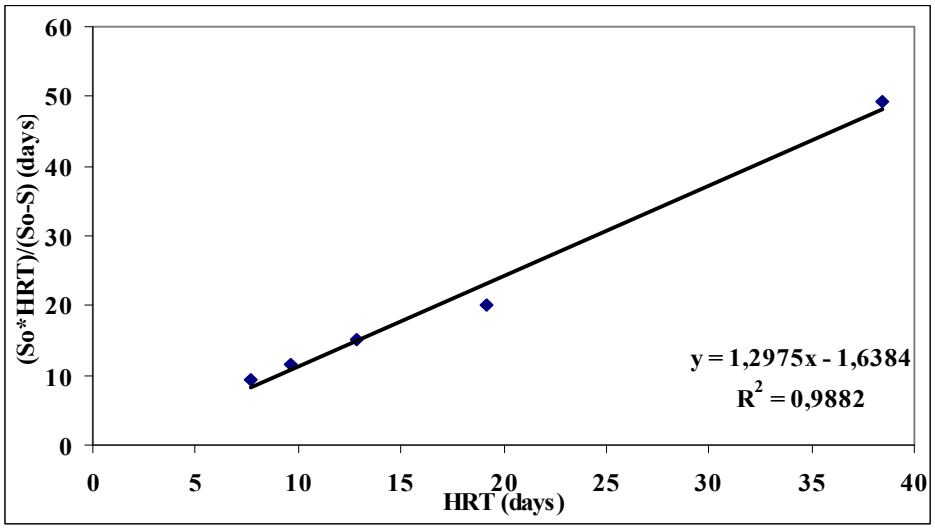


Figure 4.70 Determination of kinetic constants (a, b and k_s) for COD for Grau second order multicomponent substrate removal model.

4.2.3.2.3 Modified Stover-Kincannon Model: Figure 4.71 shows the graph plotted between reciprocal of total removed organic loading removal rate, [V/(Q*(Si-Se)], against to the reciprocal of total organic loading rate, V/(Q*Si) using Eq (3.29). Since the pilot of [V/(Q*(Si-Se)] versus V/(Q*Si) was found to be linear, linear regressions (least squares method) were used to determine the intercept ($1/R_{max}$) and the slope (K_B/R_{max}). Saturation value constant (K_B)(g/L*day) and maximum utilization rate (R_{max}) (g/L*day) for COD was calculated from the line plotted on graph given in Figure 4.71. K_B and R_{max} was found as 1,97 gCOD/L*day and 1,91 gCOD/L*day, respectively with high regression coefficient (R^2=98; y= 1.0304x+0.5239) for COD.

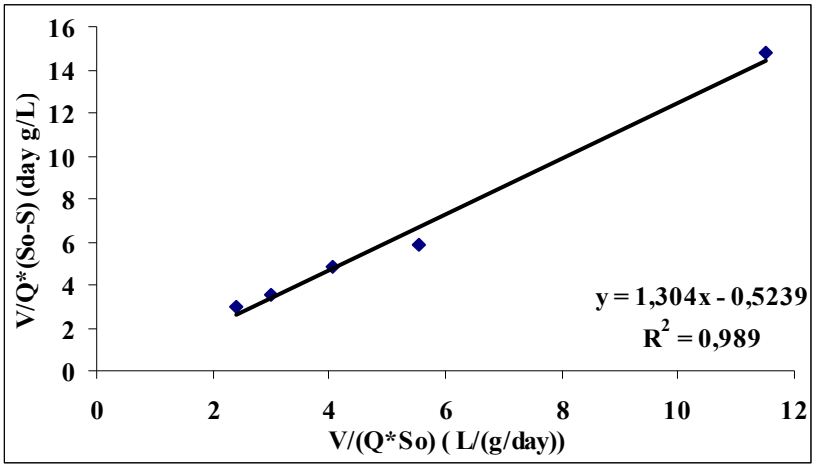


Figure 4.71 Determination of kinetic constants (R_{max} and K_B) in Stover Kincannon model for COD

4.2.3.2.4 Contois Kinetic Model. Figure 4.72 was plotted from the Eq 3.23 for determining the values of μ_{max} and β in this model. Maximum specific grow rate (μ_{max})(day^{-1}) and kinetic constant (β) (g COD/g biomass)values calculated from the intercept and the slope of the straight line illustrated in Figure 4.72 with regression coefficient of R^2=0.037,(y=-0,04887x+44,644) for COD. μ_{max} and β values was calculated as 0,022 day^{-1} and 0,001 (dimensionless), respectively.

