DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

EFFECT OF BIOSURFACTANTS ON THE BIODEGRADATION OF HYDROCARBONS IN WASTEWATER

by
Ayla UYSAL

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İZMİR

EFFECT OF BIOSURFACTANTS ON THE BIODEGRADATION OF HYDROCARBONS IN WASTEWATER

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Ph.D. THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"EFFECT OF BIOSURFACTANTS ON THE BIODEGRADATION OF HYDROCARBONS IN WASTEWATER"** completed by **Ayla UYSAL** under supervision of **Prof. Dr. Ayşen TÜRKMAN** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy.

Prof. Dr. Ayşen TÜRKMAN

Supervisor

Prof. Dr. Ayşe FİLİBELİ

Committee Member

Prof. Dr. Sümer PEKER

Committee Member

Prof. Dr. Günay KOCASOY

Jury Member

Prof. Dr. Delya SPONZA

Jury Member

Prof. Dr. Cahit HELVACI

Director

Graduate School of Natural and Applied Sciences

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Ayla UYSAL

EFFECT OF BIOSURFACTANTS ON THE BIODEGRADATION OF HYDROCARBONS IN WASTEWATER

ABSTRACT

The release of specific industrial wastewaters, including nonbiodegradable and/or toxic pollutants, to receiving environments is one of the most significant type of environmental pollution by hazardous wastes. Biological treatment of wastewater is often the most economical alternative when compared with other treatment options. However, industrial effluents are known to contain toxic and/or non-biodegradable organic substances and conventional biological treatment processes are not efficient in these cases. Thus, they require some enhancements due to the presence of refractory or toxic compounds in the wastewaters. For this reason, the use of surfactants in the biodegradation of persistent organic pollutants by biological treatment was investigated as an enhancement technique for the biological treatment process.

In this study, the effect of biosurfactant on biodegradability of 2,4-dichlorophenol (2,4-DCP) and 4-chlorophenol (4-CP) with using acclimated culture was investigated by activated sludge bioreactor with changing chlorophenols loading rate and sludge retention time (SRT), and the effect of biosurfactant on 4-CP degradation in unacclimated culture was studied. Glucose as growth substrate and JBR 425 rhamnolipid as biosurfactant were used. Control and test reactors were operated through parallel experiments.

During the experimental study, enhanced biodegradation of 2,4-DCP and 4-CP was observed in biosurfactant added activated sludge systems (test reactors). Especially, effect of biosurfactant was much higher at lower sludge ages and unacclimated culture. COD, 2,4-DCP and 4-CP biodegradation rates were much higher in biosurfactant added systems relative to control reactor. Since biosurfactant existence attenuated chlorophenol toxicity on the microorganisms, bioactivity of

activated sludge was maintained during operation periods. Also, the presence of biosurfactant stimulated bacterial growth.

Keywords: Activated sludge; Bacterial growth; Biodegradation; Biosurfactant; 2,4-Dichlorophenol (2,4-DCP); 4-Chlorophenol (4-CP); Sludge retention time (SRT); Toxicity.

ATIKSUDA HİDROKARBONLARIN BİYOLOJİK AYRIŞMASI ÜZERİNE BİYOSURFAKTANLARIN ETKİSİ

ÖZ

Biyolojik olarak parçalanamayan ve toksik kirleticiler içeren endüstriyel atıksuların çevresel ortamlara tam olarak arıtılmadan verilmesi, günümüzde tehlikeli atıklardan kaynaklanan çevre kirliliklerinin en önemli sebeplerinden biridir. Atıksuların arıtımında uygulanan diğer yöntemlerle karşılaştırıldığında, biyolojik arıtma sistemleri en ekonomik alternatif olarak karşımıza çıkmaktadır. Bununla beraber, konvansiyonel biyolojik arıtma sistemlerinin toksik ve /veya biyolojik olarak zor ayrışan organik maddeler içeren endüstriyel atıksuların arıtımında zaman zaman yetersiz kaldığı da bilinmektedir. Sonuç olarak, biyolojik arıtma sürecinde parçalanmayı hızlandıracak modifikasyonları gerekmektedir. Bu çalışmada, zor ayrışabilir kirleticileri içeren atıksuların biyolojik olarak arıtımında alternatif bir hızlandırıcı yöntem olarak surfaktan kullanımı incelenmiştir.

Bu çalışmada, aklime edilmiş çamur kullanılarak 2,4-diklorofenol (2,4-DKF) ve 4-klorofenolün (4-KF) biyolojik ayrışabilirliği üzerine biyosurfaktanın etkisi, klorofenol yükleme hızı ve çamur alıkonma süresinin (ÇAS) değiştirilmesi ile aktif çamur biyoreaktöründe incelenmiş, ve aklime edilmemiş kültürde 4-KF ayrışması üzerine biyosurfaktanın etkisi çalışılmıştır. Büyüme maddesi olarak glikoz ve biyosurfaktan olarak JBR 425 rhamnolipid kullanılmıştır. Kontrol ve test reaktörleri paralel olarak işletilmiştir.

Çalışmanın sonuçlarında, biyosurfaktan eklenilen aktif çamur sistemlerinde (test reaktörleri) 2,4-DKF ve 4-KF'ün biyolojik ayrışmasının arttığı gözlenmiştir. Özellikle, biyosurfaktanın etkisi düşük çamur yaşlarında ve aklime edilmemiş çamurda çok daha fazla etkili olmuştur. KOİ, 2,4-DKF ve 4-KF biyolojik ayrışma hızları biyosurfaktan eklenilen sistemlerde kontrol reaktörüne göre daha yüksektir. Biyosurfaktan mevcudiyeti mikroorganizmalar üzerine klorofenol toksisitesini azalttığından dolayı, aktif çamur biyoaktivitesi işletim periyodu boyunca muhafaza

edilmiştir. Biyosurfaktan mevcudiyeti aynı zamanda bakteriyel büyümeye de neden olmuştur.

Anahtar Kelimeler: Aktif çamur; Bakteriyel büyüme; Biyolojik ayrışma; Biyosurfaktan; 2,4-Diklorofenol (2,4-DKF); 4-Klorofenol (4-KF); Çamur alıkonma süresi (ÇAS); Toksisite.

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CHAPTER ONE
INTRODUCTION

1.1 Introduction

The presence of toxic and/or refractory organic compounds in the discharge of wastewaters and in some cases in water supplies is a topic of global concern. One important group of such chemicals is the halogenated aromatic compounds. Halogenated aromatics, particularly chlorinated phenolic hydrocarbons are generated from a number of industrial manufacturing processes, mainly including pulp and paper, dyestuffs, pesticides, herbicides and fungicides. Chlorophenols are widespread toxic compounds that are included in the U.S. Environmental Protection Agency (EPA) list of priority pollutants.

Chlorophenols are a group of chemicals in which chlorines (between one and five) have been added to phenol. Phenol is an aromatic compound derived from benzene, the simplest aromatic hydrocarbon, by adding a hydroxy group to a carbon to replace a hydrogen. There are five basic types of chlorophenols: monochlorophenols, dichlorophenols, trichlorophenols, tetrachlorophenols, and pentachlorophenols.

The fate of chlorophenolic compounds in the environment is really important issue nowadays because these compounds are found to be toxic, recalcitrant and bioaccumulating in organisms. The recalcitrant structure of chlorophenols results form the carbon-halogen bond, which is cleaved with great difficulty and the stability of their structure, resulting in their accumulation in nature (Jianlog, Yi, Horan, & Stentiford, 2000; Farrell & Quilty, 2002). Therefore, their discharge into the environment must be regulated.

Phenol and its chlorinated forms are used extensively as pesticides, wood preservatives and its intermediates in the manufacture of pesticides, paper, and as chemical components in the process of fossil fuel extraction and beneficiation (Kuraman & Paruchuri, 1997). Chlorophenolic compounds are often found in the

waste discharges of many industries including petrochemical, oil refinery, plastic, pesticides, biocides, wood preservers, pulp and insulation materials (Liu, Wang, Pen, Hsu, & Chou, 1991; Raung, 1984).

Due to their high toxicity, recalcitrance, bioaccumulation, strong odour emission, persistence in the environment and suspected carcinogenity and mutagenity to the living, chlorophenols pose serious ecological problem as environmental pollutants (Armenante, Kafkewitz, & Lewandowski, 1999; Puhakka & Jarvinen, 1992). Their fate in the environment is of great importance. Hence, the removal of phenol and chlorinated organic compounds from wastewater is necessary task to conserve the water quality of natural water resources (Ha, Qishan, & Vinitnantharat, 2000).

Several physical, chemical and biological methods including activated carbon adsorption, ion exchange, air stripping, chemical oxidation, incineration and biological degradation have been proposed for treating or recovering chlorophenolic compounds (Raung, 1984). The high cost and low efficiency of physical and chemical processes limit their applicability (Quan, Shi, Zhang, Wang, & Qian, 2003). The biological treatment of chlorophenols attracts more attention than physicochemical methods such as activated carbon adsorption and incineration because the latter have high treatment costs and possibilities of causing a secondary pollution (Raung, 1984; Quan et al., 2003).

In general, biological treatment of wastewater, i.e. completely mixed activated sludge process, is often the most economical alternative when compared with other treatment options. Although biological treatment methods have been generally found most effective alternatives in the removal of persistent compounds, they require some enhancements due to the presence of refractory or toxic compounds in the wastewater. In recent years, however, the use of surfactants in the removal of persistent organic pollutants by biological treatment has been investigated as an enhancement technique for the biological treatment process.

Surfactants can either be chemically synthesized (synthetic) or microbially produced (biosurfactants). Synthetic surfactants are of petrochemical origin, whereas biosurfactants or biogenic surfactants are produced by bacteria, yeast, and fungi. For specific applications, biological surfactants have advantages over synthetic surfactants due to their structural diversity, biodegradability, and effectiveness at extreme temperatures, pH and salinity. Microbial degradation of certain hydrocarbon contaminants has been demonstrated to be facilitated by the simultaneous production of a biosurfactant. In contrast, synthetic surfactants have been shown to inhibit microbial activity when added to the environment at high concentrations (Thangamani & Shreve, 1994).

A number of researchers indicated surfactant enhancement of the microbial degradation of organic contaminants (Aronstein & Alexander, 1993; Bury & Miller, 1993; Zhang, Valsaraj, Constant, & Roy, 1998; Diehl & Borazjani, 1988; Mulligan & Eftekhari, 2003; Royal, Preston, Sekelsky, & Shreve, 2003; Cort, Song, & Bielefeldt, 2002). However, limited number of these studies was on enhanced biodegradation of chlorophenols using a surfactant in an activated sludge. Activated sludge process is being applied worldwide in municipal and industrial wastewater treatment. In general, it is recommended to use the activated sludge to treat toxic compounds due to its microbial diversity (Spain & Van Veld, 1983; Watson, 1993).

In this study, the effect of biosurfactant on biodegradability of 2,4-dichlorophenol (2,4-DCP) and 4-chlorophenol (4-CP) with using acclimated culture was investigated by activated sludge bioreactor with changing chlorophenols loading rate and sludge retention time (SRT), and the effect of biosurfactant on 4-CP degradation in unacclimated culture was studied.

1.2 Objectives and Scope

The rapidly increasing costs of new wastewater treatment technologies and/or their limited effectiveness in the removal of persistent pollutants and regulations becoming more restrictive each year are fostering interest in the development and the

use of alternative cost-effective and environmentally acceptable approaches. One approach being investigated in this study is the use of biosurfactants to enhance the biodegradation of persistent pollutants in wastewaters.

The use of surfactants has been found as an effective and feasible alternative in the bioremediation of contaminated soil and groundwater environments and oil spill clean up at sea and inland. On the other hand limited studies were performed to investigate the effect of biosurfactants in the removal of persistent organic pollutants in industrial wastewaters.

In the light of related studies on microbial decomposition of chlorinated phenolic compounds, it was found that 2,4-DCP and 4-CP were two of those toxic and/or refractory organic compounds which were most difficult to biodegrade by both aerobic and anaerobic microorganisms and they were reported as an accumulating compound in biological treatment systems due to the dechlorination of highly chlorinated phenolic compounds.

Literature survey has shown that majority of biological treatability studies of 2,4-DCP and 4-CP were carried out batch-wise and with pure cultures. When practical application of engineering systems is considered, however, the fate and effect of 2,4-DCP and 4-CP in continuously operated systems, i.e. completely mixed activated sludge systems, with a mixed culture, gains importance.

Major objective of the proposed study is to investigate the effect of biosurfactant on chlorophenol biodegradation in an activated sludge bioreactor system.

Based on this approach, major objectives of this thesis can be summarized as follows:

1. To investigate the effect of biosurfactant on 2,4-DCP biodegradation in an activated sludge bioreactor at various 2,4-DCP loading rates (0.007-0.212 g 2,4-DCP/l.day) and various sludge ages (3-25 days).

2. To investigate the effect of biosurfactant on 4-CP biodegradation in an activated sludge bioreactor at various 4-CP loading rates (0.007-0.635 g 4-CP/l.day) and various sludge ages (3-25 days).

3. To investigate and evaluate the potential utility of biosurfactant on 4-CP degradation in unacclimated activated sludge system to enhance 4-CP removal and system stability under toxic loading conditions.

CHAPTER TWO
BIOLOGICAL REMOVAL OF CHLOROPHENOLS

2.1 Theoretical Background

A variety of biological treatment processes, aerobic as well as anaerobic, such as facultative basin, aerated stabilization basin, aerated lagoon system, decanted aerated reactors, fluidized bed bioreactors and upflow anaerobic sludge blanket (UASB) have been used for treatment of wastewaters containing chlorophenols. Despite the recalcitrant nature of chlorophenols, their biodegradation by aerobic or anaerobic treatment methods is more specific and relatively inexpensive (Armenante et al., 1999; Atuanya, Purohit, & Chakrabarti, 2000; Annachhatre & Gheewala, 1996; Bali & Sengul, 2002). Aerobic microorganisms are more efficient in degrading toxic compounds because they grow faster than anaerobes and usually achieve complete mineralization of toxic organic compounds, rather than transformation, as in the case of anaerobic treatment (Kim, Oh, Lee, Kim, & Hong, 2002). In aerobic conditions majority of chlorophenols are resistant to biodegradation because chlorine atoms interfere with the action of many oxygenase enzymes, which normally initiate the degradation of aromatic rings (Copley, 1997). Most of the investigations on biodegradation of chlorophenols focused on suspended pure culture studies using different bacteria and fungi (Dapaah & Hill, 1992; Fahr, Wetzstein, Grey, & Schlosser, 1999; Farrell & Quilty, 2002; Hill, Milne, & Nawrocki, 1996; Kim & Hao, 1999; Li, Erberspacher, Wagner, Kuntzer, & Ligens, 1991; Steinle, Stucki, Stettler, & Hanselmann, 1998; Wang, Lee, & Kuan, 2000; Wang & Loh, 1999; Yee & Wood, 1997).

A limited number of studies have been reported on biological treatment of wastewaters containing chlorophenols using activated sludge system with mixed culture. Recent investigations on biodegradation of chlorophenols focused on the use of immobilized cells or biofilm reactors (Shieh, Puhakka, Melin, & Tuhkannen, 1990; Radwan & Ramanujam, 1996; Shin, Yoo, & Park, 1999; Swaminathan & Ramanujam, 1998; Kim et al., 2002). Biofilm reactors are more resistant to high

concentrations of chlorophenols, because of high biomass concentrations and diffusion barriers within the biofilm for the toxic compounds. However, it is difficult to control some parameters such as the biofilm thickness, dissolved oxygen concentration, pH, and redox potential in biofilm reactors due to the heterogeneous nature of such reactors. Suspended culture systems (i.e. activated sludge processes) offer major advantages such as better control and operation as compared to the biofilm reactors and may yield high removal efficiencies for COD, chlorophenols, and toxicity if operated with high sludge recycle at high sludge ages. Mixed cultures are particularly important when the emphasis is placed on complete mineralization of toxic organics to CO_2. Many pure-culture studies have shown that toxic intermediates accumulate during biodegradation, because a single organism may not have the ability to completely mineralize the xenobiotic (Buitron & Gonzalez, 1996). Therefore, the treatment of chlorophenols using an activated sludge process in which a mixed culture is in action in the absence of a special growth substrate would be more meaningful, informative, and practical.

Kim et al. (2002) reported that the differences in structure and toxicity of phenolic compounds require that various bacteria with specific qualities to degrade each compound or a mixed culture should be used. Mixed culture is divided into defined and undefined types. The activated sludge process is one example of undefined mixed cultures. Activated sludge is a complex group of microorganisms that have the ability to oxidize organic compounds in wastewater under aerobic conditions. The main advantage achieved by the microbial consortium formed by activated sludge is the interaction between all the species present in the flocs. Also, it is well known that the capacities of an activated sludge system can be enhanced by acclimation (Buitron, Gonzalez, & Lopez-Marin, 1998; Kim et al., 2002). Pre-adaptation of the activated sludge cultures to chlorophenols was reported to improve the rate and the extent of biodegradation of those compounds (Bali & Sengul, 2002; Sahinkaya & Dilek, 2002)**.** Many factors may affect the length of the acclimation such as the inoculum size, nature of the inoculum, the initial culture conditions, toxicity of chlorophenol, and especially some undefined factors (Alexander, 1994).

The influence of the inoculum source and the acclimation strategy on the 4-CP degradation in a sequencing batch reactor was studied by Moreno & Buitron (2004). Three different sources of inocula were obtained from the aeration tank of domestic, municipal and industrial wastewater treatment plants. The acclimation was performed using two strategies, the first one fixing the reaction time, independent of the removal efficiency (fixed time) and the second one fixing a removal efficiency of 90% as 4-CP (variable time). The degradative activity was followed for each condition. Variable time strategy produced a microbial community with higher specific activity compared with those obtained for the fixed time strategy. The microbial activity was dependent of the origin of the inoculum. Each inoculum presented different specific activity to 4-CP degradation. It was observed that the use of the fixed time strategy for the acclimation reduced the diversity of bacterial community. The origin of the inoculum and the acclimation strategy have an influence on the specific substrate removal rate obtained after acclimation. Activated sludge originating coming from a municipal wastewater treatment plant presented an initial higher bacterial diversity and thus a better adaptability to the toxic compound.

Chlorophenols are not good substrate for biomass and they have a strong inhibitory effect on the biomass growth (Sahinkaya & Dilek, 2006). Similarly, Rutgers, Breure, Andel, & Duetz (1997) reported that the growth yield coefficients on chlorinated phenols are lower than those of heterotrophic growth on non-chlorinated compounds. It has been reported that chlorinated solvents generally cannot serve as a carbon and energy source for microbial growth, but rather must be biodegraded by cometabolism (Wang & Loh, 1999, 2000; Bali & Şengül, 2002). Usually, a carbohydrate substrate was used as the primary metabolite and the chlorophenols were the co-metabolite in the biodegradation of chlorophenols (Hill et al., 1996; Kim & Hao, 1999; Wang & Loh, 1999)**.** It is quite common that an organic compound is chosen as a growth substrate because it can support cell growth of the cometabolizing bacterium naturally (Wang & Loh, 1999; Hill et al., 1996). The effect of the presence of conventional organic substrates on the biodegradation of toxic waste components through cometabolic pathways is of great practical importance because a toxic waste component not only may inhibit its own

biodegradation but the biodegradation of other nontoxic organics as well. For biological degradation of toxic compounds degraded, a suitable growth substrate, which serves as sources of carbon and energy to support cell growth, is required.

The nongrowth substrate, then, can only be transformed in the presence of a growth substrate, a phenomenon called cometabolism. The growth substrate not only serves to sustain biomass production but also acts as an electron donor for degradation of the nongrowth substrate. However, nongrowth substrates have been shown to inhibit the oxidation of the growth substrate (Loh & Wang, 1998). As such, the rate and efficiency of cometabolism are always dependent on a complex interaction between the growth substrate and nongrowth substrate. Neverthless, because some of the most common chlorinated organics are known to be biodegraded through cometabolic pathways (Alexander, 1994), the biodegradation behaviour of cometabolised compounds is of great importance to the biological treatment of polluted groundwater, industrial effluent, hazardous waste sites, and so on. It is therefore necessary to devote attention to study the interaction between the growth substrates and nongrowth substrates in order to enhance the rate of the cometabolism. This being the case, growth substrates can be optimally chosen from a wide range of carbon sources, including nontoxic, readily degradable organic compounds. Especially when the selected growth substrate is a conventional carbon source, the design of cometabolic systems can be facilitated with reduced cost and risks associated with the addition of toxic growth substrates such as phenol (Si-Jing & Kai-Chee, 2000).

In cometabolic transformation of chlorophenols, phenol is a good primary substrate. Strong competitive inhibition between phenol and chlorophenol, however, inhibits chlorophenol transformation significantly. It has been found that chlorophenol was transformed rapidly only after phenol was almost fully depleted (Loh & Wang, 1998). Furthermore, phenol is an environmentally toxic compound, the use of which may result in additional pollution. The results of above mentioned experiments, however, show that chlorophenol can also be degraded in the absence of phenol by cells grown on glucose as the sole growth substrate. Si-Jing & Kai-

Chee, (2000) have suggested that in this case, the cometabolic enzymes required for 4-CP transformation were most probably induced by 4-CP. This is likely since phenol and 4-CP are structurally analogous. As a result of using glucose as the growth substrate, competitive inhibition with 4-CP can be avoided. Moreover, the use of glucose would not result in additional environmental pollution as opposed to using phenol.

Chlorophenols are less readily biodegradable than phenol and their rate of biodegradation decreases with increasing number of chlorine substituents on the aromatic ring (Banarjee, Howard, Rosenberg, Dombrowsky, Sikka, & Tullis, 1984). Some researchers have proposed that the position of the substituent has also an effect on the degradability of the compound (Genthner, Price, & Pritchard, 1989; Mcleese, Zitko, & Peterson, 1979) although such observations often lack uniformity. For example, the relative order of biodegradability for chlorophenols was found to be ortho⟩ meta⟩ para during anaerobic conditions in aquifer sediments (Genthner et al., 1989). However, the degradation of chlorophenols in anoxic natural marine sediments (Abrahamsson & Klick, 1991) and in soil (Namkoong, Loehr, & Malina, 1988) showed the order of dehalogenation to be ortho⟩ para⟩ meta. These observations suggest that differences in the ability of microbes to degrade chlorophenols could exist based on the environment under which they act. History of biomass acclimatization could also be a possible reason for such variations.

The mechanisms of aerobic degradation differ amongst chlorophenols depending on the degree of chlorination, and there is a clear division of the bacterial isolates into two groups: (i) strains that degrade mono and dichlorophenols, but will not attack more highly chlorinated phenols, and (ii) strains that degrade pentachlorophenol and other highly chlorinated phenols, but will not degrade mono and dichlorophenols. For complete degradation of chlorinated aromatic compounds to occur, two steps are necessary, cleavage of the aromatic ring and the removal of chlorine atom (Häggblom, 1990). The initial step in the aerobic degradation of chlorophenols is, generally, their transformation to chlorocatechols by a phenol monooxygenase. Folllowing transformation of chlorophenols to chlorocatechols, ring

cleavage by dioxygenases may proceed. Dehalogenation takes place as a fortuitous reaction only after cleavage of the aromatic ring. Degradation of mono and dichlorophenols has been demonstrated with bacteria belonging to the genera Pseudomonas, Alcaligenes, Arthrobacter, Nocardia, Rhodococcus, Mycobacterium, Achromobacter, and Bacillus (Gorlatov, Mal'tseva, Shevchenko, & Golovleva, 1989; Engelhardt, Rast, & Wallnöfer, 1979; Knackmuss & Hellwig, 1978).

