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Biological treatment of *para*-chlorophenol containing synthetic wastewater using rotating brush biofilm reactor

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Abstract

A novel rotating brush biofilm reactor (RBBR) was used for *para*-chlorophenol (4-chlorophenol, 4-CP), COD and toxicity removal from synthetic wastewater containing different concentrations of 4-CP. Effects of major operating variables such as the feed 4-CP and COD concentrations and A/Q (biofilm surface area/feed flow rate) ratio on the performance of the biofilm reactor were investigated. A Box–Wilson statistical experiment design method was used by considering the feed 4-CP (0–1000 mg l^{-1}), COD (2000–6000 mg l^{-1}) and A/Q ratio (73–293 m^2 day m^{-3}) as the independent variables while the 4-CP, COD and toxicity removals were the objective functions. The results were correlated by a response function and the coefficients were determined by regression analysis. Percent 4-CP, COD and toxicity removals determined from the response functions were in good agreement with the experimental results. 4-CP, COD and toxicity removals increased with decreasing feed 4-CP and increasing A/Q ratio. Optimum conditions resulting in maximum COD, 4-CP and toxicity removals were found to be A/Q ratio of nearly 180 m^2 day m^{-3}, feed COD of nearly 4000 mg l^{-1} and feed 4-CP of less than 205 mg l^{-1}.

Keywords: 4-Chlorophenol (4-CP); Rotating brush biofilm reactor (RBBR); Toxicity removal

1. Introduction

Chlorophenols are toxic chemicals present in many industrial effluents from pulp and paper, pesticides, petrochemicals and plastics industries. Due to toxic effects of chlorophenols on microorganisms biological treatment performance of wastewaters containing chlorophenols is usually low resulting in toxic effluents. Therefore, in treatment of chlorophenol containing wastewaters effluent toxicity levels need to be controlled along with the other effluent parameters such as COD, nutrients and chlorophenols.

Different physical, chemical and biological methods such as activated carbon adsorption, chemical oxidation and aerobic/anaerobic biological degradation were used for removal of chlorophenols from industrial wastewater [1–3]. Adsorption and ion exchange methods are usually used to concentrate the chlorinated phenols on the solid phase, but not for complete mineralization. Chemical or biological oxidation methods can be used for complete mineralization of chlorophenols and may be used in combination. Chemical oxidation methods are fast, but may result in formation of undesirable by products and also are expensive. Biodegradation of chlorophenols are more specific, relatively inexpensive and can be realized under aerobic and anaerobic conditions as reported in literatures [2–5]. Most of the investigations on biodegradation of chlorophenols focused on suspended pure culture studies using different bacteria and fungi [6–15]. Biodegradation of chlorophenols was accomplished by using a carbohydrate substrate as the primary metabolite and the chlorophenols as the cometabolite [9,10,14]. Limited number of studies was reported on biological treatment of wastewaters containing chlorophenols [16,17]. Pre-adaptation of the activated sludge cultures to the chlorophenols was reported to improve the rate and the extent of biodegradation of those compounds [5,16]. Recent investigations on biodegradation of chlorophenols focused on the use of immobilized cells or biofilm reactors [17–21]. Suspended culture systems such as conventional activated sludge processes are not resistant to shock loadings of toxic compounds. However, biofilm reactors are more resistant to high concentrations of chlorophenols because of high biomass concentrations and diffusion barriers within the biofilm for the

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Nomenclature

A	total biofilm surface area of rotating tubes and brushes (m^2)
Q	flow rate of wastewater (m^3 day^{-1})
COD_o	chemical oxygen demand in the feed wastewater ($mg\,l^{-1}$)
$4\text{-}CP_o$	4-chlorophenol concentration in feed wastewater ($mg\,l^{-1}$)
E_{COD}	percent removal of COD (%)
$E_{4\text{-}CP}$	percent removal of 4-chlorophenol (%)
$E_{Toxicity}$	percent removal of toxicity (%)
L_{CP}	4-CP loading rate ($Q \cdot 4\text{-}CP_o$, g 4-CP h^{-1})
L_{COD}	COD loading rate ($Q \cdot COD_o$, g COD h^{-1})
RBBR	rotating brush biofilm reactor
Θ_H	hydraulic residence time (HRT) V/Q (day)

toxic compounds. Therefore, biofilm systems usually perform better than the suspended growth cultures and yield high treatment efficiencies [26–28].