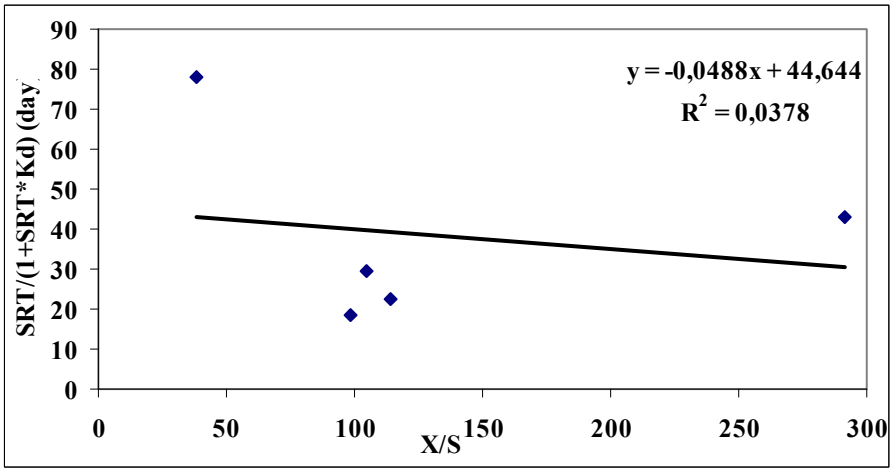


Figure 4.72 Determination of kinetic constants (μ_{max} and β) in Contois Kinetic Model for COD

4.2.3.2.5 Evaluation of the Kinetic Models Thought Anaerobic Degradation of Chloramphenicole in ABR Reactor. All kinetic coefficients calculated from the models are summarized in Table 4.10 with regration coefficients. The kinetic data showed that monod kinetic was more appropriate model than other models for predicting the performance of the lab scale ABR reactor when the regression coefficients and kinetic coefficients were compared with each other.

The yield coefficiency (Y) was higher compared to the death rate coefficient (k_d) in Monod kinetic model. Half saturation constant (K_s) was lower compared to initial COD concentration of 3000 mg/L. Maximum specific grow rate (μ_{max}) was higher compared to the death rate constant (k_d). This can be explained by the long HRT and sludge retention time. Low K_s value indicates a higher affinity to COD by the anaerobic methanogens.

The regression coefficients for COD under five different HRTs were higher in Stover Kincannon kinetic model (R^2=0.9890), Monod kinetic model (R^2=0.9883) and Grau second order model (R^2=0.9882) compared to Contois kinetic model (R^2=0.0378). Furthermore the kinetic constants determined in Stover Kincannon model, Monod kinetic model (R^2=0.9883) and Grau second order model are more meaningful than that observed in Contois kinetic model. Both the maximum substrate utilization rate (R_{max}=1,91 gCOD/L*day) and the saturation value constant (K_B=1,97 gCOD/Lday) are higher during COD degradation. Although high COD utilization rate (R_{max}) increase the reactor efficiency high substrate saturation constant (K_B) indicates the non- utilization of COD by the methanogens in the ABR.

In this study, the saturation value constant (K_B) (1,97 g/L*day) and maximum utilization rate (R_{max}) (1,91g/L*day) values obtained from the Modified Stover-Kincannon model are lower than those obtained by Işık & Sponza (2005) (7.5 g/L*day and 8.2 g/L*day, respectively) in UASB reactor treating simulated textile wastewater and than those obtained by Kapdan, (2005) (12.9 g/L*day and 37.7 g/L*day, respectively) in packed colum treating textile dye stuff in modified Stover-Kincannon model. The multicomponent substrate rate constant (k_s) ($0.055day^{-1}$)

value obtained from the Grau second order model in this study was lower than those obtained by Işık & Sponza (2005) in a UASB reactor (k_s=0.337 day^{-1}). Furthermore, the k_s value was 0.217 day^{-1} in a study performed by Ubay, (1989) in a UASB reactor treating municipal wastewater using Grau second order kinetic model.