2.2 Literature Survey

The fate of chlorophenolic compounds in the environment is of great importance as these compounds are found to be toxic, recalcitrant and bioaccumulating in organisms and hence their discharge into the environment must be regulated. Literature survey has shown that treatment of 2,4-DCP and 4-CP has been studied so far aerobic biological conditions by using different types of microorganisms.

Experiments with 2,4-DCP concentrations between 10-200 mg/l in batch studies showed that higher concentrations of 2,4-DCP (50-200 mg/l) are inhibitive to the growth of either suspended or immobilized *Bacillus insolitus* by Wang et al. (2000). At lower concentrations of 2,4-DCP, immobilized mixed culture may have the same removal efficiency of 2,4-DCP as immobilized pure culture of *Bacillus insolitus*. But with regard to the overall 2,4-DCP removal efficiency, immobilized pure culture is considered to be superior to immobilized mixed culture.

Kargi, Eker, & Uygur (2005) reported that inhibition effects of 2,4-DCP were pronounced for the feed 2,4-DCP contents above 150 mg/l in activated sludge unit. Biomass concentration in the aeration tank decreased with feed 2,4-DCP concentrations above 150 mg/l resulting in lower COD and 2,4-DCP removal rates.

Treatment performance of COD in the presence of 2,4-DCP was explored by using a biological activated carbon-sequencing batch reactor (BAC-SBR) system by Ha et al. (2000). Although effluent concentration was increased with reduction of SRT from 8 days to 3 days, treatment efficiency was indicated to be higher than 90%

for COD at all SRT applied. Reactors operated with acclimated sludge could be expected to cope with quite high loading of inhibitory substances.

Quan et al. (2003) studied that microorganisms, identified as *Achromobacter* sp. and capable of degrading 2,4-DCP, were immobilized in the ceramic carrier and used for biodegradation of 2,4-DCP in an air-lift honeycomb-like ceramic reactor. Semi-continuous biodegradation of 2,4-DCP as a single substrate and in the presence of phenol as co-substrate was investigated. When phenol was used as a co-substrate, the existence of phenol could inhibit the biodegradation of 2,4-DCP and the biodegradation rate of 2,4-DCP decreased gradually. In addition, continuous degradation of 2,4-DCP was also investigated. The results indicated that 2,4-DCP at the concentration range of 6.86 to 102.38 mg/l could be degraded at a dilution rate of 0.16 h^{-1} and the removal percentage ranged between 84 and 100%.

It has been reported that 4-CP could be degraded and mineralized by aerobic bacteria (Puhakka & Melin, 1996) within a wide range of concentrations, ranging from 10 mg/l in a continuous activated sludge reactor (Ellis, Smets, Magbanua Jr, & Grady Jr, 1996) to 350 mg/l using a pure culture of *Arthrobacter chlorophenolicus* A6 (Elvang, Westerberg, Jernberg, & Jansson, 2001).

4-CP degradation was investigated by suspended and immobilized white rot fungus *Phanerochaete chrysosporium* in static and agitated cultures (Zouari, Labat, & Sayadi, 2002). The use of *P. chrysosporium* immobilized on polyurethane foam and polyethylene discs resulted in an efficient degradation of 4-CP in a rotating biological contactor (RBC). However, 4-CP can not be used as substrate by the fungus and an additional carbon source, glucose or glycerol was required for growth. At 300 mg/l of 4-CP, *P. chrysosporium* growth was totally inhibited.

Katayama-Hirayama, Tobita, & Hirayama (1994) studied the biodegradation of phenol and monochlorophenols by a yeast strain of *Rhodotorula glutinis*. 4-CP was well degraded and stoichiometric release of chloride ion was observed. Biodegradability of 4-CP was increased by the addition of phenol.

Bae, Lee, & Lee (1996) investigated the aerobic biodegradation of 4-CP by *Arthrobacter ureafaciens*, strain CPR706, in batch cultures and found that it exhibited much higher substrate tolerance and degradation rate than other strains. They concluded that it degrades 4-CP via new pathway, in which the chloro-substituent was eliminated in the first step and hydroquinone was produced as a transient intermediate. The maximum degradation rate was found to be 0.054 mM/h when the initial 4-CP concentration was between 0.9 and 1.6 mM. The degradation was completely inhibited when the initial concentration of 4-CP increased to 2 mM.

Kim et al. (2002) examined the aerobic biodegradation of phenol and chlorophenols in shake-flask and a packed-bed reactor (PBR). The degradation capacity of PBR was higher than that of the continuous stirred tank reactor. *Pseudomonas testosteroni* was able to degrade phenol and 4-CP simultaneously via meta-cleavage pathway but degradation rates of these compounds were affected by 4-CP.

The effect of varying phenol concentration on cometabolic transformation of 4-CP by *Pseudomonas putita* in the presence of a conventional carbon source, sodium glutamate, was investigated in batch cultures (Wang & Loh, 2000). When the sodium glutamate was provided as the sole growth substrate, both the extent and efficiency of 4-CP transformation were severely reduced compared with that when phenol was the sole growth substrate. However, although sodium glutamate was not an efficient sole growth substrate for cometabolic transformation of 4-CP, its presence attenuated the toxicity of 4-CP and consequently enhanced the transformation rate of 4-CP significantly when used together with phenol. In an other study carried out by Wang & Loh (1999), glucose was used instead of sodium glutamate as the growth substrate and only 78% and 43% of the initial 4-CP concentrations of 100 and 200 mg/l, respectively, were transformed before the pH dropped to below 4.5 and stopped all reactions. By maintaining the medium pH, complete removal of 4-CP was achieved even at the high initial concentration of 200 mg/l.

Bali & Sengul (2002) reported that total treatment efficiency of 5000 mg/l glucose, in terms of dissolved organic carbon (DOC), decreased from ⟩99% for a 4-CP free cycle to 66% for an initial 4-CP concentration of 300 mg/l in a fed-batch reactor. However as the concentration of glucose and the rate of feeding were decreased to 2000–3000 mg/l and 450 ml/h respectively, complete removal of 300 mg/l 4-CP with a low residual DOC was achieved. Phenol induction prior to inoculation was not a prerequisite to ensure transformation of 4-CP when glucose was the added growth substrate.

Effect of a biogenic substrate (peptone) concentration on the performance of sequencing batch reactor (SBR) treating 220 mg/l 4-CP and 110 mg/l 2,4-DCP mixtures was investigated by Sahinkaya & Dilek (2006). It was observed that decreasing peptone concentration associated with decreasing biomass concentration led to the observation of lower degradation rates, which caused accumulation of chlorophenols within the reactor. Accumulation of chlorophenols further decreased the removal rate due to self inhibitory effect of chlorophenols on their own degradation and strong competitive inhibition of 2,4-DCP on 4-CP degradation. Although peak chlorophenol concentrations within the reactor showed an increasing trend with decreasing peptone concentrations, complete removal of chlorophenols and associated intermediates along with high COD removals were observed even when chlorophenols were fed to the reactor as sole carbon sources.

CHAPTER THREE
BIOSURFACTANTS

3.1 Surfactants

Surfactants, which are amphipathic molecules with both hydrophilic (water-soluble) and hydrophobic (water-insoluble) functional groups, act at the surface, or interface, between polar and nonpolar phases to modify the surface properties of both phases due to presence of the hydrophobic group.

Surfactants can either be chemically synthesized (synthetic) or microbially produced (biosurfactant). Synthetic surfactants are of petrochemical origin whereas biosurfactants or biogenic surfactants are produced by bacteria, yeast, and fungi. Synthetic surfactants may be cationic, anionic, nonionic or amphoteric although only anionic and nonionic surfactants have been used as oil dispersants. Biosurfactants are produced mainly by aerobically growing microorganisms in aqueous media from a carbon source feedstock, e.g. carbohydrates, hydrocarbons, oils and fats or mixtures thereof (Bognolo, 1999).

3.2 Classification of Biosurfactants

Biosurfactants are usually classified based on their biochemical nature and the microbial species producing them. According to Zajic & Seffens (1984), biosurfactants may be classified into five groups:

1. Glycolipids, e.g. threalose, sophorose and rhamnose lipids and mannosylerithritol lipids. They are involved in the uptake of low polarity hydrocarbons by micro-organisms.

2. Liposaccharides, e.g. the high molecular weight, water soluble extracellular emulsifiers produced by hydrocarbon degrading bacteria like *Acinetobacter calcoaceticus* (emulsans).

3. Lipopeptides, e.g. ornithine lipids and the subtilysin produced by *Bacillus subtilis*, claimed to be the most effective biosurfactant reported to date.

4. Phospholipids: although they are present in every micro-organism, there are very few examples of extracellular production, the most notable one being the biosurfactants produced by *Corynebacterium lepus*.

5. Fatty acids and neutral lipids, e.g. ustilagic acid, the corynomycolic acids, the lipo-theichoic acids (sometimes classified as glyco-lipids) and the hydrophobic proteins.

3.3 Advantages of Biosurfactants

Almost all surfactants currently in use are chemically derived from petroleum; however, interest in microbial surfactants has been steadily increasing in recent years due to their diversity, environmentally acceptable nature, the possibility of their production through fermentation, and their potential applications in the environmental protection, crude oil recovery, health care and food-processing industries (Desai & Banat, 1997). Biosurfactants can be produced using relatively simple and inexpensive procedures (Kosaric, 1992; Lang & Wullbrandt, 1999).

Biosurfactants with surface active and emulsifying properties can exceed the performance of their surfactant synthetic equivalents in terms of efficiency. Potential environmental advantages of such biologically based surfactants include their biocompatability and hence decreased likelihood of cellular toxicity relative to synthetic surfactants. Other advantages of microbial surfactants compared with synthetic counterparts are as follows (Vardar-Sukan & Kosaric, 2000; Bognolo, 1999);

1. Biodegradability: Biosurfactants are biodegradable, which is positive ecological aspect. Because of this characteristic, biosurfactants can be readily and fully degraded if released to the environment after its function is completed.

2. Having low or no toxicity: Because biosurfactants are produced by living organisms on environmentally acceptable substrates (hydrocarbons and/or carbohydrates) they are non-toxic or less toxic than chemical surfactants.

3. Acceptable production economics: At present many types of biosurfactant are being utilized but they have been unable to compete economically with their chemically synthesized counterparts in the market, due to high production costs involved. However, this problem can be overcome by improving the efficiency of current bioprocessing methodology and strain productivity, and the use of cost-effective substrates such as using sterilized or pasteurized fermentation broth without any need for extraction, concentration or purification of the biosurfactant may significantly reduce the cost of production.

4. Biocompatibility: That many biosurfactants especially those produced by yeast such as sophorolipids are compatible with living tissues allow them to be used extensively in industrial application such as food processing, pharmaceuticals, and cosmetic industries.

5. Availability of raw material: Biosurfactants can be produced from cheap raw material, which are available in large quantities. The hydrophilic and hydrophobic moieties of biosurfactants are synthesized by two metabolic pathways: the hydrocarbon, carbohydrates and/or lipids. These pathways constitute carbon source and may be used separately or in combination with each other. Because industrial and municipal wastewaters contain organic pollutants, they can be utilized as substrate for the production of biosurfactants. With the use of wastewaters as organic matter source, a double benefit is expected: (a) The wastewaters utilized for the biosurfactant production is treated. (b) Valuable product is emerged.

6. Use in the environmental control: Due to their environmental friendly, composition biosurfactants are considered as a feasible approach to resolve certain environmental related problems caused by mankind. Some areas in which biosurfactants are effectively used are bioremediation of contaminated soil and

groundwater, biodegradation and detoxification of industrial effluents and control of oil spills.

7. Specificity: Different biosurfactants characterized so far exhibit a rich diversity of chemical structure. Having a wide range of functional characteristics, biosurfactants are often specific in their action. Due to this property, biosurfactants have gained particular interest in detoxification of organic or inorganic contaminants, deemulsification of industrial emulsions, and other specific food, cosmetic and pharmaceutical applications (Kosaric, 1992)

8. Extreme temperature, pH, and salinity tolerance: Compared with synthetic surfactant, biosurfactants show stable activity under extreme environmental conditions such as extreme temperature, pH and salinity values (Thangamani & Shreve, 1994).

3.4 Production of Biosurfactants

Biosurfactants are produced by microbial biosynthesis using organic matter, containing carbon and oil sources. Most of the biosurfactants are high molecular weight lipid complexes which are normally produced under highly aerobic conditions. The production of microbial biosurfactants can be achieved in their ex-situ production in aerated bioreactors. When their large-scale application is encountered, their in-situ production or action (production of biosurfactants in the application site directly) would be advantageous. Low oxygen availability in their in-situ production conditions requires maintenance of anaerobic microorganisms and aerobic biosynthesis of biosurfactants (Kosaric, 1992).

3.5 Application of Biosurfactants

Biosurfactants are amphiphilic compounds of microbial origin with considerable potential in commercial application with in various industries.

Biosurfactants have potential applications in agriculture, cosmetics, pharmaceuticals, detergents, personal care products, food processing, textile manufacturing, and laundry supplies. At present, biosurfactants are also used in studies on enhanced oil recovery and hydrocarbon bioremediation. The solubilization and emulsification of toxic chemicals by biosurfactants have also been reported. Appendix 1 summarizes the different applications of biosurfactants with respect to industrial sectors.

Several oil spill accidents, reaching petroleum the oceans and deliberate releases of soil have caused considerable contamination. Such accidents have increased attempts to advance various chemicals, procedures and techniques for resisting oil pollution both at sea and along the shoreline. Biosurfactants are such chemicals and applied to such contaminated area due to their ability to emulsify hydrocarbons in the environment by increasing the bioavailability of the compound. Some microorganisms such as *Pseudomonas aeruginosa* SB30 is capable of hydrocarbon degradation by quickly dispersing oil into fine droplets.

3.6 Potential Limitations of Biosurfactants Applications

Existing problem about biosurfactants are related with their application areas. For environmental applications, large amount of biosurfactants is required due to the bulk use. Therefore amount of biosurfactant used can be expensive. Using nontraditional and relatively cheap raw materials for the production of biosurfactants, such as waste organic substrate, the production costs might be decreased. Another problem about biosurfactant is their purity, which is particular importance in pharmaceutical, food and cosmetic applications (Kosaric, 1992). This problem seems to have very slight effects on the environmental applications, because biosurfactants are used as an enhancement tool in the contaminated soil and groundwater bioremediation or oil spill clean-up.

3.7 Mechanisms of Surfactant-Enhanced Biodegradation

Surfactants are capable of enhancing the apparent solubility of hydrophobic compounds in water. Surfactant molecules, above the critical micelle concentration (CMC), form aggregates in water that are called micelles. These aggregates have a hydrophobic core and a hydrophilic outer surface. Micelles are capable of dissolving HOC's in their hydrophobic cores resulting in an increased apparent aqueous solubility of the compound. Solubilization depends on the type and dose of the surfactant. Conceivably, in the absence of any inhibition, the enhanced solubility of hydrocarbons in the presence of surfactants should lead to an enhanced biodegradation if the contaminant in the micellar phase is directly bioavailable (Guha & Jaffe, 1996).

A possible way of enhancing the bioavailability of hydrophobic organic compounds is the application of (bio)surfactants, molecules which consist of a hydrophilic part and a hydrophobic part. Because of this property these molecules tend to concentrate at surfaces and interfaces and to decrease levels of surface tension and interfacial tension. The effect of surfactant on the bioavailability of organic compounds can be explained by three main mechanisms: (i) dispersion of nonaqueous-phase liquid hydrocarbons, leading to an increase in contact area, which is caused by a reduction in the interfacial tension between the aqueous phase and the nonaqueous phase; (ii) increased solubility of the pollutant, caused by the presence of micelles which may contain high concentrations of hydrophobic organic compounds, a mechanisms which has been studied extensively previously (Edwards, Liu, & Luthy, 1992; Edwards, Luthy, & Liu, 1991; Liu, Edwards, & Luthy, 1992); and (iii) "facilitated transport" of the pollutant from the solid phase to the aqueous phase, which can be caused by a number of phenomena, such as lowering of the surface tension of the pore water in soil particles, interaction of the surfactant with solid interfaces, and interaction of the pollutant with single surfactant molecules (Volkering, Breure, Andel, & Rulkens, 1995).

Consequently, there are many mechanisms that are effective for the enhancement of hydrocarbons biodegradation with the addition of biosurfactants. An important one is solubilization of hydrophobic compounds. In this thesis study, since 2,4-DCP and 4-CP are added below solubility level, solubilization effect is not present.

The presence of surfactants affects the biological process due to interactions between surfactant, organic compounds and microorganisms. Since biosurfactant structure is a characteristic of the producing species and the available carbon source during growth, biosurfactant structure may play different roles in hydrocarbon metabolism (Zhang and Miller, 1995). The three-way interaction among the biosurfactant, substrate, and microbial cells that is crucial to achieving enhanced biodegradation rates (Zhang and Miller, 1995). Biosurfactant molecules form aggregates in water called micelles. Biosurfactant seems to bind pollutants tightly in the micelle (Mata-Sandoval et al., 2000). Because structure of microorganisms is similar to biosurfactant structure, microorganisms cells are able to take up the pollutant from the micelle, ―to a certain extent― by fusion with the cell membrane (Miller and Bartha, 1989). This event could have implications for microbial uptake.

3.8 Literature Survey

Environmental applications of biosurfactant included enhancing solubilization and biodegradation, soil treatment (in situ and ex situ) and water and waste treatment. However, enhanced biodegradation studies of contaminants in liquid medium limited relative to in soil medium.

The halogenated aliphatic compounds, position, and number of halogens are important in determining the rate and mechanism of biodegradation. Some research has also focused on polychlorinated biphenyl biodegradation. The mineralization of PCBs was studied after the addition of rhamnolipid R1 (Robinson, Ghosh, & Shi, 1996). Using 4 g/l biosurfactant, 4,4′ chlorobiphenyl was mineralized by 213 times more than the control.

Pesticides are another group of contaminants that have been studied. Mata-Sandoval, Karns, & Torrents (2000) compared the ability of the rhamnolipid mixture to solubilize the pesticides, trifluralin, coumaphos and atrazine, with the synthetic surfactant Triton X-100. The synthetic surfactant was able to solubilize approximately twice as much of all pesticides as the rhamnolipid. The biosurfactant sems to bind trifluralin tightly in the micelle and releases the pesticide slowly to the aqueous phase, which could have implications for microbial uptake. This approach of utilizing micellar solubilization capacities and aqueous-micelle solubilization rate coefficients and micellar-aqueous transfer rate coefficients could be useful for future studies on microbial uptake. Addition of rhamnolipid in the presence of cadmium enabled biodegradation of the hydrocarbon naphthalene to occur as if no cadmium was present (Maslin & Maier, 2000).

Further work by Mata-Sandoval, Karns, & Torrents (2001) was performed on the biodegradation of the three pesticides in liquid cultures in the presence of rhamnolipid or Triton X-100. Trifluralin biodegradation was enhanced in the presence of both surfactants, while atrazine decreased. Coumaphos biodegradation increased at rhamnolipid concentrations above 3 mM but declined when Triton concentrations were above that of the CMC. In soil slurries, trifluralin degradation decreased as both surfactant concentrations increased. As the concentration of rhamnolipid increased, biodegradation rates of coumaphos decreased but removal increased. The concentration of rhamnolipid also decreased, indicating biodegradation of the rhamnolipid.

Surfactant mediated solubilization and simultaneous microbial degradation of phenanthrene in a completely mixed batch system has been studied by Jahan, Ahmed, & Maier (1999). The results also indicated that the most significant effect of surfactant addition was the increase in the dissolution rate of phenanthrene to the aqueous phase. The study showed that oxygen uptake, substrate concentration and cell mass versus time data can be utilized simultaneously to evaluate the relative rates of solubilization and biodegradation for substrates with low aqueous solubility.

Cort et al. (2002) investigated that pentachlorophenol (PCP) biodegradation, glucose degradation, and oxygen uptake during endogenous conditions and during glucose degradation were measured for batch systems in the presence of the nonionic surfactant Tergitol NP-10 (TNP10). TNP10 reduced the substrate inhibition effect of PCP at high PCP concentrations, resulting in faster PCP degradation rates at higher concentrations of TNP10. However, inhibitory effects of surfactants on the biodegradation process have frequently been reported (Rouse, Sabatini, Suflita, & Harwell, 1994). In other study, Cort & Bielefeldt (2000) studied that several potential mechanisms of surfactant-induced inhibition of PCP biodegradation were tested using a pure bacterial culture of *Sphingomonas chlorophenolicum* sp. Concentrations of the surfactant TNP10 over 200 mg/l inhibit biodegradation of PCP at concentrations below 100 mg/l. At PCP concentrations above 200 mg/l, TNP10 reduced the substrate inhibition effect of PCP, resulting in faster PCP degradation rates at higher concentrations of TNP10.

Triton X-100 and JBR 425 (a rhamnolipid biosurfactant) were then used to investigate the removal efficiency in soils contaminated with PCP by Mulligan & Eftekhari (2003). Triton X-100 showed better results in terms of final removal efficiency. Triton X-100 (1%) removed 85% and 84% of PCP from fine sand soil and sandy-silt, respectively, contaminated with 1000 mg/kg PCP. These values were 60% and 61% for JBR 425 (1%).

Zhang et al. (1998) studied to evaluate the potential effects of selected surfactants on the biodegradation of chlorinated hydrocarbons in the wastewater in an aerobic reactor. Results from this study showed that biodegradation of a real word waste containing a broad array of hazardous contaminants was significantly enhanced by the amendment of mineral nutrients and surfactants, especially a nonionic surfactant Witconol. The enhancement based on TOC reduction was 49% higher for the mixture of PPI wastewater with Witconol than the combined biodegradation of PPI wastewater and Witconol alone, whereas a similar enhancement was observed with ananionic surfactant sodium dodecylsulfate (SDS).

A summary of some of the studies involving the use of biosurfactants is shown in Table 3.1. It can be seen that most of the studies have involved rhamnolipids, and were done in the soil medium and specific culture.

Table 3.1 Summary of biodegradation studies involving biosurfactants (Mulligan, 2005)

Biosurfactant	**Medium**	**Microorganism**	**Contaminant**
Rhamnolipid	Soil slurry	*P. aeruginosa* UG2	Hexachlorobiphenyl
Rhamnolipid	Soil	*P. aeruginosa* UG2	Aliphatic and aromatic hydrocarbons
Rhamnolipid	Soil slurry	*P. aeruginosa* UG2	Phenanthrene
Rhamnolipid	Soil	*P. aeruginosa* UG2	Phenanthrene and hexadecane
Rhamnolipid	Soil	*P. aeruginosa* #64	Phenanthrene, fluoranthrene, pyrene, pentachlorophenol benzo[*a*]prene
Rhamnolipid	Soil	*P. aeruginosa* ATCC 9027	Napthalene and phenanthrene
Rhamnolipid	Soil	*P. aeruginosa* ATCC 9027	Octadecane
Rhamnolipid	Soil	*P. aeruginosa* ATCC 9027	Phenanthrene and cadmium
Mono-rhamnolipid	Soil	*P. aeruginosa* ATCC 9027	Napthalene and cadmium
Di-rhamnolipid	Liquid	*Pseudomonas*	Toluene, ethyl benzene, butyl benzene
Sophorolipid	Liquid	Mixed culture	$^{14\text{-}16}$C alkanes, pristane, phenyldecane, naphthalene
Sophorolipid	Soil	*C. bombicola* ATCC 2214	Phenanthrene
Crude surfactin	Soil	*B. subtilis* ATCC 2423	Hexadecane and kerosene
Crude surfactin	Soil	*B. subtilis* ATCC 2423	Endosulfan
Crude surfactin	Seawater	*B. subtilis* 09	Aliphatic and aromatic hydrocarbons
Alasan	Liquid	*Acinetobacter radioresistens* KA 53	Phenanthrene, fluoranthene and pyrene

All these studies showed a positive effect of the biosurfactant on biodegradation.

