Enhanced biodegradation of 4-chlorophenol using biosurfactant in an activated sludge

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Biosurfactants enhance the removal of some recalcitrant pollutants in contaminated water and soil. The production and usage of man-made chemicals in industry has led to the entry of any xenobiotics into the environment. One such group of xenobiotics is chlorinated phenols. In this study, the treatment performance of 4-chlorophenol (4-CP) was investigated using a biosurfactant added activated sludge bioreactor system with changing food to mass (F/M) ratio. JBR 425 rhamnolipid was used as biosurfactant. A control reactor (without biosurfactant; R1) and a test reactors (with biosurfactant addition; R2 and R3) were used in parallel tests. Three lab-scale continuous reactors were run in parallel with the same chemical oxygen demand (COD) and 4-CP loading rates. The effect of F/M ratio on the COD and 4-CP removal efficiencies was investigated in the reactors. F/M ratio lower than 0.5 day^{-1} has no significant effect on the 4-CP volumetric removal rates in all reactors. At the high F/M ratios ()0.5 day^{-1}), volumetric removal rate of 4-CP was greatly enhanced in R2 and R3 as compared to R1 due to higher biomass concentrations.

Keywords Activated sludge; biosurfactant; 4-chlorophenol; F/M ratio; sludge retention time.

1. Introduction

Chlorophenols are introduced to the environment through man-made activities, such as waste incineration, uncontrolled use of wood preservatives, pesticides, fungicides and herbicides as well as bleaching of pulp with chlorine. Due to their high toxicity, strong odor emission and persistence in environment and suspected carcinogenic and mutagenic characteristics to the living organisms, chlorophenols pose a serious ecological problem as environmental pollutant [1]. Their fate in the environment is of great importance. Hence, the removal of phenol and chlorinated organic compounds from wastewater is a necessary task to conserve the water quality of natural water resources [2]. 4-CP is formed from chlorination of wastewater, from chlorine bleaching of pulp and from breakdown of phenoxy herbicides such as 2,4-dichlorophenoxyacetic acid [3]. 4-CP is used as a disinfectant in homes, farms, hospitals, and as an antiseptic for root canal treatment.

Several physical, chemical and biological methods including activated carbon adsorption, ion exchange, incineration and biological degradation have been proposed for treating or recovering chlorophenolic compounds [4]. Despite the recalcitrant nature of chlorophenols, there are still some efforts being made toward their biological treatment with specialized culture conditions, because of economical reasons and a low possibility of by-product formation. The microorganisms used are usually aerobes. Aerobes are more efficient at 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 [5].

Surfactants can either be chemically synthesized (synthetic) or microbially produced (biosurfactants). Biosurfactants are usually classified based on their biochemical nature and the microbial species producing those [6]. For specific applications, biological surfactants have advantages over synthetic surfactants due to their structural diversity, biodegradability, and effectiveness at extreme temperatures, pH and salinity [7]. Biosurfactant applications in the environmental industries are promising due to their biodegradability, low toxicity and effectiveness in enhancing biodegradation and solubilization of low solubility compounds. 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 [8]. Microorganisms' cells are able to take up the pollutant from the micelle – to a certain extent – by fusion with the cell membrane [9]. This event could have implications for microbial uptake.

The F/M ratio and sludge retention time (SRT) are the important factors in determining the state of the biomass in activated sludge system, and is also important parameter affecting effluent wastewater quality, COD and chlorophenols removal efficiency. The concentration of mixed liquor suspended solid (MLSS) in the bioreactor increases with decreasing F/M ratio and increasing SRT.

A number of researchers indicated surfactant enhancement in microbial degradation of organic contaminants [10-15]. However, no study was on enhanced biodegradation of 4-chlorophenol using a surfactant in an activated sludge.

The main purpose of this study was to investigate the potential effects of F/M ratio and SRT on the treatability 4-CP and COD in the biosurfactant added activated sludge bioreactor systems.