Table 4.10 Kinetic parameters of ABR reactor treating chloramphenicole

Kinetic models	**Kinetic parameters**	**Values**	**Regression coefficients(R^2)**
Monod	Y (mgVSS/mgCOD)	13,532	0.9883
	k_d (day^{-1})	0,0024	0.9883
	μ_{max} (day^{-1})	0,3559	0.8809
	k_{max} (μ_{max}/Y) (day^{-1})	0,026	0.8809
	Ks (mg/l)	0,0071	0.8809
Grau second order	k_s (day^{-1})	0,055	0.9882
	a (day)	1,2975	0.9882
	b (dimensionless)	1,6384	0.9882
Modified Stover-Kincannon	K_B (g COD/Lday)	1,97	0.9890
	R_{max} (g COD/Lday)	1,91	0.9890
Contois	μ_{max} (day^{-1})	0,022	0,0378
	β(dimensionless)	0,001	0,0378

Although the regression coefficient obtained in the Stover- Kincannon model (R^2=98) is high the saturation value constant (K_B) found according this kinetic is extremely high. The kinetic constants found in the Monod and Grau kinetic models were found to be meaningfull. Therefore, it can be concluded that the chloramphenicole antibiotic is removed according to the Monod and Grau kinetic model with COD as substrate under anaerobic conditions in ABR reactor.

CHAPTER FIVE

CONCLUSIONS

5.1 Conclusions

The streptomycin and chloramphenicole concentrations caused 50% decreases in the methanogenic activity (decrease of methane gas production) (IC_{50}) were calculated as 292,06 mg/L and 252,49 mg/L, respectively.

ABR reactor reached to steady-state conditions after an operation period of 92 days at a streptomycin concentration of 25 mg/L. The COD removal efficiency was found as 84% after 92 days of the start-up period. The daily methane gas production, total gas production and methane percentage remained stable at 69,12 L/day, 100,8 L/day and 45% ,respectively.

ABR reactor reached to steady-state conditions after an operation period of 12 days at a chloramphenicole concentration of 50 mg/L. The COD removal efficiency was found as 82% after 12 days of the start-up period. The daily methane gas production, total gas production and methane percentage remained stable at 439,2 L/day and 48%, respectively.

5.1.1 The removal of Streptomycin in ABR and ABR/CSTR Reactor System

1. The COD removal efficiency was 67,55% at a streptomycin concentration of 400 mg/L. The maximum COD removal efficiency was 89,27% at a streptomycin concentration of 200 mg/L. The ABR reactor exhibited high COD (E=94-95%) removal efficiencies until a HRT of 19,2 days.

2. The maximum total, methane gas and methane percentage were found as 432 L/day, 288 L/day and 58%, respectively, for the streptomycin concentration of 200 mg/L. The total gas and methane gas production rates increased from 259,2 to 504 L/day and from 144 to 446,4 L/day, respectively as the HRT decreased from 38,4 days to 9,60 days. The methane percentages of the

biogas were approximately 38-40% at a HRT of 38,4 days. The methane percentages increased from 36% up to 53% at a HRT of 19,2 days. The methane percentages increased from 35% up to 46% as the HRT decreased from 19,2 to 7,68 days.

3. The highest VFA concentration (191 mg/L) was found at a HRT of 38,4 days. The VFA concentrations in the effluent decreased to 9 mg/L at a HRT of 7,68 days. VFA was 0 mg/L at all streptomycin concentrations until a streptomycin concentration of 400 mg/L. TVFA/Bic.Alk. ratios in the effluent and in the compartments of ABR were lower than 0.4. These results indicated the stability of ABR reactor at increasing streptomycin concentrations and decreasing HRTs.