It has been well established that surfactant can enhance the solubility of HOC's in contaminated soil. But it is not yet clear how structure affects biodegradation rates in wastewaters. The current literature contains little information on the mechanism of surfactant-aided biodegradation of chlorophenols in aerobic reactors with mixed culture.

CHAPTER FOUR
MATERIALS AND METHODS

4.1 Experimental Setup

Schematic diagram of the experimental setup is depicted Figure 4.1. An activated sludge bioreactor made up stainless steel was used during the study. The activated sludge bioreactor consisted of aeration and settling tanks. Volume of aerobic reactor was 8.75 l and volume of settling unit was 1.15 l. The influent was continuously fed through the top of reactor by a feed pump. Aerobic reactor was aerated by an air pump. Aeration and sedimentation tanks were separated by an inclined plate. Effluent wastewater passage from the aeration tank to sedimentation tank was through the holes on the inclined plate. The effluent of sedimentation tank was collected in an effluent tank and it was regularly discharged. The sludge age was adjusted by discarding certain volume of activated sludge from aeration step of the aerobic reactor every day.

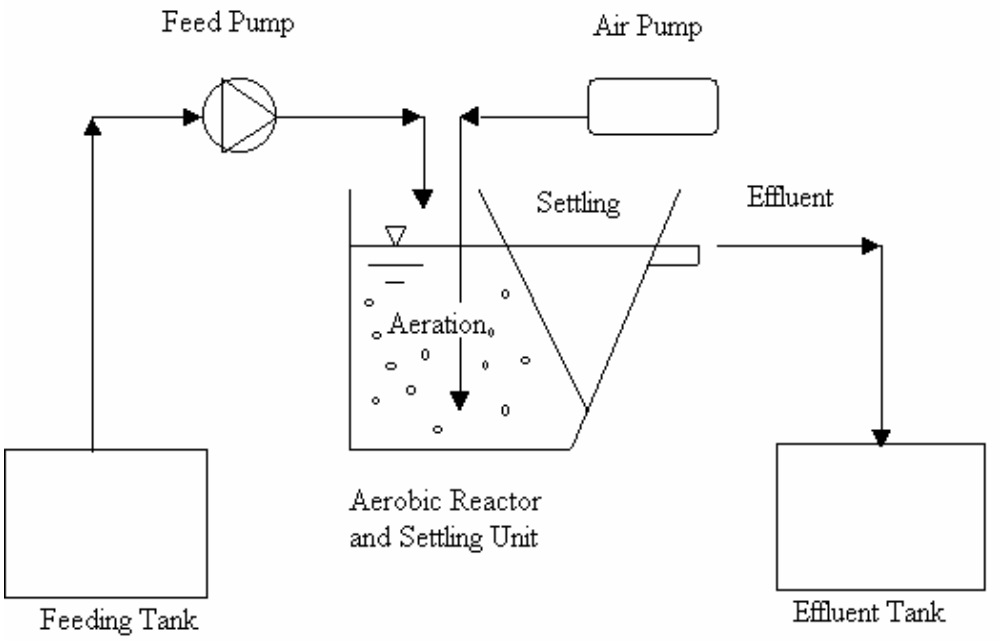

Figure 4.1 A schematic diagram of the activated sludge bioreactor used in experimental studies

4.2 Organisms and Wastewater Composition

Mixed culture was used in aerobic reactors. Activated sludge culture was obtained from the wastewater treatment plant of Pak Maya Bakers Yeast Company in İzmir, Turkey. The aerobic reactors were inoculated with this culture.

The synthetic wastewater used throughout the studies was composed of glucose as carbon source, urea as nitrogen source, KH_2PO_4 as phosphorus source, $MgSO_4.7H_2O$ (75 mg/l), $CaCl_2$ (50 mg/l), $FeCl_3$ (2 mg/l) and various concentrations of chlorophenol as either 2,4-DCP or 4-CP (1 mg 2,4-DCP=1.2 mg COD, 1 mg 4-CP=1.68 mg COD). The concentrations of nitrogen and phosphorus were adjusted to maintain COD/N/P=100/10/2 in all the experiments.

COD concentration was kept constant at the beginning of experimental study, by adjusting glucose concentration depending on the other additions. When biosurfactant was added to the tests reactors at critical micelle concentration (15

mg/l) or 2CMC (30 mg/l), it means that organic matter was also added since COD value of biosurfactant was determined as 50 mg/l COD (for CMC) and 100 mg/l COD (for 2CMC). Consequently, adding glucose value to synthetic wastewater was determined by both chlorophenol and biosurfactant amounts.

2,4-DCP and 4-CP were dissolved in hot water at 50 ºC to prepare stock solution and added directly to the synthetic wastewater from stock solution to give the desired initial concentration. The general properties and safety data of the toxic substances used, (2,4-DCP and 4-CP), are given in Table 4.1 and Table 4.2.

In order to determine the effect of 2,4-DCP loading rates on 2,4-DCP and COD removal efficiency in the control and test reactors, 2,4-DCP loading rate was changed by adjusting the 2,4-DCP concentration. 2,4-DCP concentration was increased stepwise from 5 to 150 mg/l. Influent COD concentration was kept constant at 500 mg/l.

For the experiments performed to investigate the effects of the sludge age on 2,4-DCP and COD removal efficiency in the control and test reactors, typical composition of the synthetic wastewater was COD_0=500 mg/l, 2,4-DCP_0=250 mg/l. The concentration of 2,4-DCP was selected as 250 mg/l since toxic effect on microbial community was observed above this value.

In order to determine the effect of 4-CP loading rates on 4-CP and COD removal efficiency in the control and test reactors, 4-CP loading rate was changed by adjusting the 4-CP concentration. 4-CP concentration stepwise increased from 5 to 450 mg/l. The COD concentration was kept as 500 mg/l when 4-CP concentrations were increased up to 250 mg/l. When 4-CP concentrations were applied between 350-450 mg/l, COD concentration was kept as 850 mg/l.

In order to determine the effect of sludge age on 4-CP and COD removal performance in R1 (control reactor), R2 (CMC) and R3 (2CMC), continuous activated sludge experiments were performed at different sludge ages between 3 and

25 days. The feed COD and influent 4-CP concentration were constant throughout the experiments as COD_0=1500 mg/l and 4-CP_0=250 mg/l.

In order to determine cometabolic degradation of 4-CP using unacclimated activated sludge in R1 (control reactor), R2 (CMC) and R3 (2CMC), two experimental sets were performed. 4-CP concentration was constant at 150 mg/l and 300 mg/l, in the first and second set. The feed COD concentration was constant throughout the two sets experiments as COD_0=1500 mg/l.

Table 4.1 General properties of 2,4-Dichlorophenol (http://chemfinder.cambridgesoft.com/result.asp; Czaplicka, 2004)

Synonyms	2,4-Dichlorophenol; 2,4-DCP; 4,6-Dichlorophenol; Dichlorophenol; DCP
Molecular formula	$C_6H_4Cl_2O$
Molecular weight (g/mol)	163.00
Appearance	Colourless crystals; white solid
Melting Point (°C)	45
Boiling Point (°C)	210
Flash Point (°C)	113
Density (g/m3)	1.383
Water Solubility (20 °C)	Slightly, 0.45 g/100 ml

Table 4.2 General properties of 4-Chlorophenol (http://chemfinder.cambridgesoft.com/result.asp; Czaplicka, 2004)

Synonyms	4-Chlorophenol; 4-CP p-chlorophenol 4-hydroxychlorobenzene
Molecular Formula	C_6H_5ClO
Molecular Weight (g/mol)	128.56
Appearance	White to straw colored crystals. Hydroscopic.
Melting Point (^oC)	42-44
Boiling Point (^oC)	217-219
Flash Point (^oC)	115
Density (g/m3)	1.306
Solubility in water g/l at 20 ^oC	27

4.3 Biosurfactant

The rhamnolipid (designated JBR 425) was kindly donated by Jeneil Biosurfactant Company, Saukville, WI, USA as a mixture of R1 and R2. R1 has the chemical formula $C_{26}H_{48}O_9$, and R2, $C_{32}H_{58}O_{13}$. This product was named as JBR 425, which is an aqueous solution of rhamnolipids at 25% concentration. Critical micelle concentration (CMC) of JBR 425 is 15 mg/l. Chemically, rhamnolipids are glycosides of rhamnose (6-deoxymannose) and β-hydroxydecanoic acid. Other properties of JBR 425 are given in Appendix 2.

4.4 Experimental Procedure

Experiments were started batchwise. Activated sludge from an industrial wastewater treatment plant containing no toxic substances was added to reactor as seed source. The synthetic wastewater with a glucose and nutrients was inoculated with a mixture of activated sludge. The media was aerated vigorously for several days until a dense culture was obtained. Continuous operation was realized by

pumping the feed wastewater to the aeration tank by a feed pump with a known flow rate. Experimental procedure was summarized in Table 4.3.

Table 4.3 Summarizing of experimental procedure

Run	**Chlorophenol**	**Operational Conditions**		
		Chlorophenol Concentration (mg/l)	**COD Concentration (mg/l)**	**SRT (day)**
Run 1	2,4-DCP	ranged between 5-150	500	20
Run 2	2,4-DCP	constant 250	500	between 3-25
Run 3	4-CP	ranged between 5-450	500 and 850	10
Run 4	4-CP	constant 250	1500	between 3-25
Run 5	4-CP	150 and 300	1500	15

In the first stage of experiments, effect of biosurfactant on 2,4-DCP biodegradation in an activated sludge reactor was investigated with variation of 2,4-DCP loading rates and sludge retention time (SRT). This study was conducted through parallel experiments. Two reactors with the same structure and volume as the described above were used and activated sludge from an industrial wastewater treatment plant (Pakmaya Yeast Industry) were added to reactors as seed source. The reactor was initially fed with glucose as the carbon source in order to determine its performance in the absence of 2,4-DCP and then, gradually acclimatized to 2,4-DCP. In order to effect 2,4-DCP loading rate, 2,4-DCP concentration stepwise increased from 5 to 150 mg/l. In the control reactor, feed water did not contain any biosurfactant in order to determine whether the presence of biosurfactant influences the removal of 2,4-DCP. Feed water of the parallel reactor (test reactor) contained both 2,4-DCP and biosurfactant. When biosurfactant is added to the test reactor at

CMC (15 mg/l), 50 mg/l COD is also added. Therefore in adjusting COD concentration to 500 mg/l in the reactor, the contribution of biosurfactant was also considered. Hydraulic retention time (HRT) was kept constant at 17 h through 54 days of operation period. SRT was controlled at about 20 days through removing a certain volume of mixed liquor from the aeration zone of the activated sludge bioreactor.

In order to investigate the effect of sludge age on 2,4-DCP and COD removal efficiency in the control and test reactors, continuous activated sludge experiments were performed at different sludge ages between 3 and 25 days while hydraulic residence time was kept constant throughout the experiments at θ_H=17 h. The concentration of 2,4-DCP was selected as 250 mg/l since toxic effect on microbial community was observed above this value.

In the second stage of experiments, effect of biosurfactant on 4-CP biodegradation in activated sludge reactor was investigated with variation of 4-CP loading rates and sludge ages. Experiments were started batchwise. The synthetic wastewater containing only glucose as the sole carbon source was inoculated with a mixture of activated sludge. The media was aerated vigorously for several days until a dense culture was obtained. The reactor was initially fed with glucose as the carbon source in order to determine its performance in the absence of 4-CP and then, gradually acclimatized to 4-CP. In order to effect 4-CP loading rate, 4-CP concentration stepwise increased from 5 to 450 mg/l. HRT was kept constant at 17 h through 75 days of operation period. SRT was controlled at about 10 days.

To determine the effect of sludge age on 4-CP and COD removal, three reactors were used in parallel tests. Microorganisms were reactivated due to the inhibition at the first step of the study. In the control reactor (R1), feed water did not contain biosurfactant in order to determine the effect of biosurfactant on the removal of 4-CP. Feed water of the parallel reactors (test reactors; R2 and R3) contained both 4-CP and biosurfactant. 15 mg/l (CMC) and 30 mg/l (2CMC) biosurfactant concentration were added to R1 and R2. Continuous activated sludge experiments

were performed at different sludge ages between 3 and 25 days while hydraulic residence time was kept constant throughout the experiments at θ_H=17 h.

In the third stage of experiments, the effect of 4-CP load on unacclimated activated sludge in the control (R1) and test reactors (R2 and R3) were studied. To investigate the capacity of an unacclimated activated sludge reactor to cope with 4-CP loading, three reactors were used in parallel tests. Firstly, the three reactors were inoculated with the activated sludge. HRT was kept constant at 17 h through operation period. SRT was controlled at about 15 days. A synthetic wastewater based on glucose (1500 mg/l COD) was fed to the three reactors continuously until a stable COD removal was obtained. After stable COD removal was obtained, 150 mg/l 4-CP loading period was started. During 4-CP loading periods, influent COD concentration was kept constant at 1500 mg/l. At the end of the 150 mg/l 4-CP loading, study same experiments was conducted at 300 mg/l 4-CP.

In all the experiments, temperature and pH were kept at T= 20 ± 2 °C and pH=7 ± 0.6. Dissolved oxygen (DO) concentration was kept around 3 mg/l in the reactors. Sludge was removed from the aeration tank everyday to adjust the sludge age to the desired value.

Every experiment was conducted until the systems reached the steady-state condition, yielding the same COD and chlorophenol contents in the effluent for the last 3 days. The samples collected from the feed and effluent wastewater at steady-state were analyzed for COD and chlorophenol contents after centrifugation.

4.5 Analytical Methods

In preparing water samples for analysis, samples were centrifuged at 6000 rpm for 25 minutes to remove biomass and other solids from the liquid medium. Clear supernatant were analyzed for COD (chemical oxygen demand) and, 2,4-DCP and 4-CP. Standard methods based on digestion and reflux was used for COD analyses. The 2,4-DCP and 4-CP analyses were carried out on clear supernatant using 4-

aminoantipyrine colorimetric method based on the procedure detailed in Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1992). Biomass concentrations in the liquid phase were determined by filtering samples from 0.45 μm pore size membran filters and drying the filter paper in an oven at 103 °C until constant weight. DO and pH measurements were carried out by using the DO and pH meter probes and WTW MultiLine P3 pH/OXI-SET Analyser. The dissolved oxygen probe contains a temperature probe which was used for measuring the temperature in the aerobic tank.

All the experiments and measurements were done in duplicate and arithmetic averages were taken throughout the analysis.

CHAPTER FIVE
RESULTS AND DISCUSSION

Since the aim of the study was to enhance the biodegradation rate of persistent chemicals in AS process, to optimize the working conditions and to determine the limitations, many experimental studies have been conducted and the following evaluations were obtained.

5.1 Effect of Increasing 2,4-DCP Loading Rates on the Reactors Performance

Two reactors were used in parallel tests. In the control reactor, feed water did not contain biosurfactants in order to determine whether the presence of biosurfactants influences the removal of 2,4-DCP. Feed water of the parallel reactor (control reactor) contained both 2,4-DCP and biosurfactants.

5.1.1 COD Removal Efficiency through Start-Up Period in the Control and Test Reactor

Influent concentration of COD in the reactors was 500 mg/L. The aerobic reactor was inoculated and first fed only with glucose as the sole carbon source in order to activate the seed sludge and to determine the performance of the reactor in the absence of 2,4-DCP.

The start-up period for both control reactor and test reactor was 12 days and the influent glucose COD concentration was kept constant at 500 mg/l. When the control reactor continuously started to feed with synthetic wastewater which had a COD concentration of 500 mg/l, the effluent COD concentration was around 275 mg/l resulting in 45% COD removal efficiency. At the end of the start-up period about 91% COD removal efficiency was achieved. As can be seen in Figure 5.1, The COD concentration in the effluent decreased to about 45 mg/l after 12 days of start-up period. When the test reactor was continuously fed with synthetic wastewater which contained 500 mg/l COD concentration, the effluent COD concentration was around

325 mg/l resulting a COD removal efficiency of 35%. Through the end of the start-up period about 89% COD removal efficiency was achieved. As can be seen in Figure 5.2, the COD concentration in the effluent decreased to about 55 mg/l after 12 days of start-up period.

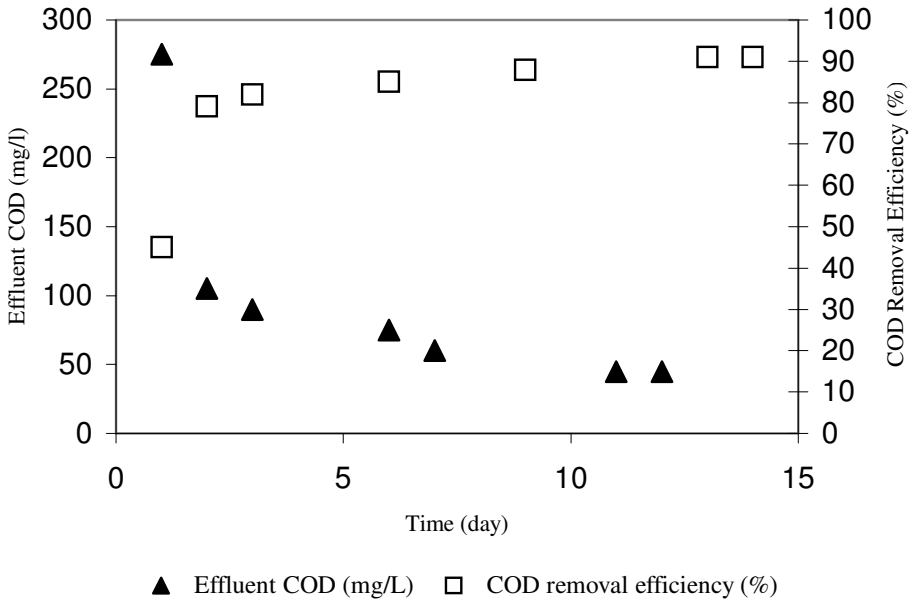

Figure 5.1 Variation of COD removal through start-up period in the control reactor

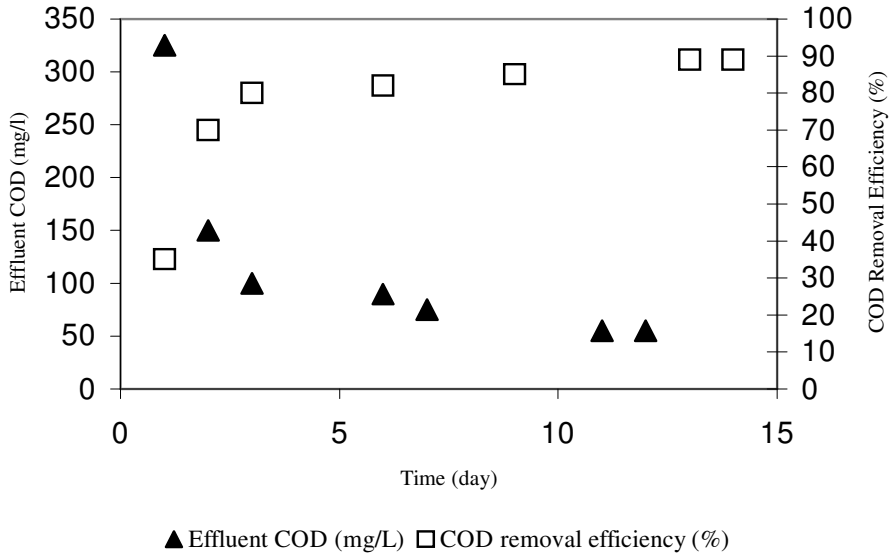

Figure 5.2 Variation of COD removal through start-up period in the test reactor

5.1.2 2,4-DCP Acclimation Period in the Control and Test Reactor

After the determination of reactor performance in the absence of 2,4-DCP and then, gradually acclimatized to 2,4-DCP. When both the control reactor and test reactor were started to operate with 5 mg/l of 2,4-DCP concentration, the 2,4-DCP removal efficiencies and the COD removal efficiencies were illustrated in Figure 5.3 and Figure 5.4. Through the end of the 2,4-DCP acclimated period about 98% 2,4-DCP removal efficiency and 92% COD removal efficiency were achieved in the reactors.

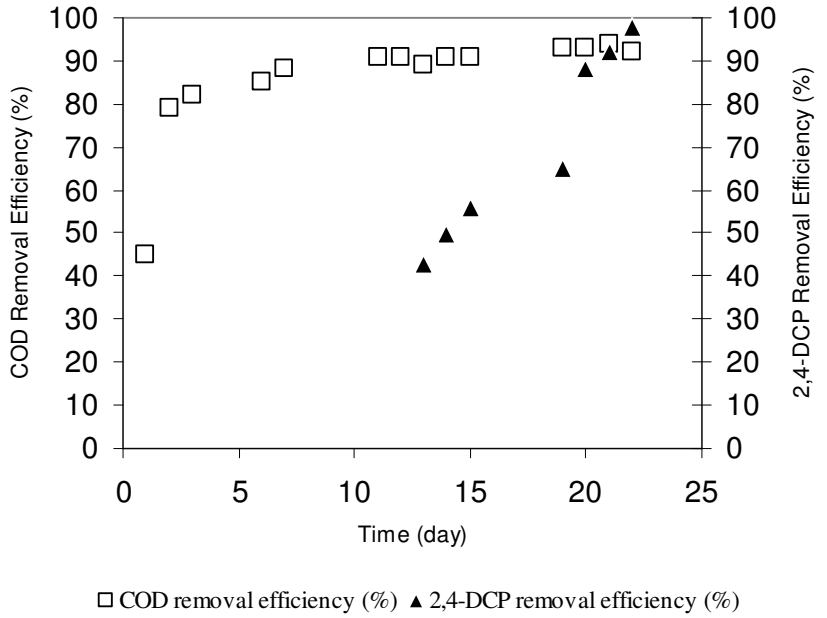

Figure 5.3 Variation of COD and 2,4-DCP removal efficiency in the control reactor during the 2,4-DCP acclimation period

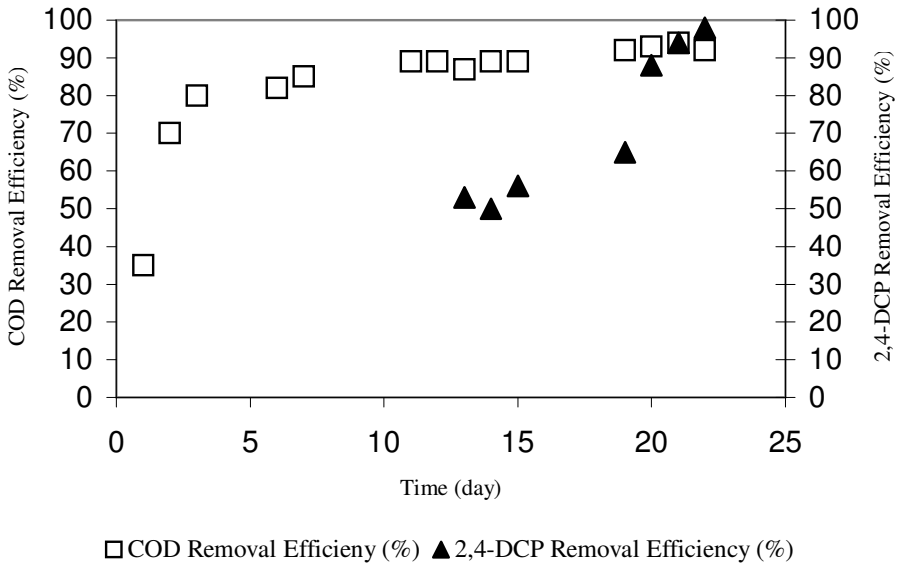

Figure 5.4 Variation of COD and 2,4-DCP removal efficiency in the test reactor during the 2,4-DCP acclimation period

5.1.3 Comparison of 2,4-DCP Removal Efficiency in the Control and Test Reactor

The reactors were operated with only glucose for 12 days of operation periods. After stable COD removal conditions were obtained, 5 mg/l 2,4-DCP was added as starting concentration. At the end of the 2,4-DCP acclimation period, about 98% 2,4-DCP removal efficiency and 92% COD removal efficiency were achieved in the reactors. After acclimation period, the reactors were operated at a range of 30-150 mg/l 2,4-DCP concentrations. At this range, the test reactor was operated with the addition of biosurfactant at critical micelle concentration.

