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Biodegradation of 4-CP in an activated sludge reactor: Effects of biosurfactant and the sludge age

Ayla Uysal [a,*], Aysen Turkman [b,c]

[a] *Department of Environmental Engineering, Suleyman Demirel University, Isparta, Turkey*
[b] *Department of Environmental Engineering, Dokuz Eylul University, Tınaztepe Campus, Buca, Izmir, Turkey*
[c] *Lefke European University, Lefke, North Cyprus*

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Abstract

The biosurfactant's effect on the biodegradation of 4-chlorophenol (4-CP) in the existence of glucose was researched under the circumstances of using unacclimated culture and various sludge ages. The removal efficiencies of chemical oxygen demand (COD) and 4-CP, the growth of biomass and specific substrate removal rates were examined under various operating conditions. When 150 mg/l concentration of 4-CP was applied, glucose and 4-CP degraded in the same period in the unacclimated bioreactors where biosurfactant was added. Nevertheless, the COD removal in the control reactor noticeably decreased and when compared with reactors which biosurfactant was added, a longer period was needed for the degradation of 4-CP in this reactor. While the complete removal of 4-CP in the control reactor eventuated on the 14th day, in the reactor which 2 × critical micelle concentration (CMC) was added the complete removal of 4-CP eventuated on the end of the 1st day. These results showed that addition of biosurfactant reduced the transient time before the steady-state. COD and 4-CP removal performances were improved by increasing the sludge age. No difference in system performance was observed at high sludge ages in the absence and presence of biosurfactant. However, the performance of the system in the presence of biosurfactant was satisfactory even at low sludge ages. That is, the system should be operated either at high sludge ages (>15 days) in the absence of biosurfactant or at low sludge ages (<15 days) in the presence of surfactants.

Keywords: Activated sludge; Biodegradation; Biosurfactant; 4-Chlorophenol; Sludge age

1. Introduction

Monochlorophenols (2-chlorophenol, 3-chlorophenol and 4-chlorophenol) which carry toxic and carcinogenic features are mainly used in the production of polychlorinated phenols and in the extraction of sulfur and nitrogen compounds from coal [1–4].

For the treatment of wastewaters that contain phenols and there derivatives; adsorption, air stripping, chemical oxidation, solvent extraction, ultraviolet light, ozone, etc., physical and chemical treatment methods are used [5]. Nevertheless, besides the difficult degradable structures of chlorophenols, because of economic reasons and the formation of by-products being low, biological treatment is much more attractive then other treatment methods.

Under aerobic conditions a lot of studies have been done on the degradation of 4-CP in the fed-batch reactor [6,7], sequencing batch reactor [8–11] and special culture [12–14]. In continuous operation activated sludge the biodegradation of 4-CP has been mentioned in a small amount of studies [15]. Nevertheless, when the practical appliances of engineering systems are considered, mixed culture and continuous operating systems in biologic treatment has won importance. In general, for the treatment of toxic compounds, because of its microbial diversity activated sludge systems are suggested to be used. Nevertheless, when wastewaters contain toxic compounds, very often operating problems occur in the activated sludge processes. Vardar-Sukan and Kosaric [16] have mentioned that biosurfactants could be effectively used for detoxification in industrial effluents.

There are a lot of mechanisms that are effective for the enhancement of hydrocarbons biodegradation with biosurfactant. The most important mechanism is which the biosurfactant increases the dissolving of hydrophobic compounds. In this study, because that 4-CP is added into the activated sludge

* Corresponding author. Tel.: +90 2462111697; fax: +90 2462111852.
E-mail address: auysal@mmf.sdu.edu.tr (A. Uysal).

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under the dissolving level, this effect is not existent. Because of the interaction between the surfactant, organic compound and microorganisms, the existence of the surfactant affects the biologic processes. Biosurfactant molecules form aggregates in water and they are called micelles. Biosurfactant seems to bind pollutants tightly in the micelles [17]. Microorganisms' cells are able to take up the pollutant from the micelle – to a certain extent – by fusion with the cell membrane [18]. This event could have implications for microbial uptake.