Biodegradability and toxic effects of chlorinated compounds depend on the number and position of chlorine groups on the aromatic ring. Usually biodegradability decreases and toxicity level increases with increasing number of chlorine groups [4]. Different biological tests were used for toxicity assessment of individual chemicals or complex effluents [22–25]. One of the toxicity assessment method used is 'Resazurin Assay' which is relatively simple, inexpensive and rapid method for assessment of the toxicity of chemical compounds and water samples [23–25]. The basic principle of the method is the measurement of percent inhibition on dehydrogenase activity of bacteria in the presence of toxic compounds. Toxicity values obtained with the resazurin assay are comparable to those obtained with the more commonly used biological methods such as *Daphnia magna*, and Microtox TM [22].

The major objective of this study is to investigate removal efficiencies of COD, 4-chlorophenol (4-CP) and toxicity from synthetic wastewater by using newly developed rotating brush biofilm reactor (RBBR) which provides better aeration by rotation and extensively large biofilm surface area because of the brush structure. *para*-Chlorophenol (4-CP) was selected as the model chlorophenol compound since it is more widely present in some industrial wastewaters and more easily degraded as compared to the other chlorophenols. A Box–Wilson statistical experiment design method was used to investigate the effects of important operating parameters such as A/Q ratio (biofilm surface area/feed flow rate), feed COD and 4-CP concentrations on percent removal of 4-CP, COD and toxicity.

2. Materials and methods

2.1. Experimental system

Fig. 1 depicts a schematic diagram of the rotating brush biofilm reactor. The experimental system consisted of a feed reservoir, wastewater tank containing a battery of brushes, driving motor, shaft and wastewater pump. The vertical discs containing the battery of brushes were rotated by using a motor and a shaft passing through the central hole on the discs. Rods containing brushes were mounted on the discs through the holes on disc surfaces. The system was placed in a stainless steel reactor in the shape of half a barrel with dimensions of 60 cm length, 30 cm width (or diameter) and 20 cm depth. The system was open to atmosphere. The feed reservoir was placed in a deep refrigerator to keep the temperature below 5 °C in order to avoid any decomposition. The system had two sections mounted on the same shaft each having 12 brush rods with total of 24 brush rods of length $L = 25$ cm and diameter of $D_o = 2.1$ cm. Each brush rod had 4200 bristles of length 0.9 cm and diameter of 0.6 mm yielding a total surface area of $A = 2.11\ m^2$ including the brush and rod surface areas on 24 tubes. Part of the brushes was completely immersed in the wastewater tank during rotation and part of them was in direct contact with air. Organisms grow in form of biofilm on the surfaces of the brush structures and rod surfaces. Total liquid volume in the tank was $V_L = 12$ l. Therefore, the biofilm area per unit wastewater volume in the tank was $a = 176\ m^2\ m^{-3}$. Biomass concentration on the rod and brush surfaces in form of biofilm was approximately $57 \pm 5\ g\,m^{-2}$ and the suspended biomass concentration in the tank was around $2.8 \pm 0.2\ g\,l^{-1}$.

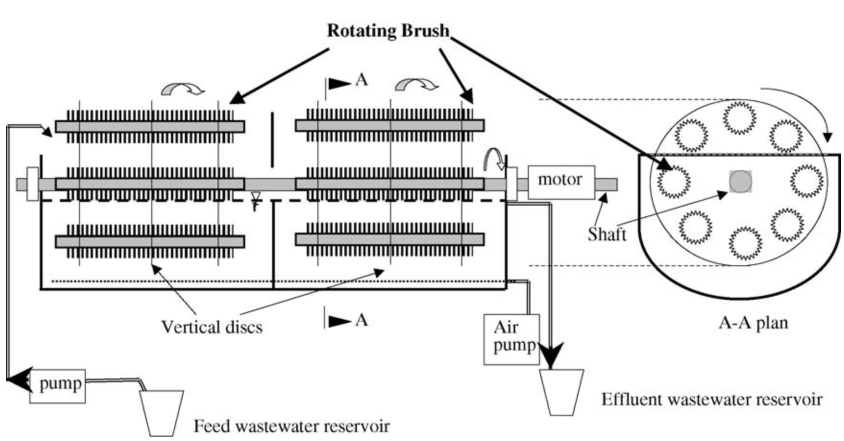

Fig. 1. Schematic diagram of the rotating brush biofilm reactor used in experimental studies.