Figure 5.5 illustrates comparison of the 2,4-DCP removal efficiencies in the control and test reactors. When the 2,4-DCP concentrations were increased from 5 to 30 mg/l between the days 23-32, 2,4-DCP removal efficiency varied between 88-97.7% and 90-99.8% in the control and test reactor, respectively. 2,4-DCP removal efficiency in control reactor varied between 90-97.5%, it was 94-99.7% in the test reactor, when the 2,4-DCP concentrations were increased from 30 to 50 mg/l between the days 33-39. When the influent concentration of 2,4-DCP was increased from 50 to 100 mg/l between the days 40-46, 2,4-DCP removal efficiency for control and test reactor ranged between 90.7-97.4% and 97.4-99.8%, respectively. The removal efficiency of 2,4-DCP was always in the range of 99.7-99.8% even when the influent concentration of 2,4-DCP ranged between 30-100 mg/l in the test reactor, in stable conditions.

Removal efficiency of 2,4-DCP decreased up to 24.2% in the control reactor and 32.9% in the test reactor when 2,4-DCP concentration was increased from 100 to 150 mg/l between the days 47-54.

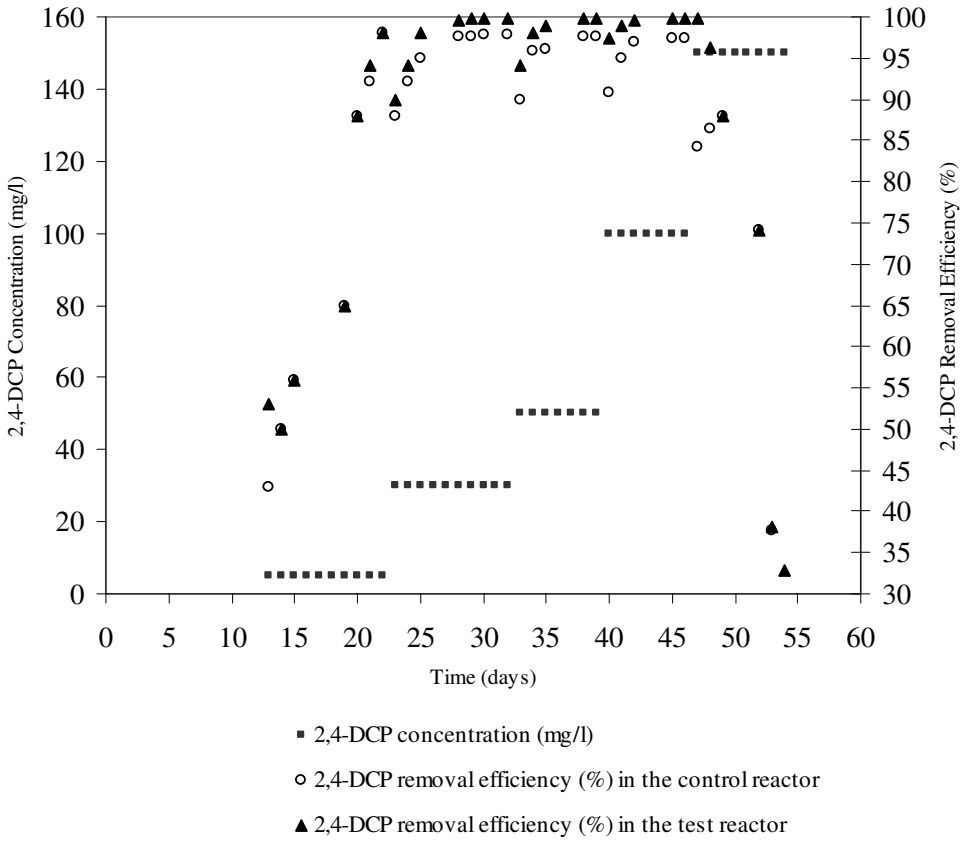

Figure 5.5 Changes of 2,4-DCP concentration and 2,4-DCP removal efficiencies through operation periods

Increase of feed 2,4-DCP concentration resulted in its elevation in the effluent, especially in the control reactor, as given in Table 5.1. During steady state conditions, effluent concentration of 2,4-DCP in the control reactor increased from 0.1 to 2.6 mg/l when the feed concentration was increased from 30 to 100 mg/l. In the test reactor, effluent concentration increased slowly as compared to the control reactor; from 0.1 to 0.2 mg/l for 30-100 mg/l feed 2,4-DCP concentration. Effluent concentration of 2,4-DCP increased up to 113.7 mg/l in the control reactor, 100.65 mg/l in the test reactor when concentration of 2,4-DCP was increased from 100 to 150 mg/l. The large amount of drop in the removal efficiency indicates the inhibitory of effect of 2,4-DCP at elevated concentrations.

Table 5.1 Removal efficiencies and effluent concentrations of 2,4-DCP and COD in the control and test reactor, after reaching stabilized condition

System	Influent Concentration (mg/l)		Effluent Concentration (mg/l)		Removal Efficiency (%)	
	COD	2,4-DCP	COD	2,4-DCP	COD	2,4-DCP
Control Reactor	500	0	45.0	0	91.0	0
	500	5	40.0	0.1	92.0	98.0
	500	30	82.5	0.69	83.5	97.7
	500	50	108.5	1.25	78.3	97.5
	500	100	119.5	2.6	76.1	97.4
	500	150	250.0	113.7	50.0	24.2
Test Reactor	500	0	55.0	0	89.0	0
	500	5	40.0	0.1	92.0	98.0
	500	30	71.0	0.06	85.8	99.8
	500	50	95.0	0.15	81.0	99.7
	500	100	98.5	0.2	80.3	99.8
	500	150	223.5	100.65	55.3	32.9

5.1.4 Comparison of Effect of Influent 2,4-DCP Concentration on the COD Removal Efficiencies in the Control and Test reactor

2,4-DCP concentration was increased from 5 to 150 mg/l in the reactors, during two months operation period. Figure 5.6 illustrates the COD removal efficiencies versus influent 2,4-DCP concentration increment. When the 2,4-DCP concentration was increased in time, the COD removal efficiencies decreased in both control and test reactors. But the efficiency decrease was more pronounced in control reactor as compared to test reactor which contains biosurfactant.

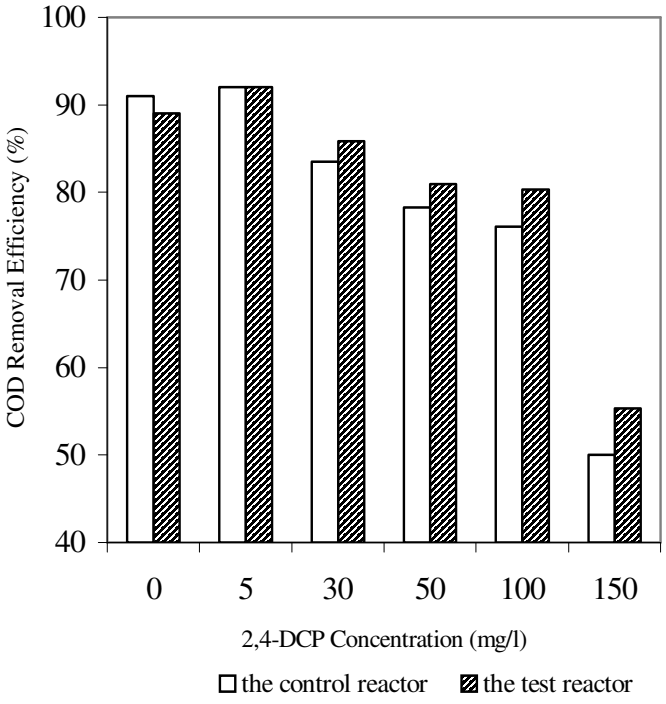

Figure 5.6 Comparison of variation of COD removal efficiencies versus influent 2,4-DCP concentrations

In the control reactor, when the 2,4-DCP concentration was increased from 5 to 150 mg/l, the COD removal efficiency decreased from 92 to 50% resulting in COD concentrations of 40-250 mg/l in effluent samples. In the test reactor, when the concentration was increased from 5 to 150 mg/l, the COD removal efficiency decreased from 92 to 55.3% resulting in COD concentrations of 40-223.5 mg/l in effluent samples (see Figure 5.7). The test reactor system could give better removal efficiency owing to the addition of biosurfactant.

As a result it is possible to evaluate that addition of biosurfactant at CMC has caused an increase in COD and 2,4-DCP removal efficiency.

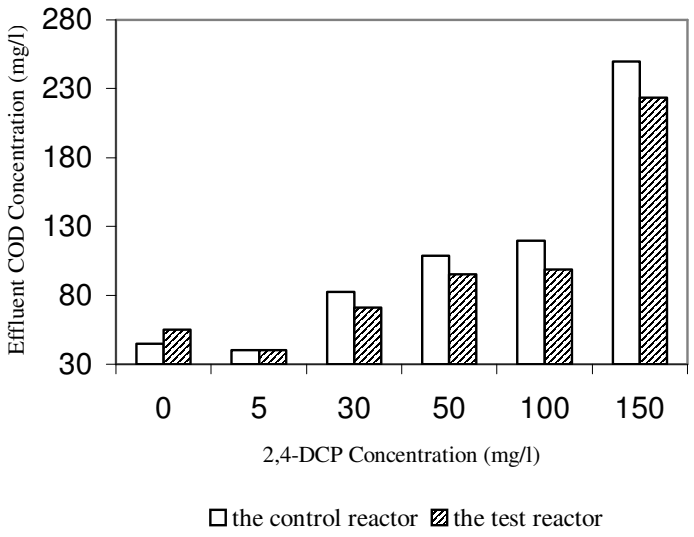

Figure 5.7 Comparison of variation of effluent COD concentrations versus influent 2,4-DCP concentrations

5.1.5 Comparison of Relationship between MLSS Concentration and Influent 2,4-DCP Concentration

Variation in MLSS concentration versus influent 2,4-DCP concentration is depicted in Figure 5.8. In the control reactor, biomass inhibition of 2,4-DCP started above 50 mg/l 2,4-DCP concentration. No inhibition effect occurred during this phase in the test reactor. The drop in MLSS concentration at the beginning of the experimental stage at low 2,4-DCP concentration may be explained by not enough time for microorganisms to acclimatize.

Applied biosurfactant gave no inhibitory effect at critical micelle concentration. This result imply that biosurfactant at the test concentration is not toxic to microorganisms. In addition to that, experimental results showed that biosurfactant could serve as a readily available carbon source, thus increasing the biomass in the medium.

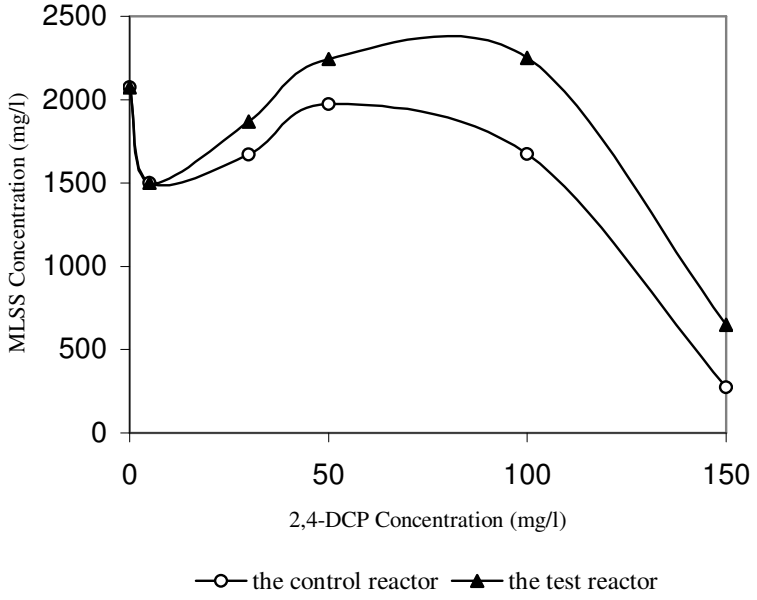

Figure 5.8 Comparison of variation of MLSS concentration by the influent 2,4-DCP concentration

The biosurfactant used in the study has been tested by the producing company for the biodegradability. Test was accomplished in accordance with OECD (Organization for Economic Cooperation and Development) 301D for ready biodegradability. According to OECD 301D "Ready Biodegradability" test, the biosurfactant showed ready biodegradability by demonstrating a biodegradability rate of 68.4% on day 10 of the 28-day test cycle. This is an excellent test result clearly demonstrating that JBR 425 is readily biodegradable (Jeneil Biosurfactant Co., LLC, 2001).

In order to evaluate the biodegradability of biosurfactant, it was used as the sole organic carbon source in the batch reactor experiment. When activated sludge was added to 300 mg/l biosurfactant solution, 80% removal was obtained after 52 hours (see Figure 5.9).

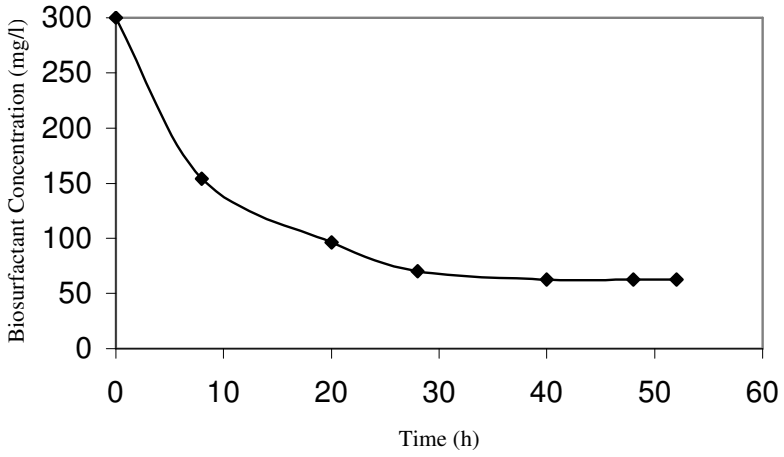

Figure 5.9 Biosurfactant biodegradation by activated sludge

5.2 Effect of Sludge Retention Time (SRT) on the Reactors Performance for 2,4-DCP Treatability

A set of experiments were performed at different sludge ages varying between 3 and 25 days while the feed COD, 2,4-DCP contents and the HRT values were kept constant throughout the experiments at COD_0=500 mg/l, 2,4-DCP_0=250 mg/l and θ_H=17 h, respectively. The concentration of 2,4-DCP was selected as 250 mg/l since toxic effect on microbial community was observed above this value.

5.2.1 Effect of SRT on 2,4-DCP Removal Efficiency

The results of the experimental study are depicted in Figure 5.10. For sludge ages between 10-25 days, 2,4-DCP removal efficiency remained above 99% in the test reactor and it was between 96.6-98% in the control reactor. 2,4-DCP removal efficiency decreased sharply from 96.6 to 88% when sludge retention time was decreased from 10 to 5 days in the control reactor. However, in the test reactor, removal efficiency of 2,4-DCP was always 99.6% even when the sludge age was as short as 5 day. Removal efficiency of 2,4-DCP decreased to 33.3% and 37.1% in the control and test reactors, respectively, when the sludge age was decreased from 5 to

3 days. The drastic decrease in 2,4-DCP removal efficiencies in the reactors could be attributed to toxic effect of 2,4-DCP on microorganisms in 3 days sludge age since the adaptation time is quite insufficient at this SRT value.

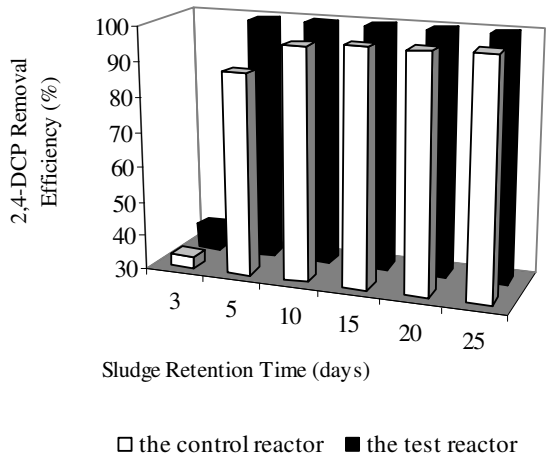

Figure 5.10 Comparison of removal efficiency of 2,4-DCP in the control and test reactor at different SRT values

Figure 5.11 shows the variation in effluent 2,4-DCP concentrations at different SRT values. Although effluent concentrations of 2,4-DCP in the test reactor ranged from 0.125 to 1 mg/l between the sludge ages 5-25 days, they ranged from 5 to 30 mg/l in the control reactor. In this range of sludge ages, effluent 2,4-DCP concentration in the control reactor was higher than that of the test reactor. Effluent concentration of 2,4-DCP increased to 166.75 and 157.25 mg/l in the control and test reactor, respectively, when the sludge age was decreased from 5 to 3 days. From these results, it can be said that addition of biosurfactant to the reactor enhances the 2,4-DCP biodegradation.

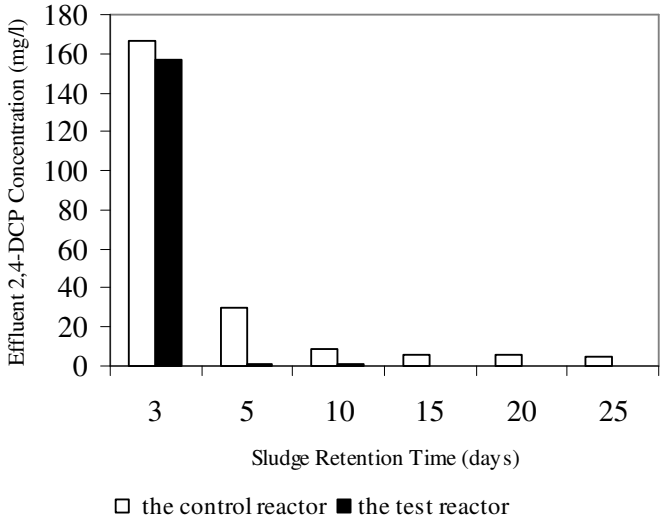

Figure 5.11 Comparison of effluent 2,4-DCP concentrations at different SRT values

Short sludge ages resulted in lower 2,4-DCP removal rates as shown in Figure 5.12, especially in the control reactor. When the sludge age was varied between 5-25 days, removal rate of 2,4-DCP in the test reactor was higher than the control reactor and approximately stable (over 351 mg/l.day) throughout the experiment. Shorter sludge ages, between 3 to 10 days, resulted in lower 2,4-DCP removal rate in the control reactor relative to the test reactor due to lower concentration of microorganisms in the control reactor. Increasing the sludge age above 10 did not give any noticeable increase in 2,4-DCP removal efficiency. 2,4-DCP removal rate was high in both of the reactors at higher sludge ages due to increase in mean cell residence time (sludge age). Increasing mean cell residence time provided enough adaptation period for microorganisms. At lower sludge ages (⟨15 days), 2,4-DCP removal rate decreased more in the control reactor than in the test reactor. Due to lower adaptation periods in lower sludge ages, 2,4-DCP toxicity affects microorganisms in the control reactor more than test reactor which contains biosurfactant. Biosurfactant existence has caused a decrease in 2,4-DCP toxicity on the microorganisms. As can be seen in Figure 5.12, standard deviation values were very small.

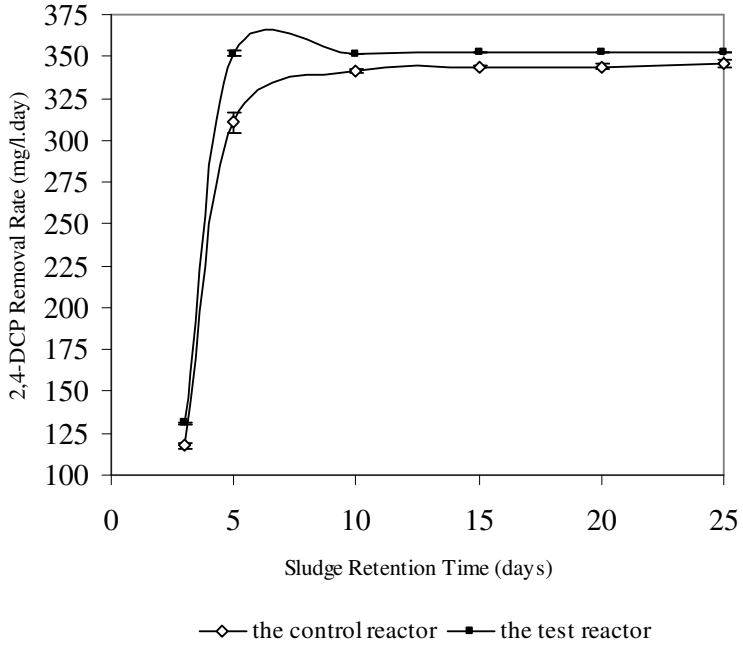

Figure 5.12 Comparison of removal rate of 2,4-DCP at different SRT values

As seen Figure 5.13, short SRT resulted in high specific biodegradation rates. The higher specific 2,4-DCP removal rates ($R_{2,4\text{-}DCP}=[(DCP_o-DCP_e)/(\theta_H \times X)]$ in short SRT conditions, between 3-5 days, were due to lower concentrations of microorganisms in the control reactor. The 2,4-DCP specific removal rates in the reactors was lower at the 3 days sludge age than 5 days because 2,4-DCP removal efficiency decreased to the lowest value at the 3 days due to toxic effects of high 2,4-DCP content. Since 2,4-DCP removal efficiency is lower in the reactors at 3 days sludge age, specific removal rates are also lower at 3 days sludge age than 5 days despite lower biomass concentrations at 3 days sludge age. In contrast, the low specific 2,4-DCP removal rates at longer SRT, above 10 days, were due to high biomass concentrations. The reactors with longer SRT had more biomass, and consequently lower specific removal rates. Specific removal rates of 2,4-DCP in the test reactor were lower relative to the control reactor as biomass concentrations were higher than the control reactor for sludge ages between 3-25 days (see Figure 5.14). This result implies that biosurfactant could serve as a readily available carbon source, causing an increase in the biomass amount in the medium. In all the experiments

biomass concentrations were higher in biosurfactant added reactor. At higher sludge ages (〉15 days), biomass was less affected from 2,4-DCP toxicity. Therefore, biomass adaptation periods were longer in both of the reactors at higher sludge ages. 2,4-DCP affected microorganisms especially at the lower sludge ages because of decrease in mean cell residence time (lower sludge age). Decrease in mean cell residence time resulted in toxic effect of 2,4-DCP on microorganisms in the control reactor due to insufficient adaptation periods.