4. The COD removal efficiency in sequential anaerobic ABR/aerobic CSTR reactor system was 94,52% at a HRT of 19,2 days. After that, the COD removal efficiency of the total reactor performance decreased from 94,52% to 85,70% when the HRT was decreased from 19,2 days to 7,68 days in sequential anaerobic ABR/aerobic CSTR reactor. For maximum COD removal efficiency (E=94,52%) the optimum HRT was found as 19,2 days.

5. In *Daphnia magna* acute toxicity test the wastewater containing 200 mg/L of streptomycin concentration was found to be toxic (% inhibition = 100%) in the influent of anaerobic ABR/aerobic CSTR reactor system. The acute toxicity reduction in sequential ABR/ CSTR reactor system effluent was 95% at a HRT of 38,4 days. The acute toxicity removal decreased from 95% to 80% as the HRTs decreased from 38,4 days to 7,68 days.

6. In this study it was found that the “ streptomycin ” antibiotic was mainly degreaded (179,57 mg/L) in anaerobic ABR reactor while the remaining small part of this antibiotic (47,54 mg/L) was removed in the aerobic CSTR reactor. The streptomycin removal efficiency was 66% at a HRT of 12,8 days in the anaerobic ABR effluent. The streptomycin concentration was measured

as 47,54 mg/L in the aerobic CSTR effluent corresponding a removal efficiency 74%.

5.1.2 The removal of Chloramphenicole in ABR and ABR/CSTR Reactor System

1. The maximum chloramphenicole concentration introduced in ABR was found as 340 mg/l, COD removal efficiency was 61,08%. The best COD removal efficiency of 130 mg/L was 94,40%. The COD (E=94-95%) removal efficiencies exhibited a good performance until HRT of 19,2 days.

2. The maximum total, methane gas and methane percentage were found as 547,2 L/day, 504 L/day and 58%, respectively, as the chloramphenicole concentration of 130 mg/L. The total gas and methane gas production rates increased from 259,2 to 547,2 L/day and from 216 to 504 L/day, respectively as the HRT decreased from 38,4 days to 19,2 days. After that methane percentages increased from 38% up to 58%, until at a HRT of 19,2 days. However the methane percentages decreased from 58% to 42% as the HRT decreased from 19,2 to 7,68 days.

3. The highest VFA concentration (0 mg/L) was found at all HRTs. TVFA/Bic.Alk. ratios in the effluent and in the compartments of ABR were lower than 0.4. These results indicated the stability of ABR reactor at increasing chloramphenicole concentrations and decreasing HRTs.

4. The COD removal efficiency in sequential anaerobic ABR/aerobic CSTR reactor system was 98,12% at a HRT of 19,2 days. After that, the COD removal efficiency of the total reactor performance decreased from 98,12% to 87,94% when the HRT was decreased from 19,2 days to 7,68 days in sequential anaerobic ABR/aerobic CSTR reactor. For maximum COD removal efficiency (E=98,12%) the optimum HRT was found as 19,2 days.

5. In this study it was found that the " chloramphenicole" antibiotic was mainly degreaded (128,42 mg/L) in anaerobic ABR reactor while the remaining small part of this antibiotic (0,63 mg/L) was removed in the aerobic CSTR reactor. The chloramphenicole removal efficiency was 98,5% at a HRT of 19,2 days in the anaerobic ABR reactor effluent . The chloramphenicole concentration was measured as 0,63 mg/L in the aerobic CSTR effluent corresponding a removal efficiency 99,5%.

5.1.3 Determintion of Kinetic Constant for ABR Reactor Treating Streptomycin and Chloramphenicole

1. The kinetic constants found in the Monod and Grau kinetic models were found to be meaningfull for streptomycin. Y and K_s was 2,137 mgVSS/mgCOD and 0,029 mg/L, respectively with high regression coefficient (R^2=0,99) for monod kinetic model. Similarly, the k_s and a was 0,062 day^{-1} and 1,0461 day, respectively with high regression coefficient (R^2=0,99) for Grau kinetic model for streptomycin.