Many of researchers indicated that surfactant enhancement in the microbial degradation of the organic contaminants [19–22]. However, there are no studies in the literature on enhanced biodegradation of 4-CP using a biosurfactant in an activated sludge bioreactor. The objective of this study is to investigate and evaluate the potential utility of biosurfactant on 4-CP degradation in unacclimated activated sludge system and determine system stability under toxic loading conditions. To investigate the effect of biosurfactant on 4-CP biodegradation in a continuously operated activated sludge bioreactor study, in different sludge ages (3–25 days) are applied in the experimental study.

2. Materials and methods

2.1. Experimental system

A continuous stirred tank reactor (CSTR) made up of stainless steel was used in the experimental study. The volume of the aerobic reactor was 8.75 l and the volume of the settling unit, 1.15 l. The influent wastewater was continuously fed through the top of the reactor by a feed pump and the reactor was aerated by an air pump. The passage of the effluent wastewater from the aeration tank to the sedimentation tank was through the holes in the inclined plate. The effluent from the sedimentation tank was collected in an effluent tank. The sludge age was adjusted by discarding a certain volume of activated sludge from the aeration step of the aerobic reactor every day.

2.2. Organisms and wastewater composition

A mixed culture was used in the aerobic reactors. The activated sludge culture was obtained from the wastewater treatment plant of Pak Maya Bakers Yeast Company in Izmir, Turkey. The aerobic reactors were inoculated with this culture.

The synthetic wastewater used throughout the studies was composed glucose as carbon source, urea as nitrogen source (50 mg/l), KH_2PO_4 as phosphorus source (10 mg/l), $MgSO_4{\cdot}7H_2O$ (75 mg/l), $CaCl_2$ (50 mg/l), $FeCl_3$ (2 mg/l) and desired concentration of 4-CP (1 mg 4-CP = 1.62 mg COD). The concentrations of nitrogen and phosphorus were adjusted to maintain COD/N/P ratio of 100/10/2 in all the experiments. Water solubility of 4-CP is about 27 g/l at 20 °C [23]. 4-CP was dissolved in water solution to prepare 5000 mg/l stock solution and added directly from stock solution to obtain the desired initial concentration. COD concentration was kept constant at 1500 mg/l in 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 2 × CMC (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 2 × CMC). Consequently, adding glucose value for synthetic wastewater was determined by both 4-CP and biosurfactant amounts.

2.3. Biosurfactant

The rhamnolipid (designated JBR 425) was kindly donated by Jeneil Biosurfactant Company, Saukville, WI, USA as a mixture of RLL and RRLL. RLL had the chemical formula $C_{26}H_{48}O_9$, and RRLL, $C_{32}H_{58}O_{13}$. JBR 425 was chosen since the biosurfactant showed no toxicity to activated sludge biomass with an effective concentration (EC_{50}) = 1000 mg/l [24].

In order to evaluate the biodegradability of biosurfactant by the authors, 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 h [25].

2.4. Analytical methods

Samples were centrifuged at 6000 rpm for 25 min to remove biomass and other solids from the liquid medium. The clear supernatant was analyzed for COD and 4-CP. The standard method based on digestion and reflux was used for COD analyses. The 4-CP analyses were carried out on the clear supernatant using the 4-aminoantipyrine colorimetric method based on the procedure detailed in standard methods for the examination of water and wastewater [26]. Biomass concentrations in the liquid phase were determined by filtering samples from 0.45 μm pore size membrane 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 a WTW MultiLine P3 pH/OXI-SET Analyser. The dissolved oxygen probe contains also a temperature probe which is 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.

2.5. Experimental procedure

Experiments were started in batch wise manner. Activated sludge from an industrial wastewater treatment plant which containing no toxic substance was added a reactor as seed source. The synthetic wastewater which including 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. Three reactors with the same structure and volume as described above were used in parallel tests.