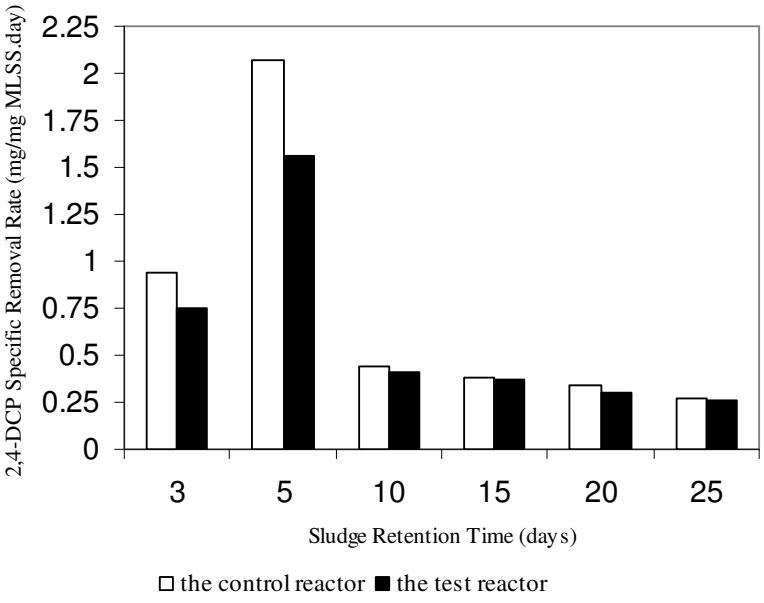

Figure 5.13 Comparison of specific removal rate of 2,4-DCP at different SRTvalues

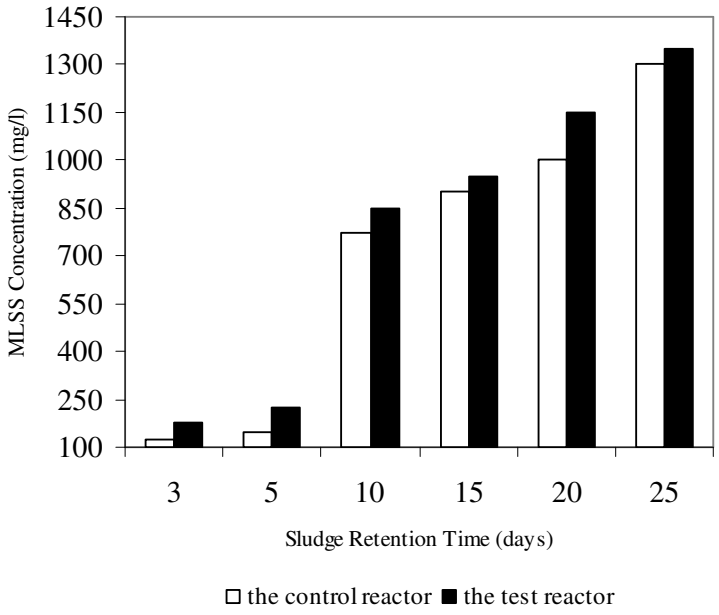

Figure 5.14 Comparison of variation of MLSS concentration in the control and test reactor at different SRT values

5.2.2 Effect of SRT on COD removal efficiency

Figure 5.15 shows the effect of SRT on COD removal efficiency. For sludge ages between 10-25 days, although COD removal efficiencies remained above 80% in the test reactor, they ranged between 77.5-80.1% in the control reactor. Further increases in sludge age above 15 days did not improve the reactor's performance. However, the COD removal efficiency decreased sharply with decreasing sludge age values from 10 to 3 days. The COD removal efficiency increases with increasing sludge age since high sludge ages resulted in larger biomass concentrations in the aeration tank resulting in better COD removal performances.

Although the difference in COD removal efficiencies between control reactor and test reactors are small, they are meaningful since standard deviation values determined in COD values are also very small, as shown in Figure 5.15.

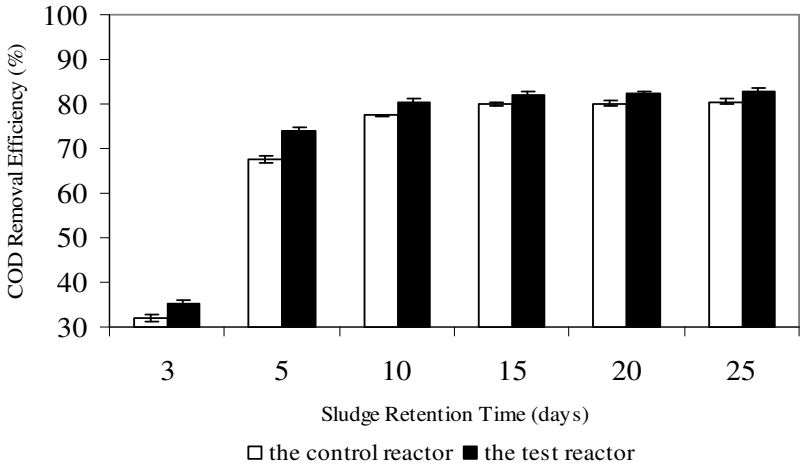

Figure 5.15 Comparison of removal efficiencies of COD at different SRT values

For sludge ages between 5-25 days, effluent COD concentrations varied from 86.5 to 130.5 mg/l in the test reactor and 97.5-162.5 mg/l in the control reactor. Effluent COD concentrations increased to 339.5 and 323 mg/l in the control and test reactor, respectively, when the sludge age was decreased from 5 to 3 days. The test reactor system was able to give lower effluent COD due to the positive effect of the biosurfactant in decay (Figure 5.16). After 15 days SRT values effluent COD values did not change much with the increase in SRT.

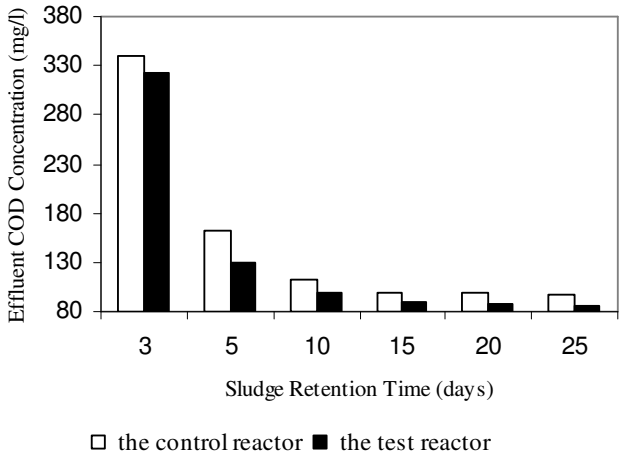

Figure 5.16 Comparison of effluent COD concentrations at different SRT values

When the sludge age was varied between 3-25 days, removal rate of COD varied between 226.59-568.24 mg/l.day in the control reactor and 249.88-583.76 mg/l.day in the test reactor (Figure 5.17). Since biomass concentration was higher in higher sludge ages due to longer adaptation periods, removal efficiencies were also high in both reactors. In lower sludge ages, removal efficiency was higher in the test reactor than in the control reactor since the presence of biosurfactant attenuated the toxicity of 2,4-DCP.

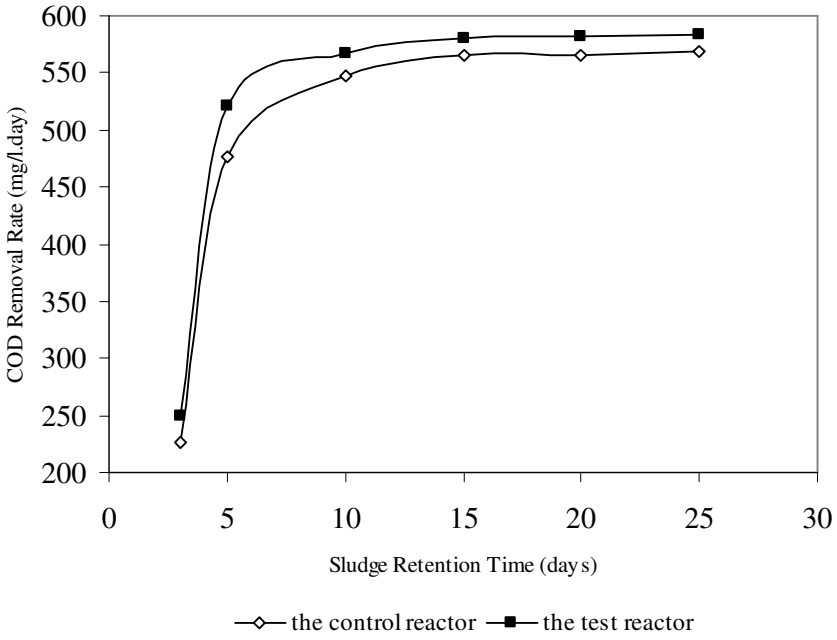

Figure 5.17 Comparison of removal rate of COD at different SRT values

5.3 Effect of Increasing 4-CP Loading Rates on the Reactors Performance

5.3.1 COD Removal Efficiency through Start-Up Period in the Control and Test Reactor

During the start-up period (day 1 to 18), to determine the reactors performance in the absence of 4-CP, glucose was fed to the reactor as the sole carbon source at an influent concentration of 500 mg/l COD. At the end of this start-up period, COD removal efficiency was 81.4% and effluent COD concentration was 93.0 mg/l in the control reactor and, 81.5% and 92.5 mg/l in the test reactor (See Figure 5.18 and Figure 5.19).

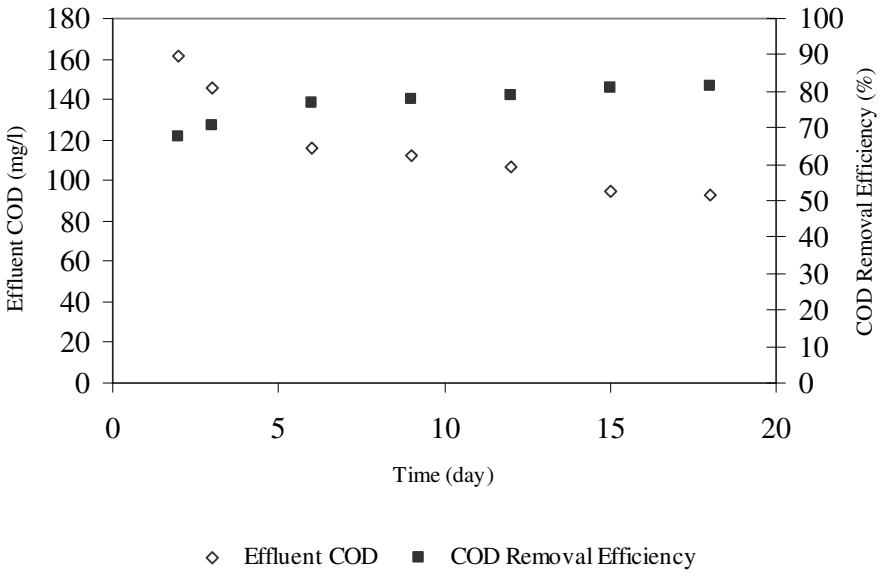

Figure 5.18 Variation of COD removal through start-up period in the control reactor

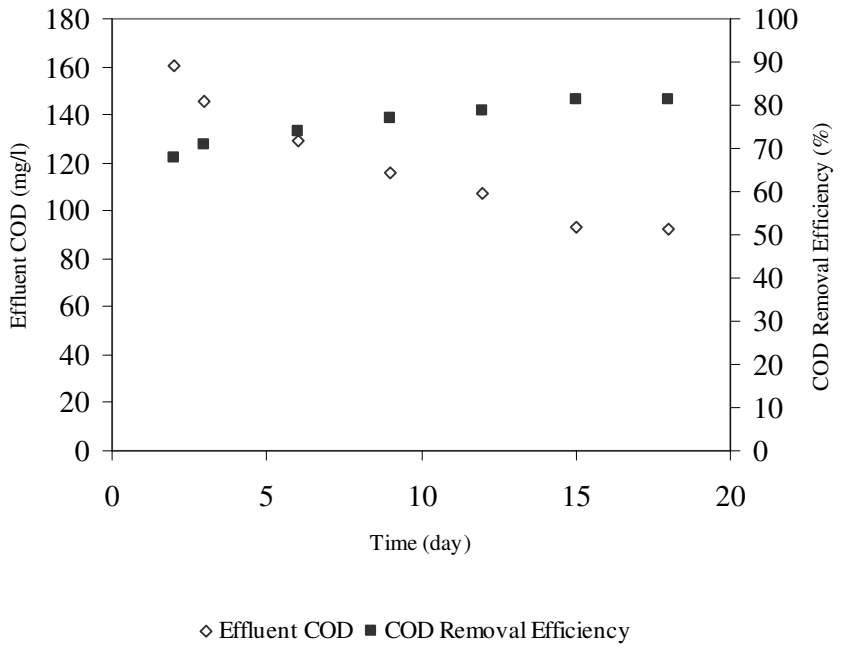

Figure 5.19 Variation of COD removal through start-up period in the test reactor

5.3.2 4-CP Acclimation Period in the Control and Test Reactor

After start-up period, 5 mg/l 4-CP concentration was applied in order to acclimatize microorganisms (day 19 to 29). When both the control reactor and test reactor were started to operate with 5 mg/l of 4-CP concentration, the 4-CP removal efficiencies and the COD removal efficiencies were illustrated in Figure 5.20 and Figure 5.21. At the end of the 4-CP acclimation period about 98.4% and 98.9% 4-CP removal efficiency in the control and test reactor, and 79.5% COD removal efficiency were achieved in both reactors. Both reactors gave similar results with respect to COD and 4-CP removal.

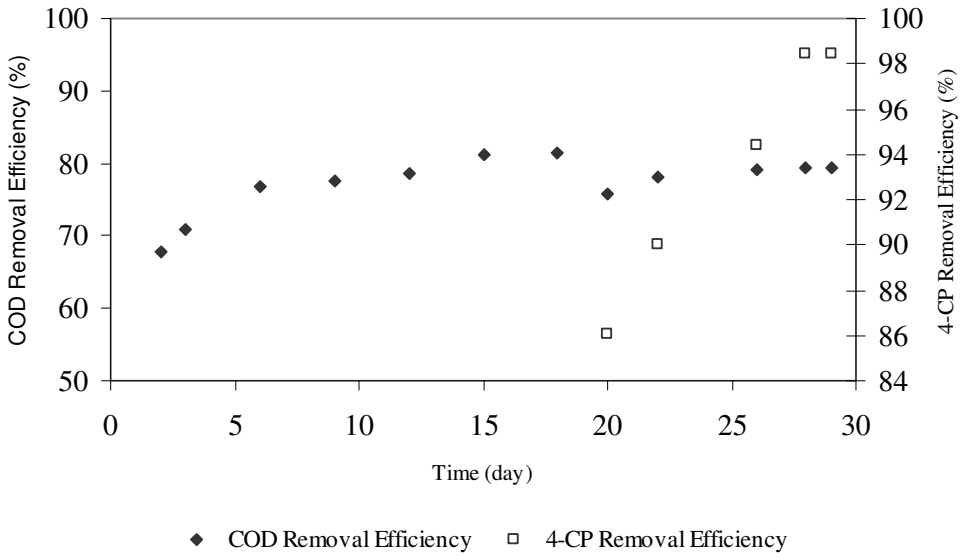

Figure 5.20 Variation of COD and 4-CP removal efficiency in the control reactor during the 4-CP acclimation period

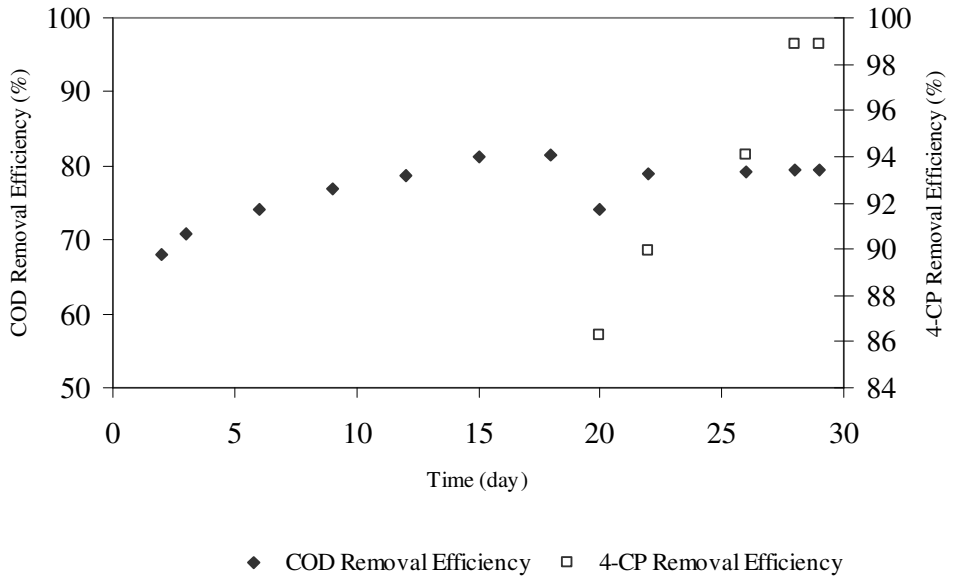

Figure 5.21 Variation of COD and 4-CP removal efficiency in the test reactor during the 4-CP acclimation periods

5.3.3 Comparison of 4-CP Removal Efficiencies in the Control and Test Reactor with Biosurfactant Addition

After 4-CP acclimation period, 15 mg/l biosurfactant concentration (CMC; critical micelle concentration) was applied in the test reactor containing 40-450 mg/l 4-CP concentrations. The feed 4-CP concentration was increased stepwise during biosurfactant application period as follows: 40, 100, 150, 250, 350 and 450 mg/l.

Figure 3.5 illustrates comparison of the 4-CP removal efficiencies in the control and test reactor (with biosurfactant). As can be seen from the Figure 5.22, no considerable increase in effluent 4-CP concentrations was observed up to 40 mg/l in the feed. At the end of operation with 40 mg/l 4-CP, the 4-CP removal efficiencies were 97.1% in the control reactor and 98% in the test reactor.

When the 4-CP concentration was increased from 40 to 100 mg/l, the 4-CP removal efficiencies decreased up to 93.2% in the control reactor. In the test reactor, 100 mg/l 4-CP concentration did not affect 4-CP removal efficiency (97.2%). When 4-CP concentrations were increased to 150 and 250 mg/l, 4-CP removal efficiency in the control reactor varied between 92.4-91.1% and it was 96.9-96.5% in the test reactor. In this range of 4-CP concentrations, the decrease in 4-CP removal efficiency was not significant in both of the reactors because of the biomass adaptation to 4-CP.

A further increase in the 4-CP concentration from 250 to 350 mg/l, however, resulted in a significant decrease in 4-CP removal efficiencies to the values of 80% and 86.5% in the control and test reactor because of inhibitory effects of high 4-CP content on microorganisms. During the last phase of operation, when the 4-CP concentration of as high as 450 mg/l was fed to the reactors, the 4-CP removal efficiencies decreased up to 76.7% in the control reactor and 84.6% in the test reactor. In the test reactor, 4-CP enhancement degradation could also be the result of cometabolism between biosurfactants, glucose and 4-CP. Biosurfactant existence

may be beneficial to promote the biodegradation of 4-CP. The positive effect of biosurfactant addition was more pronounced as 4-CP concentration was increased.

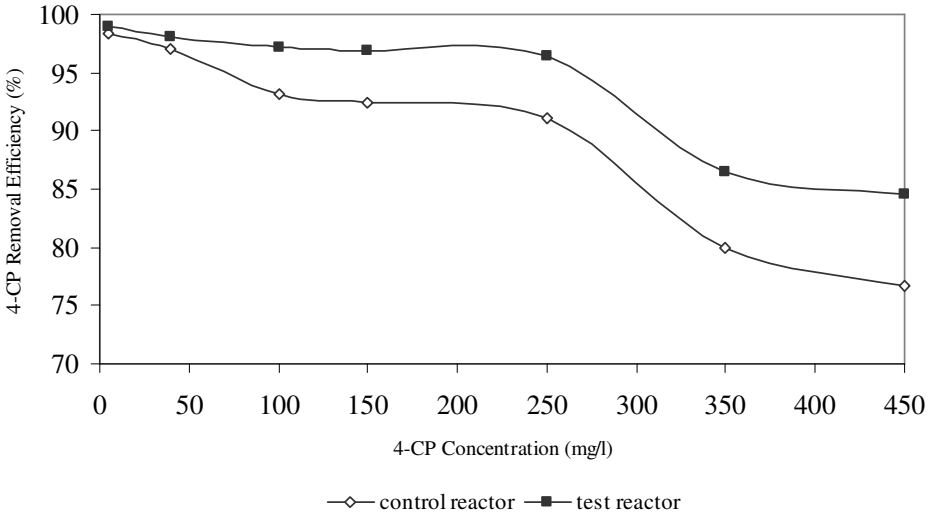

Figure 5.22 Comparison of variation of 4-CP removal efficiencies versus influent 4-CP concentrations

An increase of influent 4-CP concentration resulted in its elevation in the effluent, especially in the control reactor, as seen in Figure 5.23. Effluent concentration of 4-CP in the control reactor increased from 1.16 to 22.25 mg/l when the influent concentration was increased from 40 to 250 mg/l. In the test reactor, effluent concentration increased slowly as compared to the control reactor; from 0.8 to 8.75 mg/l for 40-250 mg/l influent 4-CP concentration. For 4-CP concentrations between 350-450 mg/l, while effluent 4-CP concentrations ranged between 70-104.85 in the control reactor, it ranged between 47.25-69.3 mg/l in the test reactor.

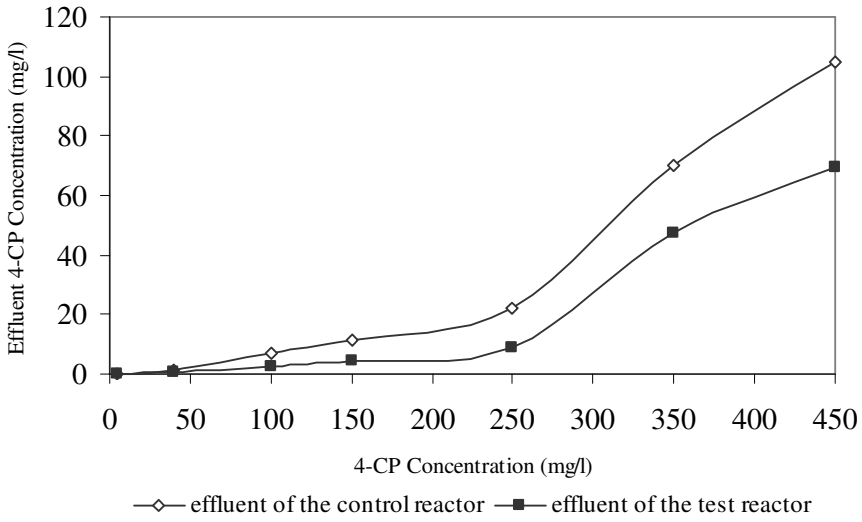

Figure 5.23 Comparison of variation of effluent 4-CP concentrations versus influent 4-CP concentrations

Effect of 4-CP concentration on 4-CP biodegradation rate is shown in Figure 5.24. 4-CP removal efficiency decreased with increasing initial concentration in the reactors, on the other hand, the 4-CP removal rate increased with increasing initial concentration. While 4-CP biodegradation rate increased from 6.95 to 487.27 mg/l.day in the control reactor, it increased from 6.98 to 537.46 mg/l.day in the test reactor when 4-CP concentration was increased from 0-450 mg/l. Degradation rates of 4-CP was more enhanced in the test reactor relative to in the control reactor.