2. The kinetic constants found in the Monod and Grau kinetic models were found to be meaningfull for chloramphnicole. Y and K_s was 13,532 mgVSS/mgCOD and 0,071 mg/L, respectively with high regression coefficient (R^2=0,98) for monod kinetic model. Similarly, the k_s and a was 0,055 day^{-1} and 1,2975 day, respectively with high regression coefficient (R^2=0,98) for Grau kinetic model for chloramphenicole.

The results of this study showed that sequential anaerobic ABR/aerobic CSTR reactor system is very useful and feasible process to treat the streptomycin and chloramphenicole antibiotics and to remove the acute toxicity.

Therefore this process system could be used in the treatment of pharmaceutical wastewaters in the future.

REFERENCES

Akunna, J. C., & Clark, M. (2000). Performance of a granular-bed anaerobic baffled reactor (GRABBR) treating whisky distillery wastewater. *Bioresource Technology.,* 74, 257–261.

Anderson, G. K., & Yang, G. (1992). Determination of bicarbonate and total volatile acid concentration in anaerobic digesters using a simple titration. *Water Environment Research*, 64, 53-59.

Aoust, D. 1994. Salmonella and the international food trade. *International Journal of Food Microbiology ., 24, 11-31.*

APHA-AWWA, (1992). *Standard Methods for the Examination of Water and Wastewater, 17th edition. American Public Health Association/American Water Works Association/Water Environment Federation,* Washington DC, USA.

APHA-AWWA, (2005). *Standard Methods for the Examination of Water and Wastewater, 21th edition. American Public Health Association/American Water Works Association/Water Environment Federation,* Washington DC, USA.

Barber, W. P, & Stuckey, D. (1999). The use of the anaerobic baffled reactor (ABR) for wastewater treatment. A Rewiew, *Water Research,* 33,, 7, 1559-1578.

Behling, E., Diaz, A., Colina, G., Herrera, M., Gutierrez, E., Chacin, E., (1997). Domestic wastewater treatment using a UASB reactor. *Biores. Technol*., 61, 239-245.

Bell, (2002). Treatment of dye wastewaters in the anaerobic baffled reactor and characterization of the associated microbial populations. Ph.D. Thesis, *School of Chem. Eng. Univ. of Natal*. Durban.

Bell, J., & Buckley, C. A. (2003). Treatment of a textile dye in the anaerobic baffled reactor. *Water S.A*, 29(2), 129–134.

Bell, J., Plumb, J., Buckley, C., & Stuckey, C. (2000). Treatment and decolorization of dyes in an anaerobic baffled reactor. *J Environ Eng*., 126 (11), 1026-1032.

Beydilli, M. I, Pavlosathis, S.G., & Tincher, W.C. (1998). Decolorization and toxicity screening of selected reactive azo dyes under methanogenic conditions. *Water Scienc. and Technology* 38(4-5), 225-32.

Bodik, I., Kratochvil, K., Gasparikova, E., & Hutnan, M. (2003). Nitrojen removal in an anaerobic baffled reactor with aerobic post–treatment. *Bioresource Technology*., 86,79-84.

Boopathy, R. (1998). Biological treatment of swine waste using ABR. *Bioresource Technology., 64, 1-6.*

Chelliapan S., Wilby, T. & Sallis, P. (2006). Performance of an up-flow anaerobic stage reactor (UASR) in the treatment of pharmaceutical wastewater containing macrolide antibiotics. *Walter Research,* 40, 507-516

Contois, D. E. (1959). Kinetics of bacterial growth: relationship between population density and space growth rate of continuous cultures. *J. Gen. Microbiol*., 21, 40–50.

Dama, P., Bell, J., Faxon, K. M., Brouckaert, C. J., Huany, T., & Buckley, C. A. (2002). Pilot-scale study of an anaerobic baffled reactor for the treatment of domestic wastewater: *Water Scienc and Technology*., 26(9), 263-270.

Donlon, B., Razo-Flores, E., Lettinga, G., &. Fıeld, A. J. (1996). Continuous detoxification, transformation and degradation of nitrophenols in upflow anaerobic sludge blanket (UASB) reactors. *Biotechnoogyl. and Bioeng*., 51, 439-449.