2. Materials and methods

2.1 Experimental system

A schematic diagram of the experimental setup is depicted in Figure 1. A continuously stirred tank reactor (CSTR) was used in the experimental study. Volume of the aerobic reactor was 8.75 liter and the volume of the settling unit, 1.15 liter. 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. 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.

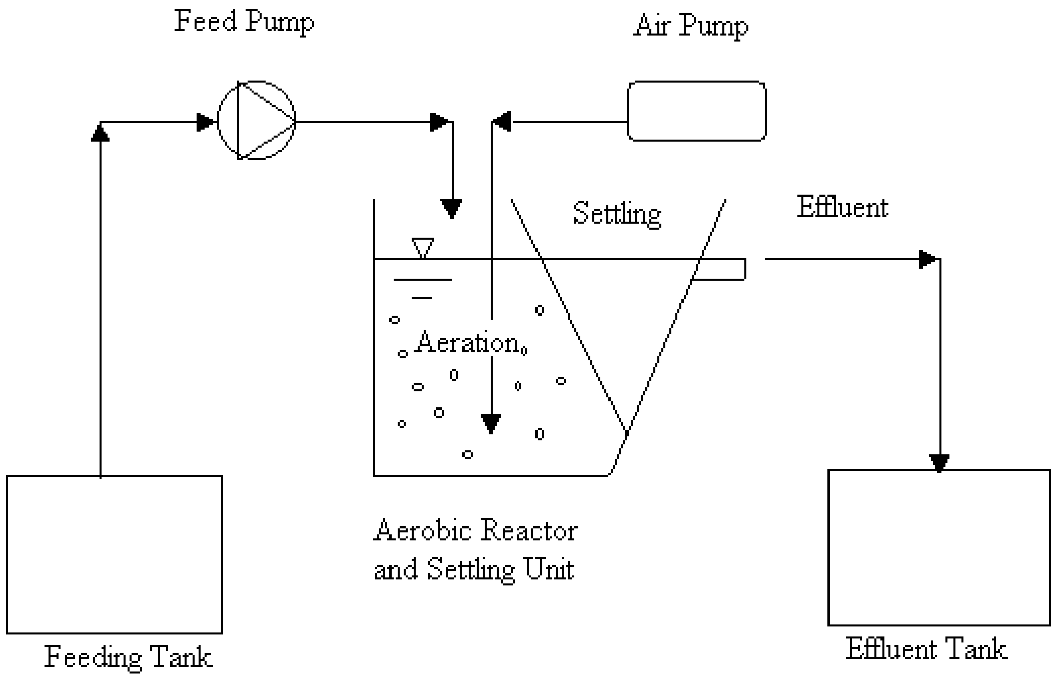

Fig. 1 A schematic diagram of the activated sludge bioreactor used in experimental studies.

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 of glucose as carbon source, urea as nitrogen source (150 mg/L), KH_2PO_4 as phosphorus source (30 mg/l), $MgSO_4.7H_2O$ (75 mg/l), $CaCl_2$ (50 mg/l), $FeCl_3$ (2 mg/l) and constant concentrations of 4-CP (250 mg/l). In experiments, influent contained 1500 mg/l COD, and nitrogen and phosphorus concentrations were adjusted to maintain C/N/P= 100/10/2.

Water solubility of 4-CP is about 27 gr/l at 20 °C [16]. 4-CP was dissolved in water solution to prepare 5000 mg/l stock solution and added directly from stock solution to obtain the desired 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 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). Therefore, in adjusting the COD concentration to 1500 mg/L in the test reactors, the contribution of the biosurfactant was also considered. Consequently, amount of glucose to be added synthetic wastewater was determined by both 4-CP and biosurfactant amounts. In this case, lower glucose concentration was added to test reactors relative to control reactor.