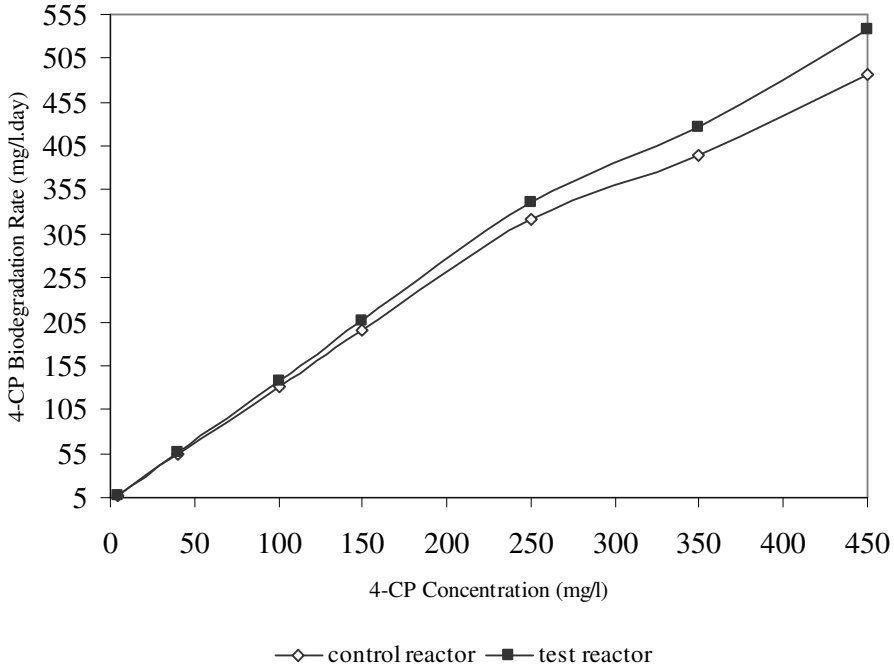

Figure 5.24 Comparison of variation of 4-CP biodegradation rate versus influent 4-CP concentrations

5.3.4 Comparison of Effect of Influent 4-CP Concentration on the COD Removal Efficiencies in the Control and Test Reactor

Figure 5.25 illustrates the COD removal efficiencies versus influent 4-CP concentrations in control and test reactors. When the 4-CP concentration was increased in time, the COD removal efficiencies decreased in both reactors.

When the 4-CP concentrations were between 40-250 mg/l, the COD removal efficiency varied between 73.0-63.1% and 76.3-68.9% in the control and test reactor, respectively. The test reactor showed lower effluent COD than the control reactor (see Figure 5.26). Although effluent concentration of COD in control reactor ranged from 135-184.5 mg/l, it ranged from118.5-155.5 mg/l in the test reactor.

COD removal efficiencies drastically decreased in both of the reactors when 4-CP concentration was increased from 250 to 350 mg/l. In the control reactor, the COD

removal efficiency decreased from 63.1 to 32.2% resulting in effluent COD concentration of 576.3 mg/l. In the test reactor, the COD removal efficiency decreased from 68.9 to 49.8% resulting in COD concentration of effluent as 426.7 mg/l. The removal efficiency of COD decreased up to 8.2% and 13.4% in the control and test reactor when the concentration of 4-CP was increased from 350 to 450 mg/l respectively, indicating the inhibitory effect of 4-CP on biomass.

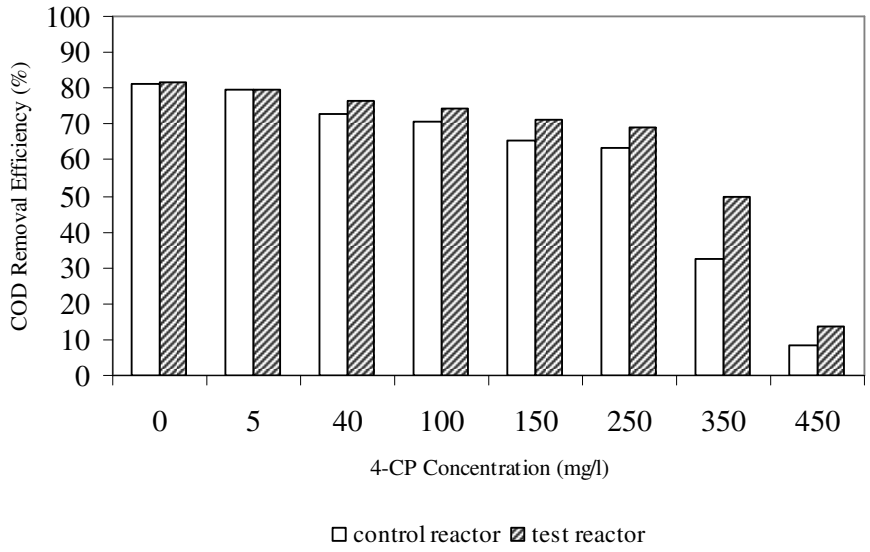

Figure 5.25 Comparison of variation of COD removal efficiencies versus influent 4-CP concentrations

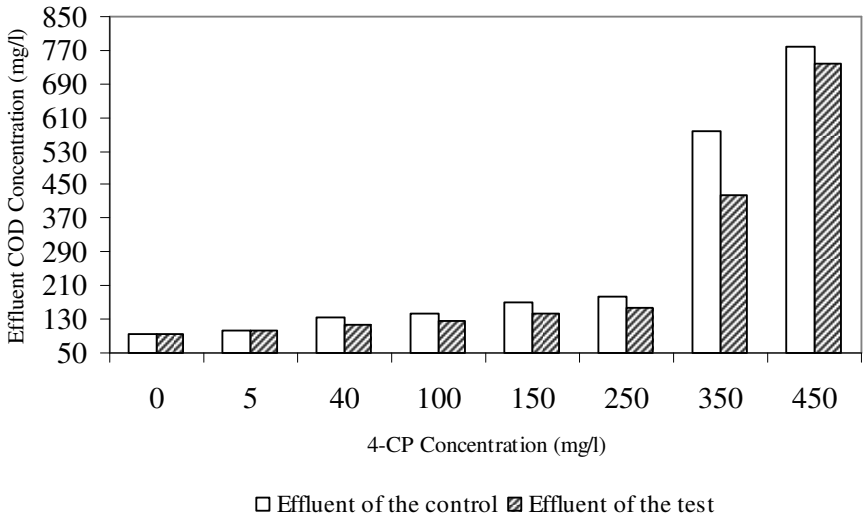

Figure 5.26 Comparison of variation of effluent COD concentrations versus influent 4-CP concentrations

In order to keep the starting COD concentration as constant at 500 mg/l (up to 250 mg/l 4-CP concentrations), glucose proportion was decreased while 4-CP proportion was increased. Increase in the proportion of 4-CP in the inlet, could enhance the degradation capacity of the biomass. In this case, the metabolic system of the cells has been adapted to 4-CP and degraded it. In order to increase 4-CP concentrations above 250 mg/l, total COD concentration should also be increased due to the contribution of 4-CP to COD. Therefore initial COD concentration was adjusted to 850 mg/l for these 350 and 450 mg/l 4-CP concentrations. Because the enzyme system was adapted to overcome the toxicity of 4-CP, oxidation of glucose in the presence of 4-CP could decrease. Relatively high concentration of 4-CP inhibited the biodegradation of glucose. Kim & Hao (1999) reported that the delay in metabolism of phenol as used growth substrate was shown at the higher 4-CP concentration. The rate of nongrowth substrate degradation has been linked to the utilization rate of growth substrate (Criddle, 1993; Saez & Rittmann, 1993).

The test reactor system gave better removal efficiency owing to the addition of biosurfactant. From these results, it is clear that addition of biosurfactant in the test reactor would increase the COD removal capacity in the presence of 4-CP.

5.3.5 Comparison of Relationship between MLSS Concentration and Influent 4-CP Concentration in the Control and Test Reactor

Variation in MLSS concentrations versus influent 4-CP concentration is depicted Figure 5.27. After the start-up period, MLSS concentrations were 1100 mg/l and 1125 mg/l in the control and test reactor. When 4-CP concentrations varied between 5-150 mg/l, biomass concentrations decreased from 1000 to 600 mg/l in the control reactor, it decreased from 1050 to 800 mg/l in the test reactor.

While 4-CP increase up to 250 mg/l resulted in a biomass production 700 mg/l in the control reactor, increase up to 350 mg/l resulted in a biomass production 1075 mg/l in the test reactor.

When higher 4-CP concentrations were applied between 250 and 450 mg/l, an obvious loss of biomass was observed in the control reactor. On the other hand, some biomass loss was observed in the 450 mg/l 4-CP concentration in the test reactor.

The first decline up to 100 mg/l 4-CP may be explained as the response of microorganisms to toxic substance and the change in the culture by elimination some microorganisms who are most effected by 4-CP. Afterwards the microorganisms who can survive under these conditions actively consume 4-CP and increase their number up to 350 mg/l after which toxic effect becomes dominant.

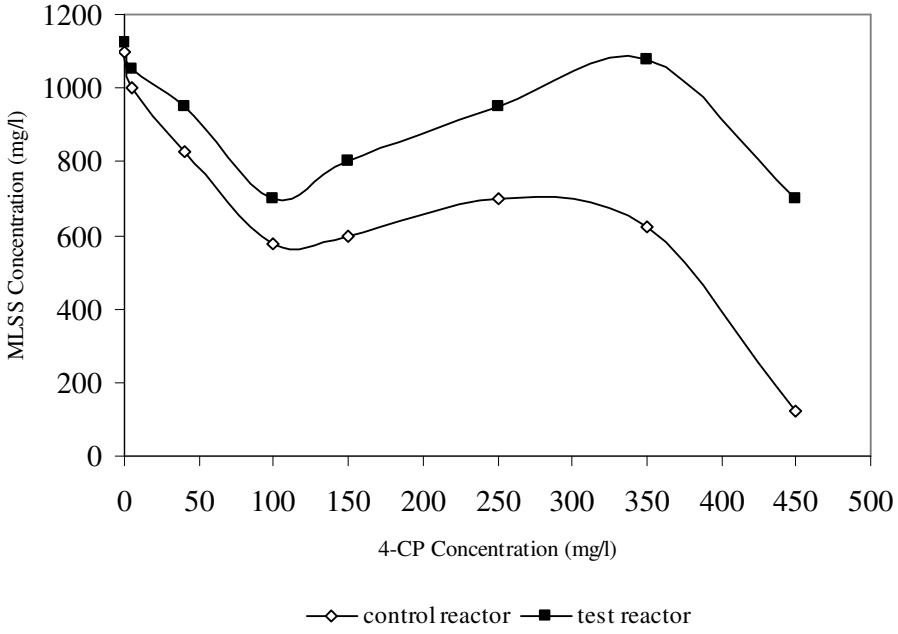

Figure 5.27 Comparison of variation of MLSS concentration between control and test reactor

5.4 Effect of Sludge Retention Time (SRT) on the Reactors Performance for 4-Chlorophenol Treatability

In order to determine the effect of sludge age on 4-CP and COD removal performance in R1 (control reactor), R2 (CMC) and R3 (2CMC), reactors continuous activated sludge experiments were performed at different sludge ages between 3 and 25 days. The hydraulic residence time, the feed COD and influent 4-CP concentrations were kept constant throughout the experiments as θ_H=17 h, COD_0=1500 mg/l and 4-CP_0=250 mg/l.

5.4.1 Effect of SRT on 4-CP Removal Efficiency

4-CP removal efficiency as a function of sludge age is given in Figure 5.28. For sludge ages between 20-25 days, 4-CP removal efficiencies remained above 99% all the reactors. 4-CP removal efficiency decreased from 99.78 to 91.85% in R1 when sludge retention time was decreased from 20 to 15 days and, it decreased sharply

59.99% in 10 days sludge age. However, for R2 and R3, removal efficiencies of 4-CP were always above 99% at the sludge ages above 10 days. While 4-CP removal efficiency was above 95% in R3 even when the sludge age was as short as 5 day, it was 31.28% and 75.25% for R1 and R2, respectively. Removal efficiencies of 4-CP decreased up to 19.15, 46.01 and 63.69% in for R1, R2 and R3, respectively, when the sludge age was decreased from 5 to 3 days. Percent 4-CP removals increased with increasing sludge age due to high biomass concentrations at high sludge ages. Also, Kargi & Eker (2006) reported that percent DCP removals were 15, 22 and 100% at the sludge ages of 5, 15 and 30 days in activated sludge unit, respectively (COD_0=2500 mg/l, DCP_o=200 mg/l, HRT=25 h). Due to lower adaptation periods in lower sludge ages, 4-CP toxicity affects microorganisms in the control reactor more than test reactors which contain biosurfactant. However, biosurfactant adding systems did not adversely affect at lower sludge ages (up to 3 days) because biosurfactant existence attenuated 4-CP toxicity on the microorganisms.

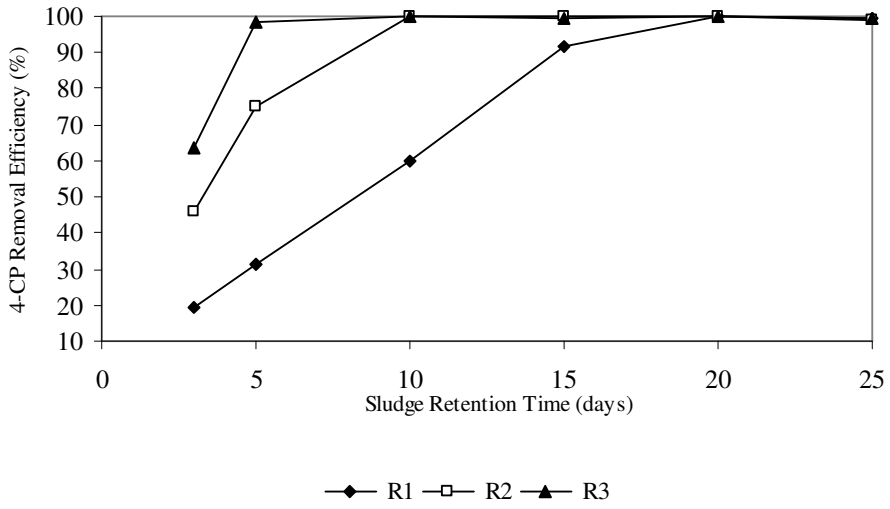

Figure 5.28 Comparison of removal efficiency of 4-CP in the control and test reactors at different SRT values

Figure 5.29 shows effluent concentrations of 4-CP at different SRT values. Although effluent concentration of 4-CP ranged from 2.125 to 61.875 mg/l and from 0.825 to 3.95 in R2 and R3 between the sludge ages 5-25, it ranged from 1.275 to 171.8 mg/l in R1. Effluent concentration of 4-CP increased to 202.125, 134.975 and

90.775 mg/l in R1, R2 and R3, respectively, when the sludge age was decreased from 5 to 3 days. Effluent 4-CP concentrations were much higher in R1 than that of R2 and R3. Biosurfactant helped to maintain activated sludge bioactivity and prevent the system from 4-CP toxicity. Addition of biosurfactant at 2CMC concentration was more efficient than CMC. This result pronounces the positive effect of biosurfactant addition in 4-CP removal in wastewater.

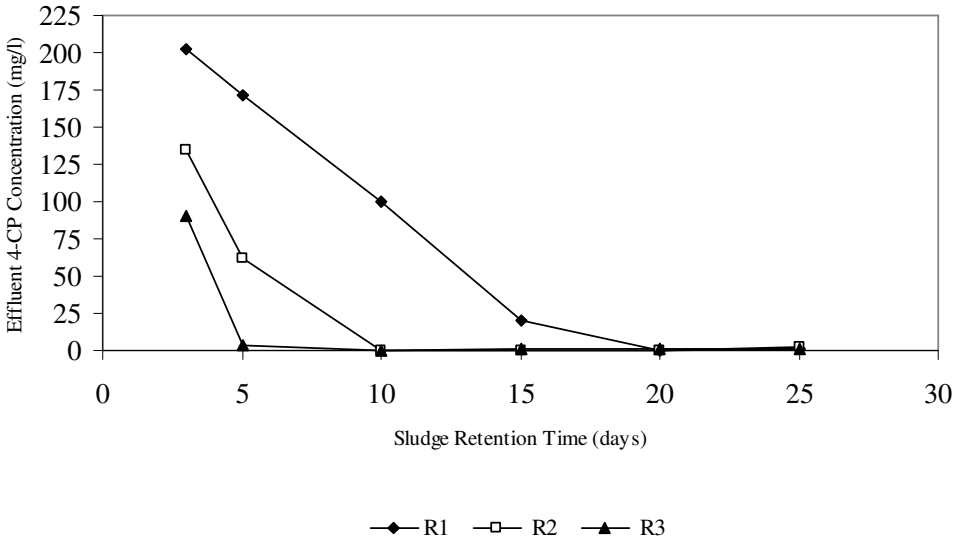

Figure 5.29 Comparison of effluent concentration of 4-CP in the control and test reactors at different SRT values

Variation of the biomass concentration with the sludge age is shown in Figure 5.30. The effect of 4-CP on biomass concentration was lower in R2 and R3 as compared to R1. R1 and R2 cope with toxicity of 4-CP due to biosurfactant addition. Increasing biosurfactant concentration led to higher biomass concentration in R3, thus, microorganisms gained more resistance against 4-CP toxicity. In lower sludge ages (⟨15 days), biomass concentration decreased more in R1 relative to R2 and R3. 4-CP affected microorganisms especially at the lower sludge ages in R1 because of decrease in mean cell residence time (lower sludge age). Decrease in mean cell residence time resulted in toxic effect of 4-CP on microorganisms in the control reactor due to insufficient adaptation periods. In the test reactors (R2 and R3),

biosurfactant addition reduced the toxic effect of 4-CP to microorganisms and caused them to maintain their bioactivity. At lower sludge ages, the positive effect of biosurfactant addition was more pronounced.

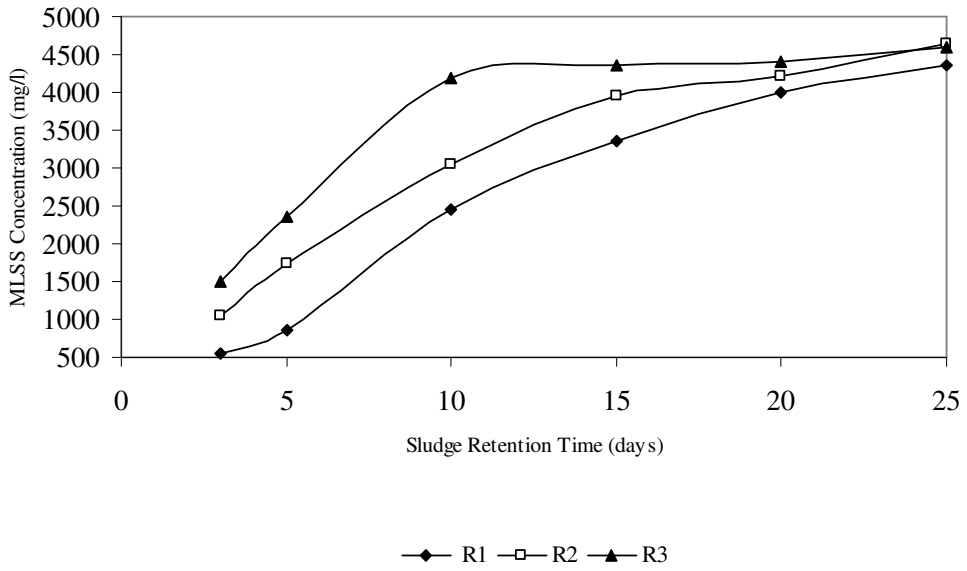

Figure 5.30 Comparison of MLSS concentration in the control and test reactors at different SRT values

Variation of specific removal rate of 4-CP [$R_{4\text{-}CP}=Q(4\text{-}CP_o\text{-}4\text{-}CP_e)/VX$)] with sludge age is depicted in Figure 5.31. Biomass concentrations and 4-CP contents in the aeration tank (which is the same as the effluent 4-CP since the aeration tank is completely mixed) affected the specific removal rate of 4-CP. Because different SRT conditions resulted in different biomass concentrations, the reactors with longer SRT had more biomass, and consequently lower specific removal rate. The short SRT resulted in a high specific biodegradation rates due to lower concentration of microorganisms in the reactors. Since 4-CP removal efficiency is lower in R1 at the low sludge ages (⟨15 days), specific removal rates are also lower in R1 than in R2 and R3 despite the lower biomass concentrations in R1.

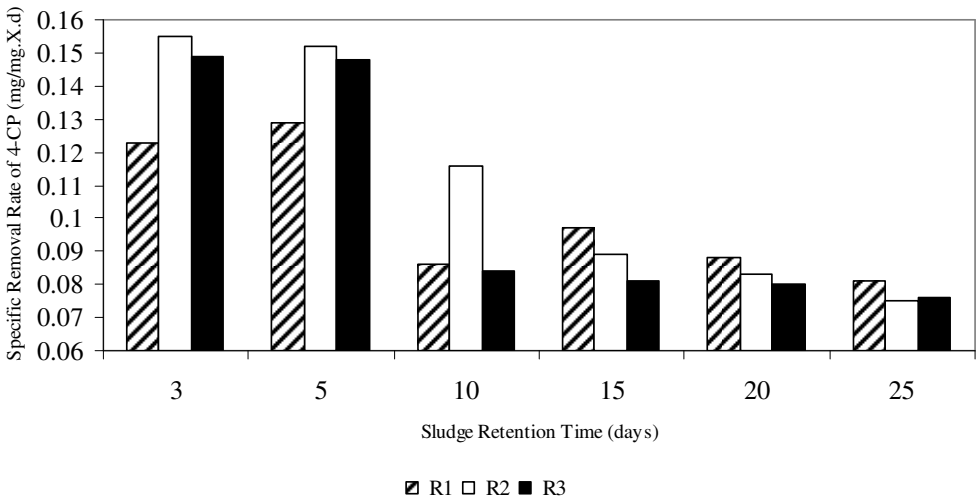

Figure 5.31 Comparison of specific removal rate of 4-CP in the control and test reactors at different SRT values

5.4.2 Effect of SRT on COD Removal Efficiency

Figure 5.32 shows the effect of SRT on COD removal efficiency. For the sludge ages between 10-25 days, although COD removal efficiency remained almost above 94% in R2 and R3, it ranged from 74.26-94.13% in R1. When sludge age decreased from 15 to 10 days, sharp decrease of COD removal efficiency was observed in R1, but the biosurfactant addition to R2 and R3 caused these reactors to work more efficiently, almost without any negative effect. COD removal efficiencies decreased to 61.53, 77.75 and 81.20% in R1, R2 and R3 at sludge age at 3 days. After 20 days sludge retention time, COD removal efficiency was almost the same in all the reactors and above this value no increase was observed in any reactor.

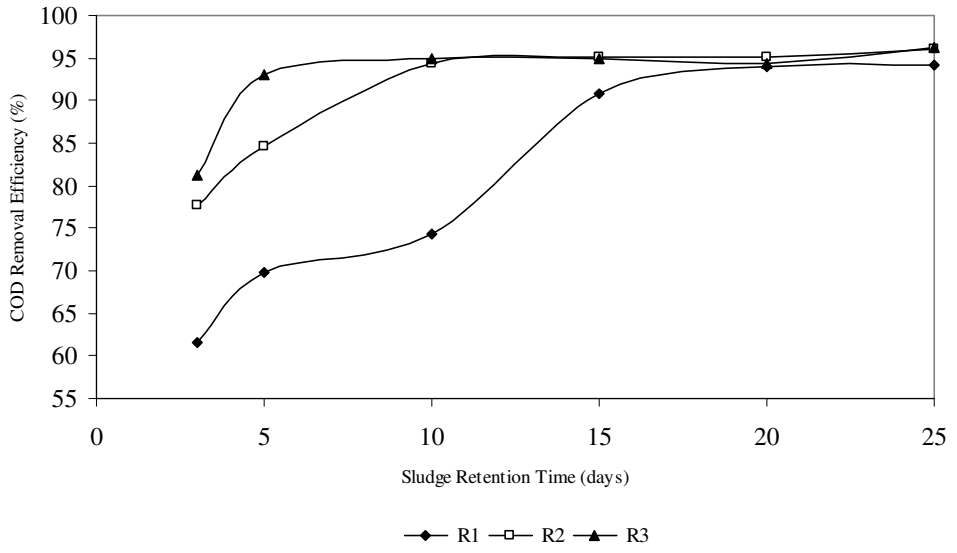

Figure 5.32 Comparison of removal efficiency of COD in the control and test reactors at different SRT values

Figure 5.33 depicts variation of specific COD removal rate [$R_{COD}=Q(COD_o-COD_e)/VX)$] with the sludge age in reactors R1, R2 and R3. Steady decrease in specific rate of COD removal with the sludge age is observed because of the increase in biomass concentration with increasing sludge age. Due to high biomass concentration in R3, specific removal rate of COD lower than R1 and R2. After 10 days sludge retention time, the difference in specific removal rate decreased sharply and at 25 days SRT, there was almost no difference.