2.2. Wastewater composition

Synthetic wastewater used throughout the studies was composed of diluted molasses, urea, KH_2PO_4 and $MgSO_4$ resulting in COD/N/P = 100/8/1.5 in the feed wastewater. $MgSO_4$ concentration in the feed was 50 mg l^{-1} in all experiments. COD and 4-CP concentrations in the feed wastewater were adjusted to desired levels specified by the Box–Wilson experimental design method. COD content of the feed included contribution of 4-CP to the COD (1.62 g COD/g 4-CP).

2.3. Organisms

The activated sludge culture used for inoculation was obtained from the Cigli wastewater treatment plant in Izmir, Turkey. The culture was cultivated for several days in growth media containing diluted molasses, urea, KH_2PO_4, $MgSO_4$ and 50 mg l^{-1} 4-CP on a shaker at 200 rpm and 25 °C and was used for inoculation of the rotating brush biofilm reactor.

2.4. Experimental procedure

Experiments were started batch wise. About 10 l of the synthetic wastewater was placed in the treatment tank containing the battery of rotating brushes and was inoculated with 2 l of the sludge culture. The system was operated batch wise for nearly 2 weeks by changing the wastewater media in every 3 days until a biofilm thickness of 1–1.5 mm was developed on the surfaces of the brush. Continuous operation was started after biofilm development. Feed wastewater was fed to the reactor with a desired flow rate between 0.3 and 1.2 l h^{-1} resulting in hydraulic residence times (HRT) between 10 and 40 h or A/Q ratios between 73 and 293 m^2 day m^{-3} and removed with the same flow rate. A/Q ratio was changed by changing the feed flow rate while the biofilm surface area was constant (2.11 m^2) throughout the experiments. Temperature and pH were approximately $T = 25 \pm 2$ °C and pH 7.5 ± 0.3 during operation. pH in the feed medium was nearly 6.9 which increased above pH 8 due to ammonia release from urea biodegradation. pH of the reactor media was controlled around 7.5 by manual addition of dilute sulfuric acid to the reactor several times a day. Biofilm thickness was controlled around 1.5 mm by removing excess biofilm from the surfaces of the rods with the aid of knives or brushes. The liquid phase in the treatment tank was aerated using diffusors and also aeration was supplied to the biofilm by direct contact of the brushes with air during rotation. Dissolved oxygen (DO) concentration in the wastewater tank was above 2 mg l^{-1} indicating no DO limitations .The brushes were rotated with a constant speed of 12 rpm. Experiments were performed in the order of increasing 4-CP loading rates ($L_{CP} = Q \cdot 4\text{-}CP_o$) to allow adaptation of the organisms to high concentrations of 4-CP. Every experiment was conducted until the system reached the steady-state with the same COD and 4-CP contents in the effluent for the last 3 days. Each experiment lasted about 2–3 weeks to reach steady-state. The samples collected from the feed and effluent wastewater at the steady-state were analyzed for COD, 4-CP contents and the toxicity levels after centrifugation.

2.5. Analytical methods

Samples were withdrawn everyday for analysis and centrifuged at 8000 rpm (7000 g) for 20 min to remove biomass from the liquid phase. Clear supernatants were analyzed for 4-CP contents. 4-Aminoantipyrene colorimetric method developed for determination of phenol and derivatives in form of phenol index was used for 4-CP analysis as specified in the Standard Methods [29]. Chemical oxygen demand (COD) was determined using the dichromate reflux method according to the Standard Methods [29]. Biomass concentrations were determined by filtering the samples through 0.45 μm milipore filter and drying in an oven at 105 °C until constant weight. The samples were analyzed in triplicates for COD and 4-CP contents with less than 3% standard deviations from the average.

Resazurin reduction method was used to determine the toxicity of the feed and effluent wastewater [23–25]. The test organisms (washed activated sludge) to be subjected to the toxic feed and effluent wastewater were cultivated on nutrient broth and were used for determination of the toxicity of wastewater samples. The test cultures were transferred every 2 days to new medium to keep the sludge age constant during the course of toxicity experiments. In the presence of active bacterial culture, as a result of dehydrogenase enzyme activity, resazurin changes color from blue to pink forming the reduced compound resorufin. Therefore, the color of the resazurin solution is an indicator of bacterial activity. A spectrometer was used at 610 nm for determination of the color intensity of the resazurin added samples.