In the first stage of experiments, the effect of biosurfactant on 4-CP load on unacclimated activated sludge in the control (R1) and test reactors (R2 and R3) were studied. In the control reactor (R1), feed water did not contain any biosurfactant in order

to determine the effect of biosurfactant on the removal of 4-CP. The feed water of the parallel reactors (test reactors; R2 and R3) contained 4-CP and biosurfactant. A 15 mg/l (CMC) and 30 mg/l (2 × CMC) biosurfactant concentration were added to R2 and R3. HRT was kept constant at 17 h through operation period. SRT was controlled at about 15 days. Firstly, the three reactors were inoculated with the activated sludge. 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 concentration period was started. During 4-CP loading period, influent COD concentration was kept constant at 1500 mg/l. At the end of the 150 mg/l 4-CP concentration, in this study same experiment was conducted at 300 mg/l 4-CP concentration.

In the second stage of experiments, the effect of biosurfactant on 4-CP biodegradation in activated sludge reactor was investigated with the variation of the sludge ages in the control (R1) and test reactors (R2 and R3). The continuous activated sludge experiments were performed at different sludge ages between 3 and 25 days while hydraulic residence time was being kept constant throughout the experiments at $\theta_H = 17$ h. The feed COD and influent 4-CP concentration were kept constant throughout the experiments as $COD_0 = 1500$ mg/l and $4\text{-}CP_0 = 250$ mg/l. Sludge was removed from the reactor periodically to adjust the sludge age to the desired level, and it was varied gradually from the highest to the lowest level. Each experiment was conducted until the systems reached the steady-state condition, yielding the same COD and 4-CP concentrations 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 4-CP contents after centrifugation.

In all the experiments, temperature and pH were kept at $T = 20 \pm 2\,^{\circ}C$ and pH 7 ± 0.6. Dissolved oxygen (DO) concentration was kept about 3 mg/l in the reactors.

3. Results and discussion

3.1. Cometabolic degradation of 4-CP in unacclimated culture

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

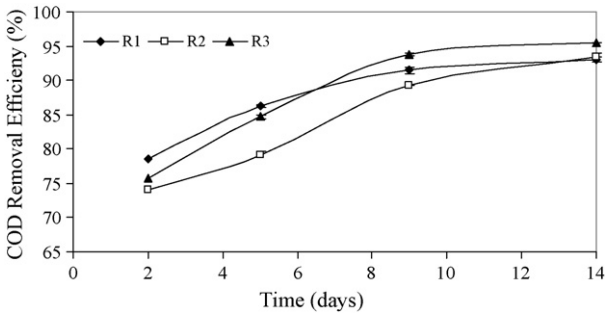

Fig. 1. COD removals in the reactors during glucose acclimation periods.

COD removal efficiencies of the reactors during the glucose acclimation periods were presented in Fig. 1. When no 4-CP loading was applied, COD removal efficiencies remained steady at 93, 93.35 and 95.42% in R1, R2 and R3.

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

Table 1 showed that the 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. Finally 4-CP removal efficiency reached to 95.79% in the 14th day in R1. 4-CP removal efficiency was increased to 93.34 and 98.59% in the 7th and 10th days in R2. However, 98.97% removal efficiency was observed even in the 1st days in R3. In unacclimated activated sludge, the complete biodegradation of 4-CP required longer time in R1 relative to R2 and R3. Reactor R3 had nearly completely abolished 150 mg/l 4-CP at the end of the 1st day. Sahinkaya and Dilek [6] reported that in acclimated activated sludge 130 mg/l 4-CP had abolished nearly completely at the end of 1st day in fed-batch reactor. According to this result, R3 system has acted like an acclimated culture in this study. The degradation rates of 4-CP ranged between 4.49–8.30, 5.17–8.80 and 8.73–8.80 mg/(l h) in R1, R2 and R3 respectively for 21 days which are operation periods. 4-CP degradation rate for 1st day in control reactor was about two times lower than in R3. Higher

Table 1
The transient time data (the time required to reach the steady-state) for the reactors under different conditions