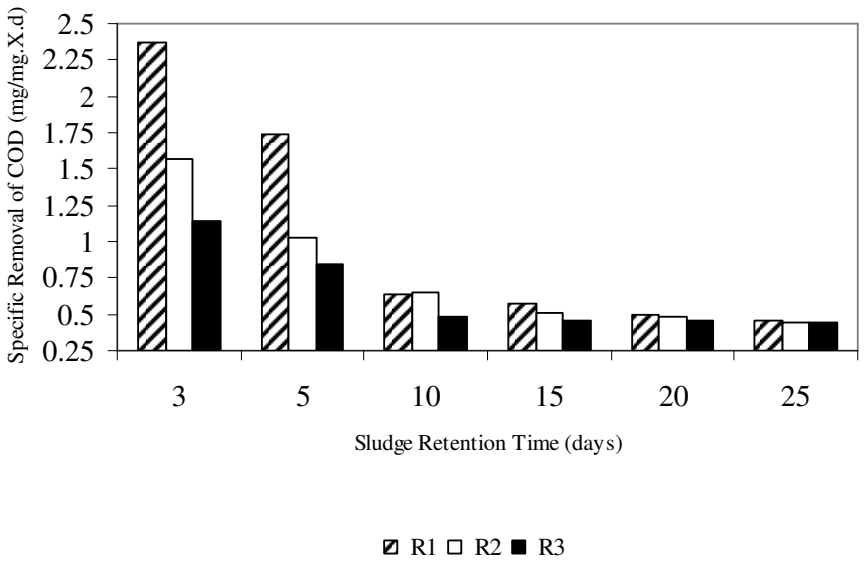

Figure 5.33 Comparison of specific removal rate of COD in the control and test reactors at different SRT values

5.5 Effect of 4-CP on Unacclimated Activated Sludge

To investigate 4-CP biodegradation capacity of unacclimated activated sludge, three reactors were used in parallel test as R1 (control reactor), R2 (CMC) and R3 (2CMC). The hydraulic residence time and the feed COD were constant throughout the experiments as θ_H=17 h and COD_0=1500 mg/l. Influent 4-CP concentration was constant at 150 mg/l and 300 mg/l in the first and second set experiment.

5.5.1 Cometabolic Degradation of 4-CP in Unacclimated Culture

The three reactors were inoculated with the activated sludge. A synthetic wastewater based on glucose (about 1500 mg/l COD) was fed to the three reactors continuously until a stable COD removal was obtained. To acclimate microorganisms to biosurfactant, R2 and R3 also included biosurfactant in this period.

COD removal efficiencies of the reactors during the glucose acclimation periods were presented in Figure 5.34. When no 4-CP loading was applied, COD removal efficiencies remained steady at 93%, 93.35% and 95.42% in R1, R2 and R3.

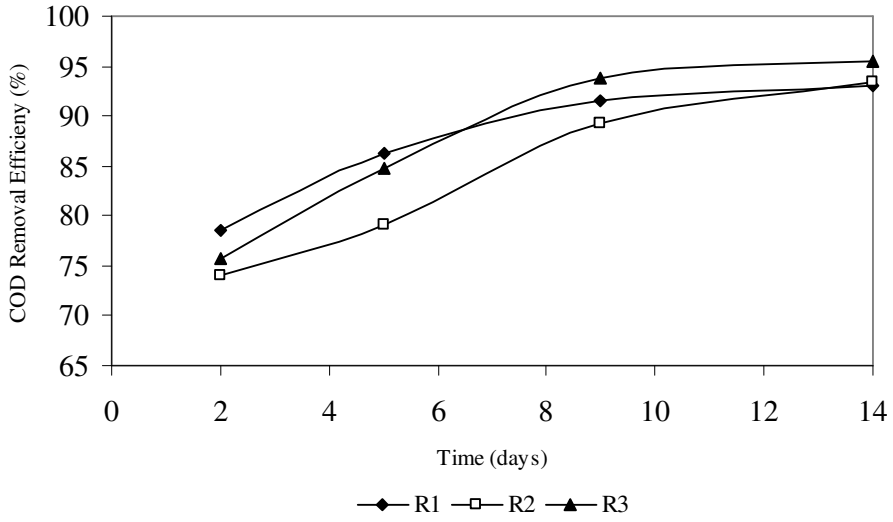

Figure 5.34 COD removals in the reactors during glucose acclimation periods

After stable COD removal values were obtained, 150 mg/l 4-CP loading period was started. During 4-CP loading period, influent COD concentration was constant at 1500 mg/l.

Figure 5.35 showed removal efficiencies of 4-CP in the reactors during 4-CP loading period. The control system (R1) could not degrade 4-CP completely at the beginning of the experimental series, so the effluent 4-CP climbed up for the first 10 days. 4-CP removal efficiency finally reached to 95.79% on the fourteenth day in R1. 4-CP removal efficiency was increased to 93.34% and 98.59% on the seventh and tenth days in R2. However, 4-CP removal efficiency of 98.97% was observed even at the first days in R3. In unacclimated activated sludge, completely biodegradation of 4-CP required longer time in R1 relative to R2 and R3. Because concentration of biosurfactant affected to 4-CP biodegradation, nearly completely 4-CP biodegradation was possible even in the first day in R3. Sahinkaya & Dilek (2005) reported that the acclimated culture (culture to acclimatize ultimately to 130 mg/l 4-

CP) was nearly reached at the end of 1 day in fed-batch reactor when the 4-CP concentration was below 200 mg/l. According to this result, R3 system behaves like acclimated culture in this study. In R2 and R3, the degradation times were shorter than control reactor. Degradation rates of 4-CP were ranged between 4.49-8.30, 5.17-8.80 and 8.73-8.80 mg/(l.h) in R1, R2 and R3 for 22 days operation periods. 4-CP degradation rate for first day in control reactor was about 2 times lower than in R3. Higher degradation rates were observed in the presence of biosurfactant. In the study of Wang & Loh (1999), when the medium pH was kept between 6.5 and 7.5, the degradation rates of 4-CP at initial concentrations of 100 and 200 mg/l by *Pseudomonas putida* were observed as 9-11 and 10 mg/(l.h), respectively, in the presence of glucose as the growth substrate. On the other hand, when pH of the medium was not controlled, the pH quickly dropped below 4.5, consequently stopping the further transformation of 4-CP, and the average transformation rate of 4-CP at an initial concentration of 200 mg/l was only about 3 mg/(l.h). Sahinkaya & Dilek (2005) reported that degradation rates of 4-CP for acclimated culture were observed as 8.15, 10.15, 10.22, and 1.03 mg/(l.h) for initial concentrations of 130, 200, 300, and 390 mg/l, respectively.

In this study the pH medium was between 6.4 and 7.6 throughout the study, the degradation rates of 4-CP at the end of loading period were fairly high despite using unacclimated activated culture. The biosurfactant systems could make a rapid response to 4-CP and degrade it effectively as the toxicity of 4-CP to the microorganisms was reduced, while activity of the microorganisms was lost in the control reactor. Consequently, the biosurfactant systems exhibited higher tolerance to loading of 4-CP than the control system.

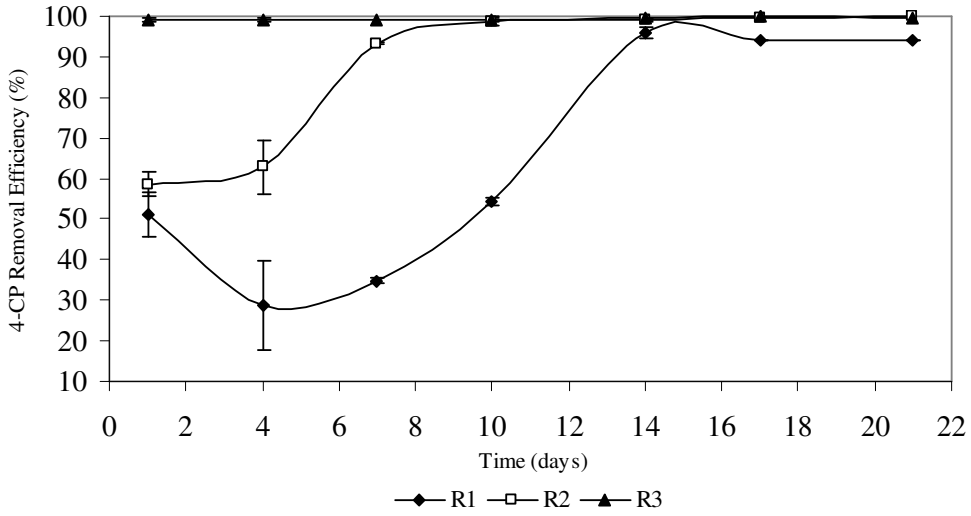

Figure 5.35 4-CP removal efficiency with time for the unacclimated activated sludge in the control and test reactors

Removal efficiencies of COD in the reactors during 4-CP loading period were demonstrated in Figure 5.36. After the start of 4-CP loading, COD removal efficiency was reduced up to 82.48% on the tenth days in R1. COD removal efficiency was decreased to 86.66% on first day 4-CP loading in R2. After first day 4-CP loading, COD removal efficiency was being increased in R2. In R3 system, COD and 4-CP were degraded simultaneously. COD removal efficiency was not adversely affected in R3 after 4-CP loading. In the end of 4-CP loading periods, COD removal efficiencies were 85.02%, 93.53% and 95.41% in R1, R2 and R3. At the end of glucose acclimation periods, while COD removal rates in the absence of 4-CP were 82.06, 82.37 and 84.19 mg/(l.h) in R1, R2 and R3, they were ranged between 72.99-75.02, 76.47-82.53 and 84.12-84.19 mg/(l.h) in R1, R2 and R3 for 22 days 4-CP loading periods. Degradation rates of COD were more enhanced in the test reactors due to the increased biomass concentrations. A more stable activated sludge bioactivity and COD removal efficiency were achieved in the biosurfactant containing units. In fact, increase in biosurfactant concentration has resulted in much better removal efficiency as compared to CMC.

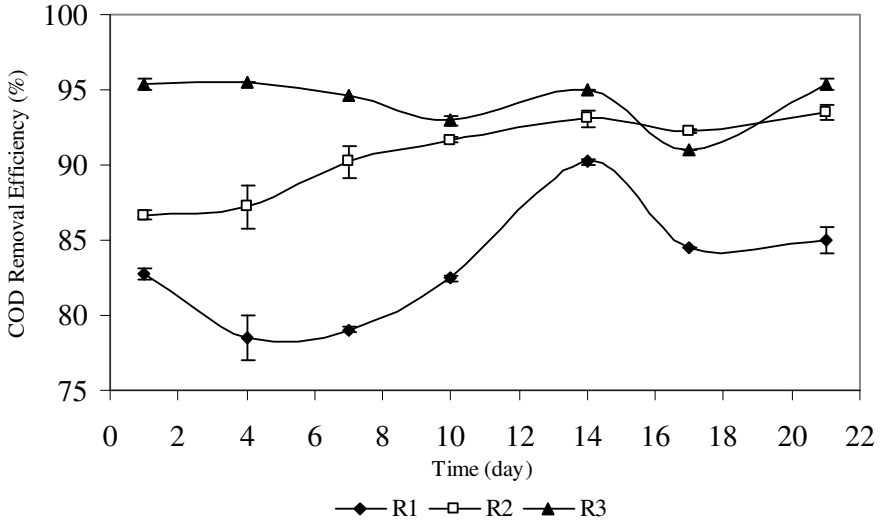

Figure 5.36 COD removal efficiency with time for the unacclimated activated sludge in the control and test reactors with the 4-CP loading

Variation of the biomass concentration in the reactors during 4-CP loading period is shown in Figure 5.37. After glucose acclimation period, biomass concentration was 2300 mg/l in all the reactors. As soon as 4-CP loading has been started, biomass concentration was reduced in R1. Biomass concentration has started to decrease since the organisms were not adapted to the toxic substance and died in R1. Sahinkaya & Dilek (2005) demonstrated that the value of IC_{50} (concentration causing 50% inhibition) was 130 mg/l when an unacclimated culture was used. However, biomass concentration gradually increased in R2 and R3. Since the presence of biosurfactant has caused a reduction in the toxicity of 4-CP and affected of cometabolism, biomass concentrations have increased in R2 and R3, more in R3 owing to the higher biosurfactant concentration.

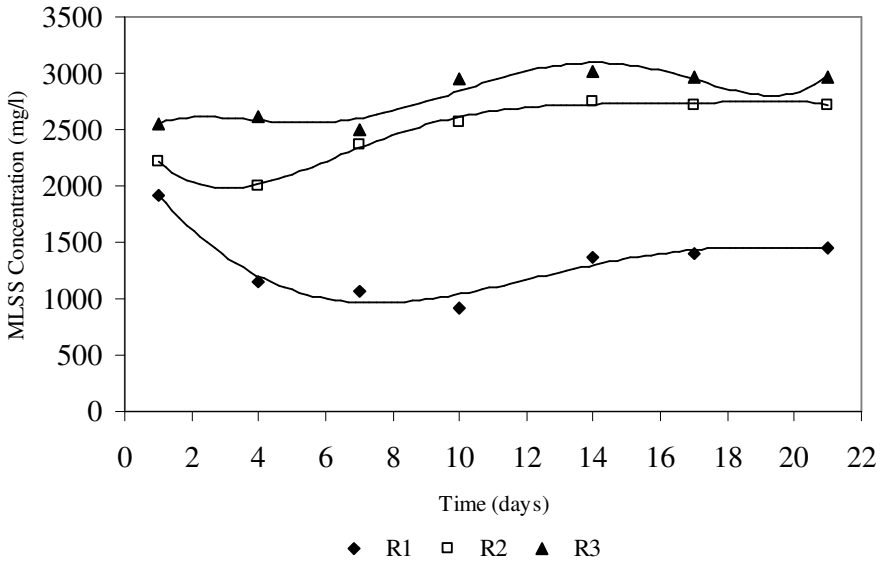

Figure 5.37 Variation of biomass concentration with time for the unacclimated activated sludge in the control and test reactors with the 4-CP loading

Figure 5.38 depicts variation of specific 4-CP removal rate (mg 4-CP/mg biomass .day) with the 4-CP loading. Specific 4-CP removal rate increased with time in R1. 4-CP concentration consumption by unit biomass was much higher in R1 due to decrease in biomass concentration. On the other hand, specific 4-CP removal rates were much lower in R2 and R3 as compared to in R1 owing to the higher biomass concentrations in R2 and R3 relative to control reactor. At the end of 4-CP loading period, specific 4-CP removal rates were 0.137, 0.078 and 0.07 mg 4-CP/mg X.day in R1, R2 and R3.

Quan, Shi, Wang, & Qian (2003) also reported that the ability of the indigenous culture to remove 2,4-DCP increased and the average removal rate was 0.01 h^{-1}, while that for the introduced culture was 0.16 h-1. Though the average specific removal rate of 2,4-DCP by the indigenous culture was still much smaller than that by the introduced special culture, the role of indigenous culture in removing 2,4-DCP was obvious due to its large biomass.

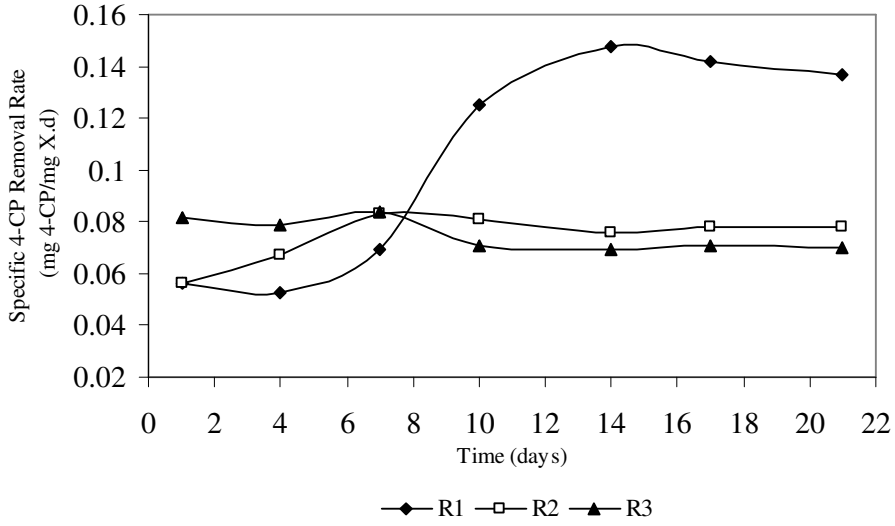

Figure 5.38 Variation of specific removal rate of 4-CP with time for the unacclimated activated sludge in the control and test reactors with the 4-CP loading

Figure 5.39 depicts variation of specific COD removal rate (mg COD/mg biomass.day) with the 4-CP loading. At the end of glucose acclimation periods, the specific COD removal rates in the absence of 4-CP were 0.856, 0.859 and 0.879 mg COD/mg X.d in R1, R2 and R3. With starting of the 4-CP loading, specific COD removal rate was increased in R1 because microorganisms in unacclimated sludge were inhibited by toxicity of 4-CP. 4-CP toxicity was less affected to microorganisms in R2 and R3 due to presence of biosurfactant. Since biomass concentration gradually increased in R2 and R3, COD concentrations consumed by unit biomass were decreased in R2 and R3. At the end of 4-CP loading period, specific COD removal rates were 1.242, 0.727 and 0.679 mg COD/mg X.day in R1, R2 and R3. Specific COD removal rate was more affected by 4-CP loading in R1 as compared to in R2 and R3. The difference between the specific COD removal rates were very small at different biosurfactant concentrations, but just a little bit higher in R2 (CMC) as compared to R3 (2CMC).

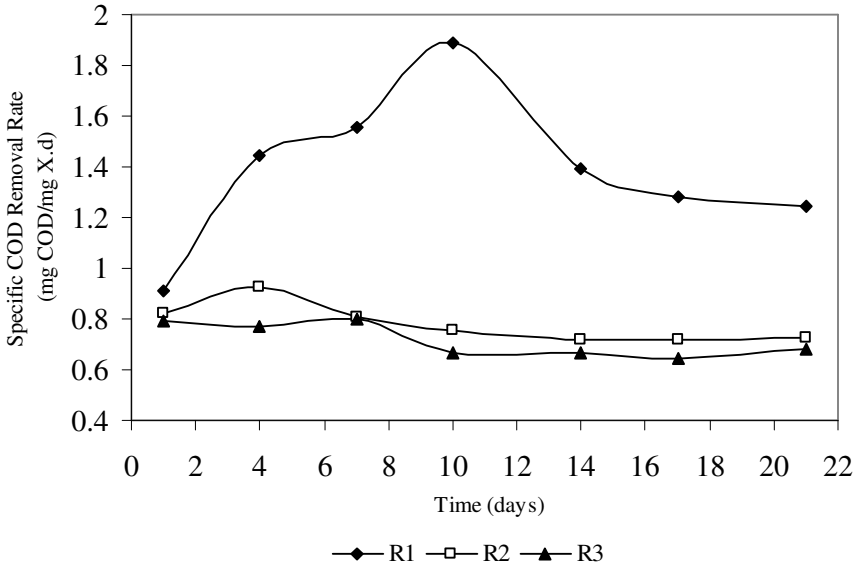

Figure 5.39 Variation of specific removal rate of COD with time for the unacclimated activated sludge in the control and test reactors with the 4-CP loading

The introduction of 4-CP load of 300 mg/l caused a total failure of the biological activity in unacclimated activated sludge. When the unacclimated culture was used, while 4-CP removals were 43.66%, 49.37% and 54.17% in R1, R2 and R3 at the end of first day, they were only 18.27%, 20.68% and 20.79% in R1, R2 and R3 for the 4-CP concentration of 300 mg/l, at the end of 20 days (Figure 5.40). The response of all the reactors to high 4-CP load were similar and the removal efficiencies were quite near.

Wang & Loh (1999) reported that when initial 4-CP concentration was increased to 300 mg/l, cells could not grow on glucose even after an extended period of incubation. Also, Sahinkaya & Dilek (2005) reported that the value of IC_{50} on the basis of μ (specific growth rate) was found to be 218 mg/l for 4-CP acclimated culture, and 4-CP removals achieved were only 44% and 30% in unacclimated culture for the initial 4-CP concentrations of 26 and 130 mg/l, respectively, at the end of 18 days.

In unacclimated activated sludge, for the 300 mg/l 4-CP load, biosurfactant containing systems failed to degrade 4-CP effectively, which caused a continuous increase of effluent 4-CP as the reactors 4-CP content gradually increased.

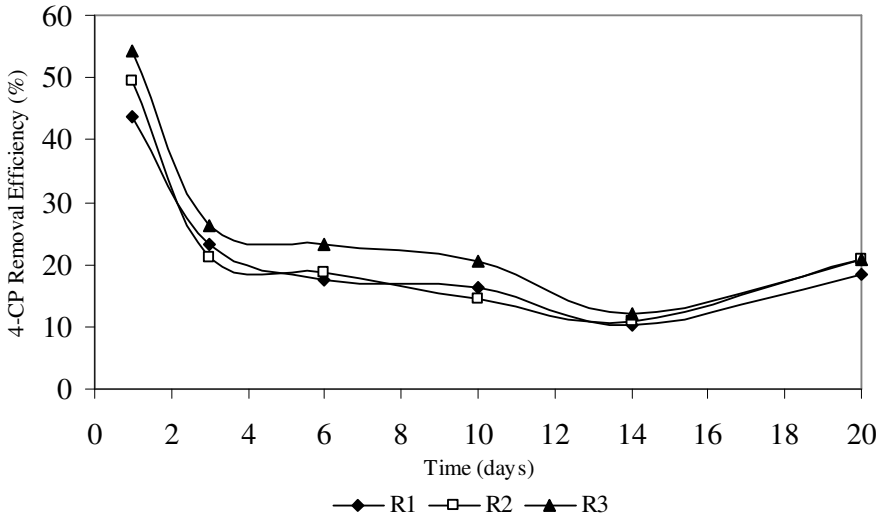

Figure 5.40 4-CP removal efficiency with time for the unacclimated activated sludge in the control and test reactors for 300 mg/l 4-CP concentration

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study was performed to investigate the effect of biosurfactant on chlorophenol biodegradation in activated sludge bioreactor. 2,4-dichlorophenol and 4-chlorophenol were selected as the model compounds and glucose was used to add COD to the synthetic wastewater. The variables considered were organic load, sludge age (SRT), biosurfactant concentration and toxic load on unacclimated sludge.

Evaluation of 2,4-DCP experiments are given below.

In the first part of the thesis, treatment performance of the mixture of glucose and 2,4-DCP by the activated sludge bioreactor (control reactor) and the biosurfactant added activated sludge bioreactor (test reactor) was studied by changing influent 2,4-DCP concentrations. When the 2,4-DCP concentration was kept between 30-100 mg/l, addition of biosurfactant in the test reactor resulted in a small increase in the biodegradation of 2,4-DCP relative to control reactor. At this concentration range, high 2,4-DCP removal efficiency in the control reactor may have resulted due to the high SRT (20 days). Since microorganisms with high SRT are acclimatized to the medium conditions, the positive effect of biosurfactant addition was negligible.