2.6. Box–Wilson statistical experiment design

Box–Wilson statistical experiment design method was used to determine the effects of operating parameters such as A/Q ratio, feed COD_o and 4-chlorophenol (4-CP_o) concentrations on percent COD, 4-CP and toxicity removals. Three important operating parameters: 4-CP_o (X_1) and COD_o (X_2) concentrations in the feed wastewater and A/Q ratio (X_3) were chosen as independent variables. 4-CP concentration (X_1) was varied between 0 and 1000 mg l^{-1} while the feed COD concentration (X_2) was between 2000 and 6000 mg l^{-1} and A/Q ratio (X_3) was between 73 and 293 m^2 day m^{-3}, resulting in hydraulic residence times between 10 and 40 h. Experimental points for Box–Wilson statistical design are shown in Table 1. The experiments consisted of six axial (A), eight factorial (F) and a centre (C) points. The centre point was repeated three times to estimate the experimental error. Experiments were conducted until the system reached the steady-state when the last 3 days measurements of COD, 4-CP and toxicity were almost the same. The performance of the system was described by the following response function:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (1)$$

where Y is the predicted response function (percent COD, 4-CP or toxicity removal) and b_0 is the offset term. The coefficients of the response functions were determined by using

Table 1
Experimental conditions of Box–Wilson statistical design

	4-CP_o (mg l^{-1}), X_1	COD_o (mg l^{-1}), X_2	Θ_H (h)	A/Q ($\text{m}^2\text{ day m}^{-3}$), X_3
Axial points				
A1	0	4000	25	183
A2	1000	4000	25	183
A3	500	2000	25	183
A4	500	6000	25	183
A5	500	4000	10	73
A6	500	4000	40	293
Factorial points				
F1	789	5156	34	249
F2	789	5156	16	117
F3	789	2844	34	249
F4	789	2844	16	117
F5	211	5156	34	249
F6	211	5156	16	117
F7	211	2844	34	249
F8	211	2844	16	117
Center point				
C	500	4000	25	183

the experimental data and the Statistica 5.0 computer program for regression analysis.

3. Results and discussion

The experimental data were correlated with the response functions by using the Statistica-5 regression program and the estimated coefficients of the response functions are presented in Table 2. The response functions with the determined coefficients were used in calculating the predicted values of percent COD, 4-CP and toxicity removals. A comparison of the experimental and predicted values for percent removals of COD, 4-CP and toxicity are presented in Table 3. Predicted and experimental values of COD, 4-CP and toxicity removals were in good agreement as shown in Table 3 indicating the accuracy of the predictions by the response functions.

Variations of percent COD removal with the feed COD (including COD content of 4-CP) and 4-CP concentrations at constant A/Q ratio of $117\text{ m}^2\text{ day m}^{-3}$ are depicted in Fig. 2. Percent COD removal decreased with increasing feed 4-CP concentration from 0 to 1000 mg l^{-1} for all feed COD concentrations, due to increasing toxic effects of 4-CP with increasing feed 4-CP. Percent COD removal was almost constant up to feed COD of 4000 mg l^{-1}, and decreased with increasing feed COD for the feed COD values above 4000 mg l^{-1}, due to adverse effects of increasing COD loading rates on the organisms at a constant A/Q ratio since the feed COD content includes COD content of 4-CP too. Nearly complete COD removal was obtained at feed COD of 3800 mg l^{-1} and A/Q ratio of $167\text{ m}^2\text{ day m}^{-3}$ when feed 4-CP concentration was between 0 and 122 mg l^{-1}.

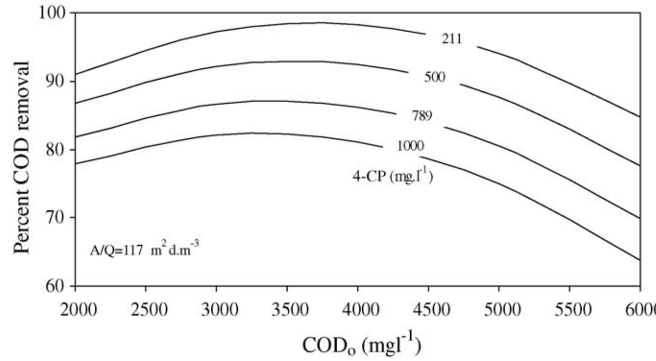

Fig. 2. Variation of percent COD removal with feed COD concentration at different feed 4-CP concentrations.