Time (days)	Set 1 (in 150 mg/l 4-CP concentration)									Set 2 (in 300 mg/l 4-CP concentration)		
	4-CP removal efficiency (%)			COD removal efficiency (%)			Biomass concentration (mg/l)			4-CP removal efficiency (%)		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
1	50.93	58.54	98.97	82.73	86.66	95.33	1925	2225	2550	43.66	49.37	54.17
4	28.69	62.92	99.08	78.49	87.19	95.52	1150	2000	2625	23.14	21.13	26.24
7	34.82	93.34	99.18	79.06	90.21	94.62	1075	2375	2500	17.55	18.73	23.12
10	54.45	98.59	99.22	82.48	91.67	92.94	925	2575	2950	16.42	14.62	20.46
14	95.79	98.89	99.75	90.21	93.07	94.96	1375	2750	3025	10.24	10.75	12.05
17	94.07	99.76	99.79	84.46	92.23	91.05	1400	2725	2975			
20										18.27	20.68	20.79
21	94.07	99.79	99.70	85.02	93.53	95.41	1450	2725	2975			

degradation rates were observed in the presence of biosurfactant. In the study of Wang and Loh [12], 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).

In this study, the pH of the 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 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 the activity of the microorganisms was being lowered in the control reactor. Consequently, the biosurfactant systems exhibited higher tolerance to loading of 4-CP than the control system.

The removal efficiencies of COD in the reactors during 4-CP loading period were demonstrated in Table 1. After the start of 4-CP loading, COD removal efficiency was reduced up to 82.48% in the 10th day in R1. COD removal efficiency was decreased to 86.66% in 1st day 4-CP loading in R2. After the 1st day 4-CP loading, COD removal efficiency was increased in R2. In R3 system, COD and 4-CP were degraded simultaneously and COD removal was not adversely affected after 4-CP loading. At the end of 4-CP loading period, COD removal efficiencies were 85.02, 93.53 and 95.41% in R1, R2 and R3, respectively. At the end of glucose acclimation periods, when 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 and they ranged between 72.99–75.02, 76.47–82.53 and 84.12–84.19 mg/(l h) in R1, R2 and R3 for 21 days in 4-CP loading periods. The 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 and increased in biosurfactant concentration has resulted in much better removal efficiency.

The variation of the biomass concentration in the reactors during 4-CP loading period is shown in Table 1. 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 and Dilek [6] demonstrated that the value of IC_{50} (concentration causing 50% inhibition) was 130 mg/l when unacclimated culture was used. 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, more in R3 owing to the higher biosurfactant concentration.

The introduction of 4-CP concentration 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 the 1st day, they were only 18.27, 20.68 and 20.79% in R1, R2 and R3 at the end of 20 days for the 4-CP concentration of 300 mg/l (Table 1). The response of all the reactors to high 4-CP load was similar and the removal efficiencies were quite near.

Wang and Loh [12] 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 and Dilek [6] 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 concentration, biosurfactant containing systems failed to degrade 4-CP effectively, which caused the increase of effluent 4-CP continuously.

3.2. Effect of sludge retention time (SRT) on the reactors performance for 4-chlorophenol treatability

A set of experiments were performed at six different sludge ages varying between 3 and 25 days when the feed COD, 4-CP concentrations and the hydraulic residence time were constant at 1500, 250 mg/l and 17 h, respectively.

3.2.1. Effect of SRT on 4-CP removal efficiency

The 4-CP removal efficiency as a function of sludge age is given in Fig. 2. For sludge ages between 20 and 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 to 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. The 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 higher biomass concentrations at high sludge ages. Also, Kargi and Eker [27] reported that percent DCP removals were 15, 22 and 100% at the

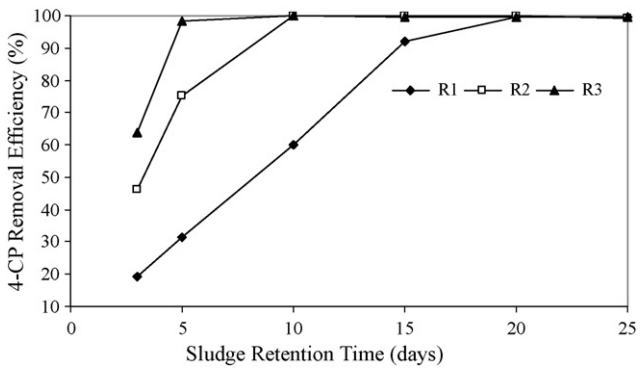

Fig. 2. Comparison of removal efficiency of 4-CP in the control and test reactors at different SRT values.