The use of surfactants has the potential to increase the biodegradation rates of hydrophobic organic compounds in contaminated environments by increasing the total aqueous solubility of these compounds. However, inhibitory effects of surfactants on the biodegradation process have frequently been reported (Cort & Bielefeldt, 2000; Rouse et al., 1994). In this study, no toxic effect of rhamnolipid biosurfactant on biomass has been observed. The presence of biosurfactant stimulated bacterial growth. As biosurfactant addition improved the system performance, the test reactor system gave lower 2,4-DCP and COD effluent concentrations relative to control reactor system.

Treatment performance of mixture of glucose and 2,4-DCP has been studied in control and biosurfactant added test reactors by changing sludge retention time. Chlorophenol and COD removals were high in higher sludge age values. In the removal of chlorophenolic compounds, lower sludge ages resulted in decreased removal capacity because the biomass was affected from the toxicity of chlorophenols due to decreasing adaptation periods. This study demonstrated that biosurfactant addition promoted 2,4-DCP and COD removals at even shorter SRT values. Long SRT, above 10 days, achieved very high removal efficiencies for 2,4-DCP and COD in both of the reactors, since operation of the reactors at high sludge ages resulted in higher biomass due to longer adaptation periods. When SRT was decreased below 10 days, 2,4-DCP and COD removal efficiencies in the control reactor decreased more as compared to test reactor. In this case, test reactor gave better results because of higher biomass concentrations.

Since biosurfactant can be utilized as an available carbon source, biosurfactant addition could be the reason of promoted microbial growth in the test reactor. Biosurfactant was also used as a carbon source in addition to glucose in activated sludge unit. Consequently, biosurfactant enhancement could also be the result of cometabolism between biosurfactant, glucose and 2,4-DCP. This effect of biosurfactant addition was particularly important in lower sludge ages as microorganisms were effected more from 2,4-DCP toxicity in lower sludge age values. Activated sludge systems are sensitive to toxic compounds. Biosurfactant presence may have attenuated the toxicity of 2,4-DCP due to increased biomass density, and consequently enhance the biodegradation rate of 2,4-DCP and COD. In this study, 2,4-DCP concentration constitutes bigger portion of 500 mg/l constant COD as compared to glucose concentration. Higher proportion of 2,4-DCP in the inlet could have caused enhancement of the degradation capacity of biomass. This may explain high 2,4-DCP removal efficiency in control reactor in higher sludge ages.

Evaluation of 4-CP experiments are given below.

In the second part of the thesis, treatment performance of the mixture of glucose and 4-CP by the activated sludge bioreactor (control reactor) and the biosurfactant added activated sludge bioreactor (test reactor) was studied by changing influent 4-CP concentrations. While 4-CP biodegradation rate was increased from 6.95 to 487.27 mg/l.day in the control reactor, it increased from 6.98 to 537.46 mg/l.day in the test reactor when 4-CP concentration was increased from 0-450 mg/l. The results of this study show that 4-CP degradation can be enhanced in the presence of biosurfactant by cells grown on glucose as the growth substrate. As a result of using glucose as the growth substrate, inhibition of 4-CP can be avoided. Moreover, the use of glucose would not result in additional environmental pollution as opposed to using phenol. Biosurfactants presence may have attenuated the toxicity of 4-CP, and consequently enhanced the biodegradation rate of 4-CP. High 4-CP concentrations have a toxic effect on biomass and thus inhibits the oxidation of both glucose and 4-CP itself.

Treatment performance of the mixture of glucose and 4-CP by the biosurfactant added activated sludge bioreactor systems (test reactors; R2 and R3) and the activated sludge bioreactor system (control reactor, R1) was studied by changing sludge retention time. The biosurfactant added systems exhibited higher tolerance to toxic effect of 4-CP at the lower sludge ages than the control system. Biosurfactant addition helped the microorganisms to cope with 4-CP toxicity. Higher biosurfactant concentration led to higher biomass concentration in R3 relative to R2. Biosurfactant adding systems did not adversely affect at lower sludge ages (up to 3 days) because biosurfactant existence attenuated 4-CP toxicity on the microorganisms. However, 4-CP removal efficiency decreased from 99.78 to 91.85% in R1 when sludge retention time was decreased from 20 to 15 days and, it decreased sharply to 59.99% at 10 days sludge age.

In the third part of the thesis, effect of biosurfactant on cometabolic 4-CP degradation was investigated by the unacclimated activated sludge cultures. In the case of 150 mg/l 4-CP loading, glucose and 4-CP was simultaneously degraded by biosurfactant added unacclimated culture. However, COD removal remarkably decreased in control reactor, and much longer time was required for 4-CP

degradation in control reactor as compared to biosurfactant added culture. Around 1 day was required to achieve complete degradation of 4-CP in R3, whereas around fourteen days was required to completely remove 4-CP in control reactor. In the case of 300 mg/l 4-CP loading, microorganisms were completely inactivated since the biomass was severely inhibited.

If the toxic substance remains for a long time in the bioreactor, then the capacity for degradation increases, whereas during abstinent periods the degrading capacity is lost. These phenomena have an important influence on the operation of wastewater treatment plants. If the presence of toxics in the reactor is only sporadic, then the efficiency of the plant will be greatly reduced, and the danger for the biomass will be higher. When biosurfactant was used in unacclimated culture, positive effect was observed without adaptation in the event of toxic loading if toxic concentration was below level to completely inhibit the microorganisms. In general, it can be said that microorganisms were able to overcome the toxicity of 4-CP due to existence of biosurfactant.

Classic activated sludge systems were operated at sludge ages 1-15 days (typically 10-15 days), not at longer sludge retention times. Biosurfactant added activated sludge systems were more effective at shorter sludge ages compared to control reactor in this study. Furthermore, it is observed that adding higher concentration of biosurfactant (up to 2CMC) showed no toxic effect to activated sludge.

Higher COD/chlorophenol ratio was applied in 4-CP applications (1500 mg/l COD) compared to 2,4-DCP applications (500 mg/l COD) at the same feeding rate and hydraulic retention time. Effect of biosurfactant was more pronounced in the test reactors at higher glucose concentrations. For relatively higher initial glucose concentrations, however, effluent chlorophenol concentration increased in the control reactor. Wastewater treatment plants generally receive influent with a mixture of recalcitrant synthetic organic chemicals (SOCs) and biogenic substrates. This means that SOCs and biogenic compounds often coexist in many wastewater reactors. Interactions among these multiple substrates are complex, partially due to the

toxicity, competition for enzymes and cofactors (Hu, Ferraina, Ericson, & Smets, 2005). Therefore, the question to be answered is how biogenic substrate concentration affect the removal efficiency of SOCs especially when SOCs are present in mixture. Many researchers claim that a specific competent biomass fraction is responsible for the degradation of specific compound, which is equal to the fraction of COD contributed to the feed by the compound (Hu et al., 2005; Hu, Ferraina, Ericson, MacKay, & Smets, 2005; Ellis, Barbeau, Smets, Grady, 1996; Tomei, Annesini, Luberti, Cento, & Senia, 2003). This means that the presence of biogenic substrate does not guarantee the enhanced biodegradation of SOCs (Hu et al., 2005). For example, Kulkarni & Chaudhari (2006) reported that degradation rate of *p*-nitrophenol decreased with the addition of glucose. Similarly, Ulusoy & Şengül (2002) reported that the removal efficiency of 4-CP decreased as the concentration of glucose was increased at the same feeding rate and reaction time, indicating competitive inhibition between glucose and 4-CP at high glucose concentrations. In another study, Hu et al. (2005) reported that at standard oxygen conditions 4-CP degradation rate decreased with the supplementation of biogenic substrate, whereas 2,4-DCP degradation rate increased in the presence of biogenic substrate. These results showed that different chemicals may give different response to the presence of biogenic substrate. However, chlorophenol removal was higher in test reactors due to presence of biosurfactant even when the glucose concentration was increased in this study. Presence of biosurfactant can enhance the transformation rate of chlorophenol significantly.

In summary, there are many mechanisms that are effective for the enhancement of hydrocarbons biodegradation with the addition of biosurfactants. An important one is solubilization of hydrophobic compounds. In this experimental study, since 2,4-DCP and 4-CP are added below solubility level, this effect does not exist. However, resolubilization of some part of adsorbed 2,4-DCP and 4-CP from the biomass is possible.

Mechanisms that are assumed to be effective in enhancement of chlorophenol degradation are summarized below:

- The positive effect of concurrent utilization of a second substrate (i.e. biosurfactant) may be mentioned. Biosurfactants cause microbial growth enhancement (Vardar-Sukan & Kosaric, 2000).

- The presence of biosurfactant does not only stimulate bacterial growth, but also induces enzymes (proteins) that may be needed to further break down contaminants (Zhang et al., 1998).

- Biosurfactant addition may retard the toxic effect of chlorophenols by acting as buffer. Vardar-Sukan and Kosaric (2000) reported that biosurfactants can be effectively used in detoxification of industrial effluents. Activated sludge systems are sensitive to toxic compounds. The presence of biosurfactant in the medium may attenuate chlorophenol toxicity on the microorganisms and help to maintain sludge bioactivity. In fact, in this study, this effect has been observed many times.

- The addition of biosurfactant increases the oxygen uptake over the control. Jahan et al. (1999) reported similar results.

- Microbial uptake of chlorophenols may become easier since biosurfactant binds chlorophenols tightly in the micelle.

- Chlorophenols are sorbed to the microorganisms flocs by a mechanism known as biosorption. Addition of biosurfactant results in resolubilization of sorbed chemicals to be consumed by bacteria decreasing the toxic effect on bacteria.

6.2 Recommendations

The interaction between biosurfactant and the chlorophenols in wastewater is a very complex phenomenon. But it is possible to say that biosurfactants are very efficient in the removal of chlorophenols as indicated in this study. More information

is required concerning the interaction of the biosurfactants and the chlorophenols, relationship of biosurfactant structure and contaminant removal, and understanding of the factors influencing the biodegradation of the compounds by enhanced biodegradation. Other recalcitrant chemicals should also be used in experimental studies before this subject becomes a concern in industrial applications.

Economical analysis should be performed in the industrial applications.

The overall production cost of the biosurfactant, which consists of biosynthesis cost and purification cost, varies very much depending on the biosurfactant type and its purity. Producing more pure biosurfactant products increases overall costs of the biosurfactant. The advantage of biosurfactants in wastewater treatment applications is that high purity is not an obligation although high purity (99% or more) is always required in the food and cosmetic applications. The rapid development in biosurfactant production and purification technologies decreases its costs.

The applications of biosurfactants in the environmental applications have potentially increased in recent years. However, more information is needed to be able to predict and model their behaviour. Full scale tests will be required. The role of biosurfactants in natural attenuation processes has not been determined. Very little information is available concerning the influence on chlorophenols biodegradation with biosurfactant. As most of the research until now has been performed with rhamnolipids, other biosurfactants need to be investigated as they may have promising properties.

Biosurfactants should be also investigated in the treatment of certain industrial wastewaters such as petrochemical, petroleum refinery wastewaters in order to determine industrial wastewaters in which biosurfactant may be effectively applied.

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NOMENCLATURE

2,4-DCP **:** 2,4-Dichlorophenol, mg/l

$2,4\text{-DCP}_0$ **:** Influent 2,4-Dichlorophenol concentration, mg/l

2,4-DCPe **:** Effluent 2,4-Dichlorophenol concentration, mg/l

4-CP **:** 4-Chlorophenol, mg/l

4-CP_0 **:** Influent 4-Chlorophenol concentration, mg/l

4-CPe **:** Effluent 4-Chlorophenol concentration, mg/l

COD **:** Chemical oxygen demand, mg/l

COD_0 : Influent chemical oxygen demand, mg/l

CODe **:** Effluent chemical oxygen demand, mg/l

COD/N/P **:** COD / NH_4-N **/** PO_4-P ratio

CMC **:** Critical micelle concentration, mg/l

DO **:** Dissolved oxygen demand, mg/l

HRT **:** Hydraulic retention time, h or day

θc **:** Sludge age, days

θ_H **:** Hydraulic retention time, h

MLSS **:** Mixed liquor suspended solids, mg/l

Q **:** Flow rate (V/θ_H), l/h

Rpm **:** Revolution per minute

SRT **:** Solid retention time, h or day

T **:** Temperature, ºC

V **:** Volume of the liquid in reactor, l

X **:** Biomass concentration in the reactor, mg/l

APPENDIX 1

Table A-1.1 Application of biosurfactants (Vardar-Sukan & Kosaric, 2000)

Area	Use	Effect
Metals	Concentration of ores, cutting, and forming, casting, rust and scale removal, plating	Wetting, foaming, emulsifying, lubrication, corrosion, inhibition in rolling oils, mold release additives in pickling and electric cleaning, electrolytic plating
Paper	Pulp treatment, paper machine, calendar	Deresinification, washing, defoaming, color leveling and dispersing, wetting and levering, coating and coloring
Paint and protective coating	Pigment preparation, latex paints, waxes and polishes	Dispersion and wetting of pigment during grinding, emulsification, stabilize latex, retard sedimentation and pigment separation, stabilize emulsions, antistat
Petroleum products and production	Drilling fluids, workover of producing wells, producing wells, second recovery, refined products	Emulsify oil, disperse solids, modify rheological properties of drilling fluids for oil and gas wells, emulsify and disperse sludge and sediment in cleanout of wells, demulsifying crude petroleum, inhibit corrosion of equipment in flooding operations, preferential wetting detergent sludge dispersant and corrosive inhibitor in fuel oils crank case oils and turbine oils
Textiles	Preparation of fibers, dyeing and printing, finishing of textiles	Detergent and emulsifier in raw wool scoaring, dispersant in viscouse rayon spin bath, lubricant and antistant in spinning of hydrophobic filaments, wetting penetration, solubilization emulsification, dye leveling, detergency, finishing formulations, softening, antistatic additive to finishes
Building and construction	Paving, concrete, ceramic	Improve bond of asphalt to gravel and sand, promote air entrainment

Area	Use	Effect
Agriculture	Phosphate fertilizers, spray application	Prevent caking during storage, wetting, dispersing, suspending of powdered pesticides and emulsification of pesticides solutions
Elastomers and plastics	Emulsion polymerization, foam polymers, latex adhesive, plastic articles, plastic coating and laminating	Wetting, solubilizaton, emulsification of monomers, introduction of air, control of cell size, improve bond strength, antistatic agents
Food and beverages	Food processing plants, fruits and vegetables, bakery and ice cream, crystallization of sugar, cooking fat and oils	For cleaning and sanitizing, improve removal of pesticides and wax coating, solubilization flower oils, control consistency, regard stalling, improve washing, reduce processing time, prevent spattering due to superheat and water
Industrial cleaning	Janitorial supplies, descaling, soft goods	Detergents and sanitizers, wetting agents and corrosion inhibitors in acid cleaning of boiler tubes and heat exchangers

Area	Use	Effect
Cosmetics and pharmaceuticals	Insect repellent, antacid, bath products, acne pads, antidandruff products, contact lense solution, hair color and care products, deodorant, nail care, body massage accessories, lipstick, lipmaker, eye shadow, mascara, soap, toothpaste and polish, denture cleaner, adhesives, antiperspirant, lubricated condoms, baby products, food care, mousse, antiseptics, shampoo, conditioner, shave and depilatory products, moisturizer, health and beauty products moisturizer, health and beauty products	Emulsifier, foaming agent, solubilizer, wetting agent, cleanser, antimicrobial agent, mediator of enzyme action
Pollution control	Soil bioremediation, oil storage tank cleanup, removal of oil spills	Emulsifiers, deemulsifiers

APPENDIX 2

JBR 425
PRODUCT DATA SHEET

Product Description:

JBR 425 is an aqueous solution of rhamnolipids at 25% concentration. It is produced from sterilized and centrifuged broth that has had all protein removed and partially decolorized. Two major rhamnolipids, RLL (R1) and RRLL (R2), are present. Chemically, rhamnolipids are glycosides of rhamnose (6-deoxymannose) and β-hydroxydecanoic acid.

Formal Chemical Names, CAS Registry Numbers and Molecular Formulae:

R1 or RLL

Decanoic acid, 3-[(6-deoxy- -L-mannopyranosyl)oxy]-, 1-(carboxymethl)octyl ester (CA INDEX NAME)

CAS Registry Number: 37134-61-5

Molecular Formula: C_{26} H_{48} O_9

R2 or RRLL

Decanoic acid, 3-[[6-deoxy-2-O-(6-deoxy- -L-mannopyranosyl)- -L-mannopyranosyl]oxy]-, 1-(carboxymethl)octyl ester (CA INDEX NAME)

CAS Registry Number: 4348-76-9

Molecular Formula: C_{32} H_{58} O_{13}

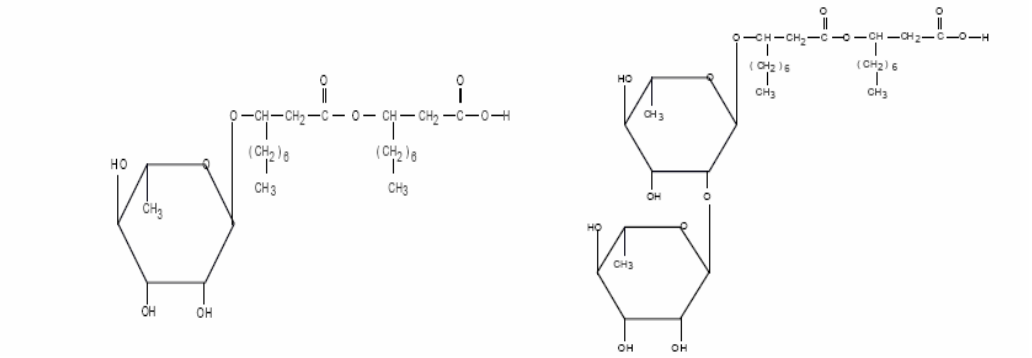
Mixture R1 or RLL and R2 or RRLL

Decanoic acid, 3-[[6-deoxy-2-O-(6-deoxy- -L-mannopyranosyl)- -L-mannopyranosyl]oxy]-, 1-(carboxymethl)octyl ester, mixt. with 1-(carboxymethyl)octyl 3-[(6-deoxy- -L-mannopyranosyl)oxy]decanoate (CA INDEX NAME)

CAS Registry Number: 14758-26-2

Molecular Formula: C_{32} H_{58} O_{13}, C_{26} H_{48} O_9

Structures:



R1 or RLL **R2 or RRLL**

Typical Properties:

Appearance: Dark amber solution

Odor: Soapy

Specific Gravity: 1.05-1.06

pH: 6.5-7.0

Solubility in Water: Soluble at neutral pH

Suitable Diluents: Water, most common alcohols

Surface and Interfacial Tension:

Surface: 29 dynes/centimeter (Surfactant diluted in water; concentration >50 ppm, Surface tension measured by ring method of du Nuoy Fisher tensiometer).

Interfacial: 0.3 dynes/centimeter (Surfactant diluted in water; concentration >40 ppm, paraffin oil layered on top of solution, interfacial tension measured by pulling ring from water phase).

Volatility: Not volatile

Stability:

Stable at room temperature

Stable to 121 ºC for at least one (1) hour

Unstable at extreme pH due to hydrolysis of the glycosidic linkage between sugar and lipid.

Environmental Characteristic:

Tests were accomplished in accordance with OECD (Organization for Economic Cooperation and Development) 301D, OECD 209, and OECD 202 for ready biodegradability, Activated Sludge Respiration Inhibition (ASRIT) and aquatic toxicity to daphnia. These results were to determine whether or not the chemical has the potential to cause problems in waste treatment plants or in the environment.

OECD 301D “Ready Biodegrdability”

The biosurfactant showed ready biodegradability by demonstrating a biodegradability rate of 68.4% on day 10 of the 28-day test cycle. This is an excellent test result clearly demonstrating that JBR425 is readily biodegradable.

OECD 209 “ASRIT”

The biosurfactant showed no toxicity to activated sludge biomass with an EC_{50} >1000 mg/l. This is the best test result possible. This means that this surfactant could be discharged to a waste treatment plants at concentrations >1000 ppm or 0.1% with no adverse effects.

OECD 202 “Aquatic Toxicity to the Water Flea, *Daphnia* Magna”

The biosurfactant demonstrated acute toxicity to *Daphnia* Magna with an EC_{50} of 36.1 mg/l. This is an extremely low toxicity for a commercial surfactant.

Toxicity:

Toxicity testing was done in accordance with U.S. EPA guidelines by an independent laboratory. A 9.5% rhamnolipid concentration aqueous solution was tested except as noted. Results follow:

Acute oral toxicity:

The Acute Oral LD_{50} is greater than 5,000 mg/kg. Toxicity Category IV.

Acute dermal toxicity:

The Acute Dermal LD_{50} is greater than 5,000 mg/kg. Toxicity Category IV.

Acute inhalation toxicity:

The Acute Inhalation LC_{50} is greater than 2.05 mg/l. Toxicity Category IV.

Dermal Irritation:

Slightly irritating. Irritation cleared by 72 hours. Toxicity Category IV.

Ocular irritation:

Moderately irritating. Toxicity Category I. At 1% rhamnolipid concentration, Toxicity Category III.

Application Data:

In aqueous solutions, JBR 425 has a very low Critical Micelle Concentration indicating the strong surface activity shown at low concentrations, characterized by low surface tension for water and electrolyte solutions with very low interfacial tensions for water/hydrocarbon systems.

JBR 425 can produce stable close-celled foams in aqueous solutions and acts as a foam stabilizer for other surfactants, particularly anionic and amphoteric compounds, JBR 425 also exhibits good tolerance to Calcium ions and is as effective foaming agent for incorporation into soap-based products.

JBR 425 is an excellent emulsifier and co-emulsifier for a wide range of organic solvents producing emulsions of greatly enhanced stability. JBR 425 also exhibits corrosion inhibition properties in aqueous solution, particularly towards ferrous metals.

The general chemical and physical characteristics of JBR 425, with the high degree of surface activity produced at very low concentrations, indicates the use of JBR 425 as a performance enhancing additive in a wide range of application areas.

Application Examples:

Typical examples utilizing the performance enhancing effects of JBR 425 in various surfactant-based application follow:

(Suggested starting concentrations: Active rhamnolipid ingredient: 1.0, 0.1, 0.01%)

Agriculture:

Anti-viral agent to control mosaic viruses
Wetter, emulsifier and adjuvant for herbicidal and pesticidal systems
Dispersant for wettable powders

Environmental Remediation:

Soil washing to remove hydrocarbons and heavy metals
Wastewater treatment to remove hydrocarbons and heavy metals
Chelating agent
Oil slick dispersant

Metal Processing:

Concentration of ores
Emulsifier and corrosion inhibitor for metal working fluids for cutting and forming

Wetting, emulsification, lubrication and corrosion inhibition in rolling oils, cutting oils and lubricants

Mold release additive in casting manufacture

Pickling and electrolytic cleaning, coating and plating

Corrosion inhibitor

Wetting and foaming in electrolytic plating.

Paper Processing:

Pulp derinsinification and washing agent

Personal Care:

Foam booster for bubble baths, shampoos, toothpastes, shaving cream/foam/gel, toothpaste, and other soap-based products.

Emulsifier for formulated skin creams, conditioners, degreasers & cleansers.

Wetter and detergent for contact lens cleaners and soaking solutions.

Other application include:

Elastomers and plastics

Electronic clenaners

Environmental remediation

Fire containment

Foam fractionation

Industrial and institutional cleaning

Leather processing

Paint and protective coatings

Petroleum production and products