Fig. 3 shows variations of percent COD removal with feed 4-CP concentration at different A/Q ratios and constant feed COD of 6000 mg l^{-1}. Percent COD removal increased with increasing A/Q ratio and reached a maximum level at A/Q ratio of around $249\text{ m}^2\text{ day m}^{-3}$. Percent COD removal was almost constant around 86% for the whole feed 4-CP concentrations at constant A/Q ratio of $249\text{ m}^2\text{ day m}^{-3}$. Further increases in A/Q ratio did not cause significant changes in percent COD removal due to presence of sufficient biomass on the surfaces of brush. Percent COD removal decreased with increasing feed 4-CP concentrations as a result of 4-CP inhibition on the organisms for A/Q ratios between 73 and $183\text{ m}^2\text{ day m}^{-3}$. At high A/Q ratio (low feed flow rates) of $293\text{ m}^2\text{ day m}^{-3}$ percent COD removal slightly increased with increasing feed 4-CP concentration due to low 4-CP loading rates at low feed flow rates yielding low level of 4-CP inhibitions.

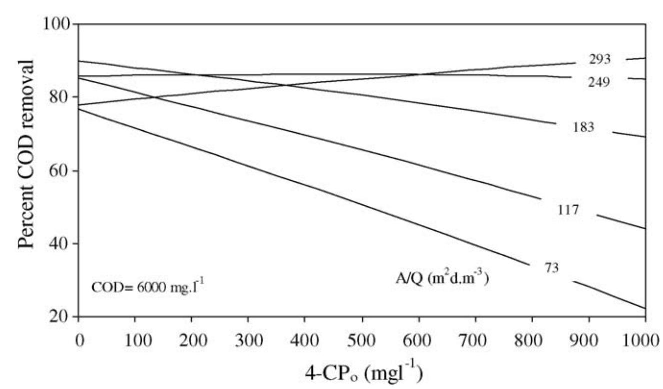

Fig. 3. Variation of percent COD removal with feed 4-CP concentration at different A/Q ratios.

Table 2
Coefficients of the response functions for COD, 4-CP and toxicity removals

	b_0	b_1	b_2	b_3	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
Y_{COD}, $R^2 = 0.93$	46.95	-5.82×10^{-2}	1.66×10^{-2}	2.83×10^{-1}	-3.66×10^{-6}	-2.58×10^{-6}	-1.05×10^{-3}	-2.51×10^{-6}	3.06×10^{-4}	1.76×10^{-5}
$Y_{4\text{-CP}}$, $R^2 = 0.96$	−50.89	-8.15×10^{-2}	1.42×10^{-2}	1.27	-1.70×10^{-5}	-9.70×10^{-7}	3.11×10^{-3}	-4.0×10^{-6}	-5.11×10^{-4}	-2.26×10^{-5}
$Y_{Toxicity}$, $R^2 = 0.97$	−61.48	-2.88×10^{-2}	1.47×10^{-2}	1.31	-7.90×10^{-7}	-1.20×10^{-6}	-3.28×10^{-3}	-1.60×10^{-6}	5.33×10^{-4}	-2.10×10^{-5}

Table 3
Comparison of experimental and predicted percent COD, 4-CP and toxicity removals for Box–Wilson design experiments

	$E_{COD\ (exp)}$	$E_{COD\ (pred)}$	$E_{4\text{-}CP\ (exp)}$	$E_{4\text{-}CP\ (pred)}$	$E_{Toxicity\ (exp)}$	$E_{Toxicity\ (pred)}$
A1	97	100	100	100	100	92
A2	90	86	93	83	89	83
A3	93	90	98	93	90	83
A4	76	80	96	93	90	83
A5	67	69	30	26	23	16
A6	96	95	96	92	88	79
F1	95	94	98	100	93	96
F2	65	65	40	47	36	42
F3	93	98	99	100	94	100
F4	70	72	39	44	36	39
F5	96	94	96	97	75	82
F6	92	87	81	79	63	67
F7	94	94	98	97	79	84
F8	92	91	71	72	55	62
C (average)	94	94	97	97	88	88

Variations of percent 4-CP removal with the feed COD content at different feed 4-CP concentrations and constant *A*/*Q* ratio of 117 $m^2\ day\ m^{-3}$ are depicted in Fig. 4. Percent 4-CP removal decreased with increasing feed 4-CP concentration due to toxic effects of high 4-CP contents on the organisms. Percent 4-CP removal increased from 32 to 78% when feed 4-CP concentration decreased from 1000 to 211 $mg\ l^{-1}$ at the feed COD concentration of 4000 $mg\ l^{-1}$. Percent 4-CP removal increased with increasing feed COD concentration for feed 4-CP values below 500 $mg\ l^{-1}$ due to negligible 4-CP inhibitions and increasing biomass concentrations with increasing feed COD contents.