2.3 Biosurfactant

The rhamnolipid (designated JBR 425) was kindly donated by Jeneil Biosurfactant Company, Saukville, WI, USA as a mixture of R1 and R2. R1 had the chemical formula $C_{26}H_{48}O_9$, and R2, $C_{32}H_{58}O_{13}$. JBR 425 was chosen because the biosurfactant showed no toxicity to activated sludge biomass with an EC_{50} (effective concentration) = 1000 mg/l. This is the best test result possible. This means that this surfactant could be added to wastewater treatment plants at concentrations <1000 ppm with no adverse effects. The biosurfactant used in the study has been tested by the producer 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 a biodegradability rate of 68.4% on the 10 th day of the 28-day test cycle. This is an excellent test result clearly demonstrating that JBR 425 is readily biodegradable [17].

2.4 Experimental procedure

Three reactors with the same structure and volume as described above were used in parallel tests.

Experiments were started batchwise. The reactors were filled with the synthetic wastewater containing 4-CP and activated sludge from an industrial wastewater treatment plant was added to the reactors as seed. The same amount of sludge was inoculated in three parallel reactors. The systems were operated batchwise to obtain a dense culture and microorganisms were firstly gradually acclimated into 250 mg/l 4-CP concentration before starting the continuous operation. After the acclimation procedure is completed, the sludge age variation period started. Continuous activated sludge experiments were performed at different sludge ages between 3 and 25 days while hydraulic residence time (HRT) was kept constant throughout the experiments at θ_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-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. Sludge age, i.e. mean cell residence time, was changed by removing a certain fraction of the sludge from the aeration tank every day. For example, 10% (1/10) of the sludge was removed from the aeration tank every day to adjust the sludge age to 10 days. Every 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 each sludge age application after obtaining stable conditions the reactors waste treatment performances were defined.

In the control reactor (R1), the feed water did not contain any biosurfactant in order to determine the effect of biosurfactant on the removal of 4-CP. Feed water of the test reactors (R2 and R3) contained both 4-CP and biosurfactant. A 15 mg/l (CMC) and 30 mg/l (2CMC) biosurfactant concentration were added to R2 and R3. Biosurfactant added to the activated sludge system during the experimental study did not cause any foaming effect.

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.

2.5 Analytical methods

Samples were centrifuged at 6000 rpm for 25 minutes to remove biomass and other solids from the liquid medium. The clear supernatant was analyzed for COD and 4-CP. Standard methods based on digestion and reflux was used for COD analyses. The 4-CP analysis 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 [18]. 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 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.

3. Result and discussion

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

Data about the operational conditions and removal efficiencies are summarized in Table 1.

F/M ratio values decreased with the increase of sludge ages as shown in Table 1. When the sludge age was increased from 3 to 25 days, F/M ratio was decreased 3.850 to 0.487 day^{-1} in R1, it was decreased 2.020 to 0.455 day^{-1} in R2 and 1.410 to 0.460 day^{-1} in R3. At the lower sludge ages, the better removal efficiencies were achieved at the lower F/M ratio in R3 relative to in R1 and R2. Their results were influenced by the fact that lower F/M ratios were achieved by an increase in biomass concentration in the R3 while the substrate feeding rate was kept constant. Biomass concentrations and 4-CP contents in the aeration tank affected the efficiency of COD and 4-CP removal.

The biosurfactant added systems exhibited higher tolerance to toxic effect of 4-CP at the higher F/M ratio 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. As shown in Table 1, biosurfactant adding systems did not adversely affect at higher F/M ratios because biosurfactant existence attenuated 4-CP toxicity on the microorganisms. Therefore, increase in removal efficiency might partly be attributed to higher biomass concentrations in R2 and R3. 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.

At the 3 days sludge age, while COD and 4-CP removal efficiencies were 61.53% and 19.15% in R1, they were 77.75% and 46.01% in R2, 81.20% and 63.69% in R3. In the study of Kargi and Eker [19], at the 5 days sludge age, COD and 2,4-DCP removal efficiencies were determined as 58% and 15% respectively in the activated sludge reactor (COD=2500 mg/l, 2,4-DCP=200 mg/l).