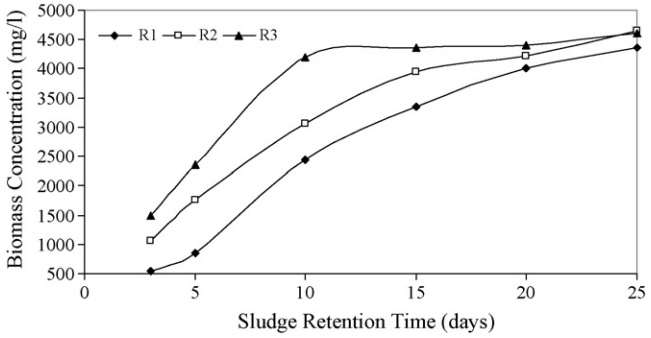

Fig. 3. Comparison of biomass concentration in the control and test reactors at different SRT values.

sludge ages of 5, 15 and 30 days in activated sludge unit, respectively ($COD_0 = 2500$ mg/l, $DCP_0 = 200$ 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. Nevertheless, the systems into which biosurfactants were added were not negatively affected by low sludge ages (up till 3 days).

The variation of the biomass concentration with the sludge age is shown in Fig. 3. The effect of 4-CP on biomass concentration was lower in R2 and R3 as compared to R1. R2 and R3 were able to cope with toxicity of 4-CP due to added biosurfactant. Increasing biosurfactant concentration led to higher biomass concentration in R3, thus, the microorganisms gained more resistance against 4-CP toxicity. In lower sludge ages (<15 days), in R1 biomass concentration more decreased than R2 and R3. 4-CP affected microorganisms especially at the lower sludge ages in R1 because of decreasing in mean cell residence time (lower sludge age). Decreasing in mean cell residence time resulted in toxic effect of 4-CP on microorganisms in the control reactor due to insufficient adaptation period. In the test reactors (R2 and R3), added biosurfactant 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.

The variation of specific removal rate of 4-CP [$R_{4\text{-CP}} = Q(4\text{-CP}_0 - 4\text{-CP}_e)/VX$] with sludge age is depicted in Fig. 4. 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 were resulted different biomass concentrations, the reactors with longer SRT had more biomass, and consequently lower specific removal rate. The short SRT resulted in a higher specific biodegradation rates due to lower concentration of microorganisms in the reactors. So, 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.

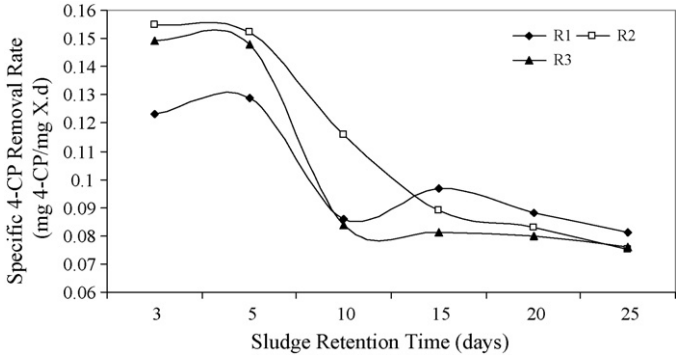

Fig. 4. Comparison of specific removal rate of 4-CP in the control and test reactors at different SRT values.