Fig. 5 depicts variations of percent 4-CP removal with feed 4-CP concentration at different *A*/*Q* ratios and constant feed COD of 6000 $mg\ l^{-1}$. Increases in feed 4-CP concentrations resulted in decreases in 4-CP removal efficiency at low *A*/*Q* ratios up to 183 $m^2\ day\ m^{-3}$ due to toxic effects of 4-CP on the organisms and low biomass concentrations at low *A*/*Q* ratios. At high *A*/*Q* ratios above 249 $m^2\ day\ m^{-3}$ (low feed flow rates) percent 4-CP removals increased with increasing feed 4-CP contents probably due to low 4-CP loading rates ($Q \cdot 4\text{-}CP_o$) at low feed flow rates or high *A*/*Q* ratios. Again, increases in *A*/*Q* ratio at constant feed 4-CP concentrations resulted in increases in percent 4-CP removals up to *A*/*Q* 249 $m^2\ day\ m^{-3}$ due to larger biofilm surface area per unit 4-CP loading rates at high *A*/*Q* values or low feed flow rates. Nearly complete 4-CP removal was obtained for feed 4-CP concentrations between 0 and 205 $mg\ l^{-1}$ at feed COD of 5170 $mg\ l^{-1}$ and *A*/*Q* ratio of nearly 170 $m^2\ day\ m^{-3}$.

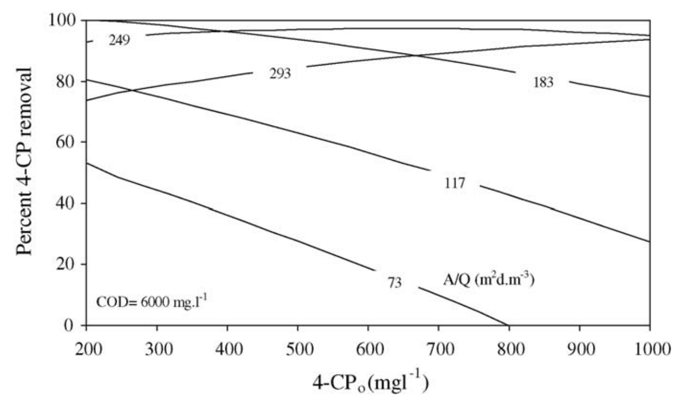

Fig. 5. Variations of percent 4-CP removal with feed 4-CP concentration at different *A*/*Q* ratio.

Removal of toxicity from the wastewater containing different concentrations of 4-CP is another important aspect of this study. Variations of percent toxicity removal with feed 4-CP concentration at different feed COD concentrations and constant *A*/*Q* ratio of 183 $m^2\ day\ m^{-3}$ are depicted in Fig. 6.

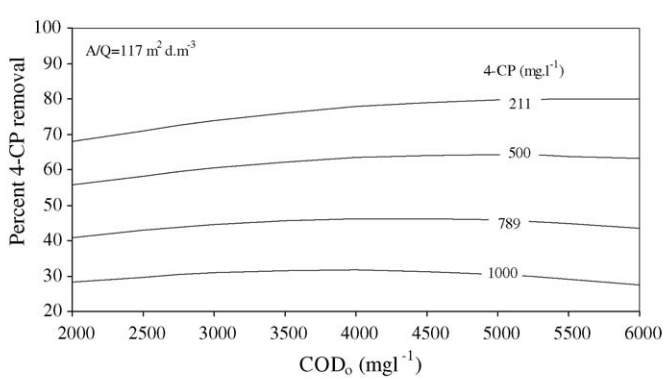

Fig. 4. Variation of percent 4-CP removal with feed COD concentration at different feed 4-CP concentration.