E.H. Allen,J.Assoc.Off.Anal.Chem.,68,(1985),990

Grau, P., Dohanyas, M., & Chudoba, J. (1975). Kinetic of mlticomponant substrate removal by activated sludge, *Water Research*, 9, 337-342.

Grover, R., Marwaha, S. S., & Kennedy, J.F. (1999). Studies on the use of an anaerobic baffled reactor for the continuous anaerobic digestion of pulp and paper mill black liquors. *Process Biochem*., 34, 653-657.

Halling, S. (2000). Algal toxicity of antibacterial agents used in intensive farming.*Chemosphere, 40, 731-739*

Huajun, F., Lifang, H., Dan, S., Chengran, F., Yonghua, H., & Dongsheng, S. (2008) Effects of Temperature and Hydraulic Residence Time (HRT) on Treatment of Dilute Wastewater in a Carrier Anaerobic Baffled Reactor. *Biomedical and Environmental Sciences*, 21, 460-466

Huajun, F., Lifang, H., Dan, S., Chengran, F., Yonghua, H., & Dongsheng, S. (2008). Effects of operatonal factors on soluble microbial products in a carrier anaerobic baffled reactor treating dilute wastewater. *Journal of Environmental Sciences,* 20, 690-695.

Işık, M., & Sponza, D. T. (2005). Substrate removal kinetics in an upflow anaerobic sludge blanket reactor decolorising simulated textile wastewater. *Process Biochemistry*, 40, 1189–1198.

Iza, J., Colleran, E., Paris, J.M., Wu, W.M. (1991). International workshop on anaerobic treatment technology for municipal and industrial wastewaters: summary paper. *Water Sci Technolgy*, 24(8),1–16.

İnce K. İ., Selcuk, A. & Ince, O. (2003). Effect of chemical synthesis-based pharmaceutical wastewater on performance, acetoclastic methanogenic activity and microbial population in an upflow anaerobic filter. *Journal of Chemical Teacnology and Biotechnology,* 77, 711-719

Kapdan, I. K. (2005). Kinetic analysis of dyestuff and COD removal from synthetic wastewater in an anaerobic packed column reactor. *Process Biochemistry*. *40, 2545–2550*

Kemper, N. (2008). Veterianry antibiotics in the aquatic and terrestrial environment. *Ecological Indicators,* 8, 1-13

Kurosawa, N.; Kuribayashi, S.; Owada, E.; Ito, K.; Nioka, M.; Arakawa, M.; Fukuda, R.; J. Chromatogr., Biomed. Appl., 11 Oct 1985, 44 (2 (J.Chromatogr., 343)), 379-385

Kuscu, O. S., & Sponza, D. T. (2005). Performance of anaerobic baffled reactor (ABR) treating synthetic wastewater containing *p*-nitrophenol. *Enzyme and Microb. Technol.*, 36, 888–895.

Kuscu, O. S., & Sponza, D.T. (2006). Performance of *p*-nitrophenol (*p*-NP) fed sequential anaerobic migrating blanket reactor (AMBR)/aerobic completely stirred tank reactor (CSTR) system under increasing organic loading conditions *Microb. Technol.*, 36, 888–895.

Kuscu, O. S., & Sponza, D. T. (2009). Effects of nitrobenzene concentration and hydraulic retention time on the treatment of nitrobenzene in sequential anaerobic baffled reactor (ABR)/ continuously stirred tank reactor (CSTR) system *Bioresource Technology.*, 100, 2162–2170.

Kümmerer, K (2009). Antibiotics in the aquatic environment – A review – Part II. *Chemosphere,* 75, 435-441

Lai, H., Hou, J., Su, C., & Chen, C. (2009). Effects of chloramphenicol, florfenicol, and thiamphenicol on growth of algae Chlorella pyrenoidosa, Isochrysis galbana, and Tetraselmis chui. *Water Research,* 72, 329-334

Liu, X., Nangi, R., & Yuan, Y,. (2009). Performance of a periodic anaerobic baffled reactor fed on chinese traditional medicine industirial wastewater. *Bioresource Technology,* 100, 104-110

Metcalf & Eddy. (1991). Wastewater Engineering: treatment, Disposal and Reuse. Mc Graw.Hill.Inc.