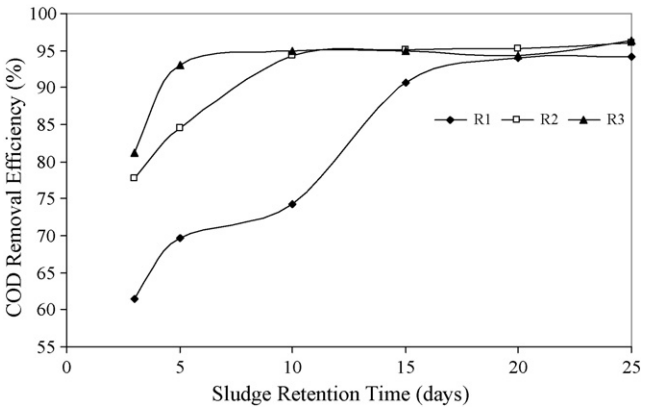

Fig. 5. Comparison of removal efficiency of COD in the control and test reactors at different SRT values.

3.2.2. *Effect of SRT on COD removal efficiency*

Fig. 5 shows the effect of SRT on COD removal efficiency. For the sludge ages between 10 and 25 days, although COD removal efficiency remained almost above 94% in R2 and R3, it ranged from 74.26 to 94.13% in R1. When sludge age decreased from 15 to 10 days, sharply decreasing 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 of 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.

Fig. 6 the depict variation of the specific COD removal rate [$R_{COD} = Q(COD_0 - COD_e)/VX$] with the sludge age in reactors

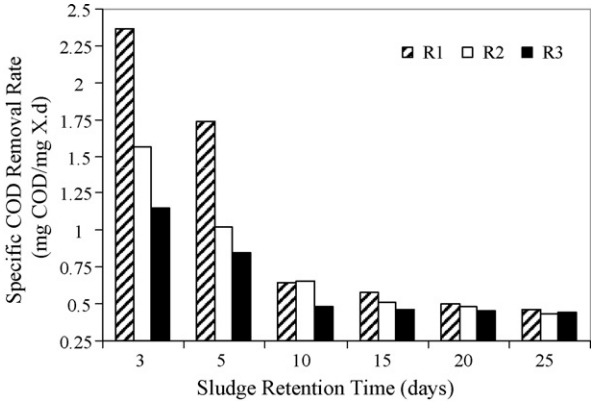

Fig. 6. Comparison of specific removal rate of COD in the control and test reactors at different SRT values.

R1, R2 and R3. Steady decrease in specific rate of COD removal with the sludge age is observed because of increasing of biomass concentration with increasing sludge age. Due to higher biomass concentration in R3, the specific removal rates 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.

4. Conclusions

There are no differences between the efficiencies of the control and biosurfactant reactors at sludge ages bigger then 15 days. Nevertheless, when activated sludge systems are operated in sludge age range of 3–15 days, they show a higher quality performance on both treatment and sludge quality. Added biosurfactant activated sludge systems were more effective at shorter sludge ages (<15 days) compared to control reactor in this study. Furthermore, it is observed that adding higher concentration of biosurfactant (up to 2 × CMC) showed no toxic effect for activated sludge. Also, addition of biosurfactant reduces the transient time before the steady-state.

Under conditions that acclimation has not been done or that when there is not enough cell retention time (at low sludge age), the biodegradation of substances containing chlorine by microorganisms is quite difficult because of the forms of substances containing chlorophenols being difficult to be degraded.

Biosurfactant molecules which are critical micellar concentration form aggregates in water and they are called micelles. Biosurfactant seems to bind 4-CP tightly in the micelles. Following mechanisms might be responsible for the transfer of 4-CP from micelles to the microorganism:

1. The addition of biosurfactant changes cell structure, facilitating the direct contact between cells and 4-CP, which occur in crystalline or droplet form.
2. Due to a close contact of micelles with the cell envelope, perhaps accompanied by membrane fusion, the microorganism are able to take up the 4-CP from the micellar core – to a certain extent – by fusion with the cell membrane.

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 4-CP as indicated in this study. Although a positive effect of biosurfactant addition can be stated, it is, however, not possible to conclude from these results, only to the increased biomass. Therefore, more information is required concerning the interaction of the biosurfactants and the chlorophenols, the relationship of the biosurfactant structure and contaminant removal, and the understanding of the factors influencing the biodegradation of the compounds by enhanced biodegradation.

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