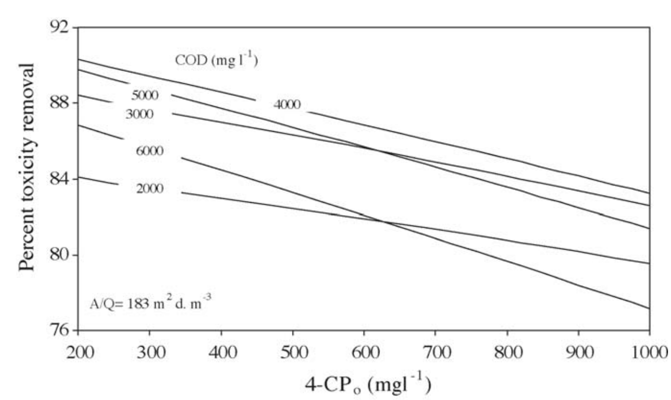

Fig. 6. Variation of percent toxicity removal with feed 4-CP concentrations at different feed COD concentrations.

Table 4
Comparison of the experimental and predicted percent 4-CP, COD and toxicity removals for different experimental points

4-CP$_o$ (mg l^{-1})	COD$_o$ (mg l^{-1})	A/Q (m^2 day m^{-3})	$E_{COD\ (exp)}$	$E_{COD\ (pred)}$	$E_{4\text{-CP}\ (exp)}$	$E_{4\text{-CP}\ (pred)}$	$E_{Toxicity\ (exp)}$	$E_{Toxicity\ (pred)}$
350	3450	147	89	93	85	86	74	76
511	2555	147	84	87	77	78	70	69
1255	6367	330	96	93	97	86	99	100

Percent toxicity removal decreased from 90 to 83% with increasing feed 4-CP content at a feed COD of 4000 mg l^{-1} due to toxic effects of high 4-CP contents on the organisms. Percent toxicity removal increased with increasing feed COD up to 4000 mg l^{-1} at constant feed 4-CP content probably due to high biomass concentrations at high feed COD contents (or high COD loading rates) while the inhibition caused by the 4-CP was unchanged. Decreases in percent toxicity removal with increasing feed COD contents above 4000 mg l^{-1} is probably due to preferential utilization of COD compounds present in molasses (sucrose) rather than 4-CP which resulted in high 4-CP contents in the reactor causing inhibitions. The optimum A/Q ratio was found to be 183 m^2 day m^{-3} for maximum percent toxicity removal. The system should be operated at optimum A/Q ratio (183 m^2 day m^{-3}) in order to obtain nearly complete toxicity removal when the feed COD is around 4000 mg l^{-1}.

In order to test reliability of the response functions predictions, three experiments different from Box–Wilson experimental points were carried out. The results are compared with the Box–Wilson response function predictions in Table 4. The first two experiments were within the range of independent variables, the third one was outside of the range. Response function predictions were in good agreement with the experimental data indicating the reliability of the response function predictions within and the outside the range of experimental points.

4. Conclusions

A Box–Wilson statistical experiment design was used to investigate the effects of major operating variables on the performance of a novel rotating brush biofilm reactor (RBBR), treating 4-chlorophenol containing synthetic wastewater. The independent variables were the feed COD and 4-CP contents and A/Q ratio while percent COD, 4-CP and toxicity removals were considered as dependent variables (objective functions). Percent COD removal decreased with increasing feed 4-CP concentration due to toxic effects of 4-CP on the organisms, but increased with increasing A/Q ratio due to larger biofilm surface area. The most favorable conditions maximizing COD removal efficiency was obtained at feed COD of 3800 mg l^{-1} and A/Q ratio of nearly 167 m^2 day m^{-3} when feed 4-CP concentration was between 0 and 122 mg l^{-1}. 4-CP contents lower than 205 mg l^{-1} and high A/Q ratios above 170 m^2 day m^{-3} were found to be more favorable for high percent 4-CP and toxicity removals at feed COD of nearly 5170 mg l^{-1}. In order to obtain complete toxicity removal, the system should be operated at optimum A/Q ratio of nearly 183 m^2 day m^{-3} (HRT = 25 h). Optimum conditions resulting in maximum COD, 4-CP and toxicity removals were found to be A/Q ratio of nearly 180 m^2 day m^{-3}, feed COD of nearly 4000 mg l^{-1} and feed 4-CP of lower than 205 mg l^{-1}.

Acknowledgements

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