Table 1 Comparison of operational parameters of the reactors with different F/M ratios.

Reactor	Run	SRT (day)	F/M (day^{-1})	COD Removal Efficiency (%)	4-CP Removal Efficiency (%)
R1	Run 1	3	3.850	61.53	19.15
	Run 2	5	2.490	69.75	31.28
	Run 3	10	0.864	74.26	59.99
	Run 4	15	0.632	90.76	91.85
	Run 5	20	0.529	93.99	99.78
	Run 6	25	0.487	94.13	99.49
R2	Run 1	3	2.020	77.75	46.01
	Run 2	5	1.210	84.55	75.25
	Run 3	10	0.694	94.38	99.85
	Run 4	15	0.536	95.18	99.81
	Run 5	20	0.501	95.20	99.83
	Run 6	25	0.455	96.11	99.15
R3	Run 1	3	1.410	81.20	63.69
	Run 2	5	0.901	93.10	98.42
	Run 3	10	0.504	94.90	99.87
	Run 4	15	0.487	95.00	99.41
	Run 5	20	0.481	94.38	99.76
	Run 6	25	0.460	96.32	99.67

Figure 2 depicts variation of 4-CP volumetric removal rates ($R_{4\text{-}CP}=(4\text{-}CP_o-4\text{-}CP_e)/\theta_H$) with the F/M ratio. Sharp decrease in 4-CP volumetric removal rate with the F/M ratio in R1 is because of high decreases in biomass concentrations with decreasing sludge age. Higher F/M ratio values were obtained in R1 relative to R2 and R3. Lowest 4-CP volumetric removal rate (67.54 mg/l.day) was obtained at highest F/M ratio (3.85 day^{-1}) in R1 because of toxic effects of high 4-CP contents on the microorganisms. Based on the Figure 2, it is apparent that the F/M ratios lower than 0.5 day^{-1} has no significant effect on the 4-CP volumetric removal rates in all reactors. At the lower sludge ages, volumetric removal rate of 4-CP was greatly enhanced in R2 and R3 as compared to R1 due to higher biomass concentrations.

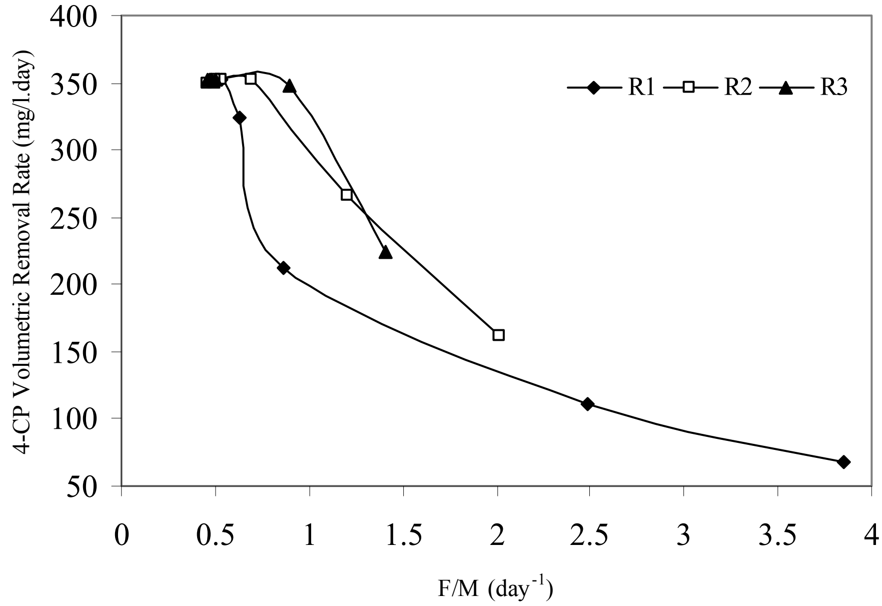

Fig. 2 The effect of F/M ratios on 4-CP volumetric removal rates in the control and test reactors.