Oktem, Y., Ince, O., Sallis, P., Donnelly, T., & Ince, K. B. (2007). Anaerobic treatment of a chemical synthesis- based pharmaceutical wastewater in a hybrid upflow anaerobic sludge blanket reactor. *Bioresource Technology,* 99, 1089-1096

Owen, W.F., Stuckey, D.C., Healy, J.B., Young, JR. L.Y., & McCarty, P. L. (1979). Bioassay for monitoring biochemical methane potential and anaerobic toxicity, *Water Research,* 13, 485-492.

Öztürk, I., Altinbas, M., Arikan, O., & Demir, A. (1998). Anaerobic UASBR Treatment of Young Landfill leachate. *1st International Workshop on Environmental Quality and Environmental Engineering in the Middle East Region, Konya, Turkey.*

Pavlostathis, S. G., & Giraldo-Gomez, E. (1991). Kinetics of anaerobic treatment. *Water Sci. and Technol*., 24(8), 35–59.

Peng, X., Wang, Z., Kuang, W,. Tan, J., & Li, K. (2006). A preliminary study on the occurrence and behavior of sulfonamides, ofloxacin and chloramphenicol antimicrobials in wastewaters of two sewage treatment plants in Guangzhou, China. *Science of the Total Environment,* 371, 314-322

Razo-Flores, E., Luijten, M., Donlon, B. A., Lettinga, G., Field, J. A. (1997). Biodegradation of selected azo dye under methanogenic conditions. *Water Science Technology*, 36 (6-7), 65-72.

Scoaris, D.O., Colacite, J., Nakamura, C.V., (2007). Virulence and antibiotic susceptibility of Aeromonas spp. İsoleted from drinking water. *Antonie Van Leewenhoek, 93, 111-122.*

Setiadi, T., Husaini, L., & Djajadiningrat, A. (1996). Palm oil mill effluent treatment by anaerobic baffled reactors: recycle effects and biokinetic parameters. *Water Science Technology*, 34(11), 59–66.

Speece, R.E. (1996). Anaerobic biotechnology for industrial wastewater. Tennessee: Archae Press, 5840 R.E. Lee, Dr. Nashville, 37215.

Ubay, G. (1989). Kinetic modelling of UASBR's. MSc thesis. Faculty of Science, Istanbul Technical University, (in Turkish).

Uyanik, S., Sallis, P. J., & Anderson, G. K. (2002). The effect of polymer addition on granulation in an anaerobic baffled reactor (ABR). Part I: process performance. *Wat. Res.,* 36, 933–943.

Vossoughi, M., Shakeri, M., & Alemzadeh, I. (2003). Performance of anaerobic baffled reactor treating synthetic wasterwater influenced by decreasing COD/SO_4 rations. *Chem. Eng and Process.*, 42, 811-816.

Wang, J., Huang, Y., & Zhao, X. (2004). Performance and characteristics of an anaerobic baffled reactor. *Bioresource Technol*ogy, 93(2), 205-208.

Weifen, W., Hong, L., Changhu, X., & Jamil, K. (2004). Elimination of chloramphenicol, sulphamethoxazole and OXYtetacycline in shrimp, Penaeus chinensis following medicated-feed treatment. *Environment Internatıonal,* 30, 367-373

Wikipedia. (n.d.). *Chloramphenicol,* Retrieved August 8, 2009, from http://en.wikipedia.org/wiki/Chloramphenicol

Wikipedia. (n.d.).*Streptomycin,* Retrieved August 8, 2009, from http://en.wikipedia.org/wiki/Streptomycin

Yu, H, Wilson, F., Tay, J. (1998). Kinetic analysis of an anaerobic filter treating soybean wastewater. *Water Research,* 32,3341–3352.

Zhu, G., F., Li, J., Z., Wu, P., Jin, H., & Wang, Z. (2008). The performance and phase separated characteristics of an anaerobic baffled reactor treating soybean protein processing wastewater. *Bioresource Technology.,* 99, 8027–8033