Low COD volumetric removal rates ($R_{COD}=(COD_o-COD_e)/\theta_H$) in R1 can be observed at high F/M ratio values and low sludge ages resulting in low microorganisms concentrations (Figure 2). At the lower F/M ratio (⟨2.5 day^{-1}), while COD volumetric removal rates was varied between 1477.06 and 1993.34 mg/l.day in R1, it was ranged between 1646.47 and 2035.27 mg/l.day, 1719.53 and 2039.72 mg/l.day in R2 and R3, respectively.

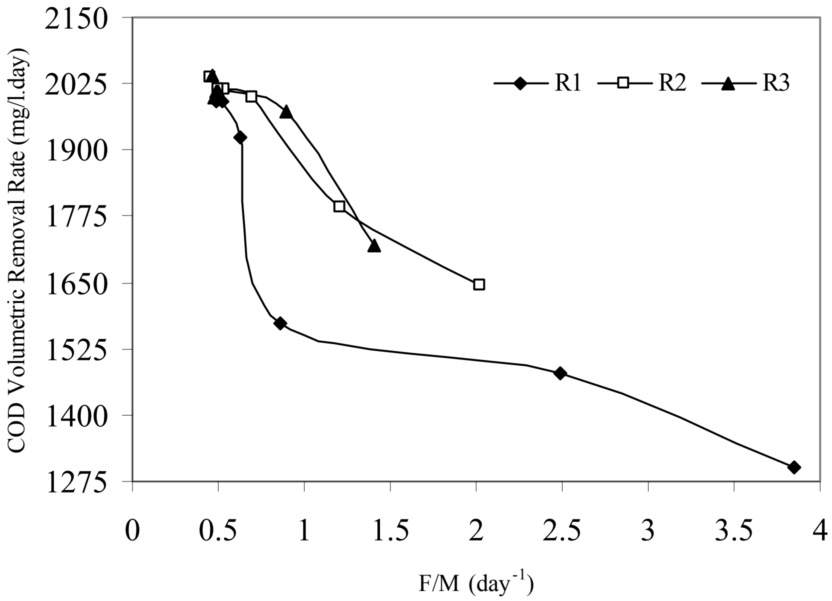

Fig. 3 The effect of F/M ratios on COD volumetric removal rates in the control and test reactors.

4. Conclusions

Treatment performance of mixture of glucose and 4-CP has been studied in control and biosurfactant added test reactors by changing F/M ratio and sludge retention time.

Operation of the all reactors at high sludge ages resulted in high biomass and low 4-CP concentrations in the aeration tanks which are the main causes for high COD and 4-CP removals at high sludge ages. Lower sludge ages resulted in decreased removal capacity in the control reactor because the biomass was affected from the toxicity of chlorophenols due to decrease in adaptation periods. This study demonstrated that biosurfactant addition promoted 4-CP and COD removals.

The results of this study show that 4-CP can be degraded in the presence of biosurfactant in the test reactors at lower sludge ages. At the low F/M ratio (〈2 day^{-1}), biosurfactant added systems could better efficiencies of COD and 4-CP removal by the positive role of biosurfactant to reduce 4-CP toxicity relative to control reactor. Low F/M ratio which resulted from long sludge ages resulted in high removal rate of substrate in R2 and R3.

Since biosurfactant can be utilized as an available carbon source, biosurfactant addition could be the reason of promoted microbial growth in the test reactors. 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 4-CP. This effect of biosurfactant addition was particularly important in lower sludge ages because microorganisms were affected more from 4-CP toxicity in lower sludge age values. Activated sludge systems are sensitive to toxic compounds. Biosurfactant presence may have attenuated the toxicity of 4-CP due to the increased biomass density, and consequently enhance the biodegradation rate of 4-CP and COD.

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