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COD, 2,4,6-trichlorophenol (TCP) and toxicity removal from synthetic wastewater in a rotating perforated-tubes biofilm reactor

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ABSTRACT

Synthetic wastewater containing different concentrations of 2,4,6-trichlorophenol (TCP) was biologically treated using a novel rotating perforated-tubes biofilm reactor (RTBR) for chemical oxygen demand (COD), TCP and toxicity removal. Performance of the reactor was investigated as function of major operating variables such as the feed TCP and COD concentrations and A/Q (biofilm surface area/feed flow rate) ratio. A Box–Behnken statistical experiment design method was used by considering the feed TCP (0–400 mg L^{-1}), COD (1000–4000 mg L^{-1}) and A/Q ratio (23–163 m^2 d m^{-3}) as the independent variables while percent TCP, COD, and toxicity removals were the objective functions. The results were correlated with the quadratic model since this was found to be the most suitable one. Response function coefficients were determined by correlating the experimental data with the response function. Percent TCP, COD and toxicity removals estimated from the response functions were in good agreement with the experimental results. TCP, COD and toxicity removals increased with increasing A/Q ratio and decreasing feed TCP concentrations. Percent toxicity removals were always lower than TCP removals indicating presence or formation of some toxic by products from TCP biodegradation. For the feed TCP of 400 mg L^{-1}, the optimum conditions resulting in maximum COD (99%), TCP (100%) and toxicity (93%) removals were A/Q ratio of nearly 165 m^2 d m^{-3} and feed COD of 2985 mg L^{-1}.

1. Introduction

Chlorophenol compounds present in some chemical industry effluents such as pulp and paper, pesticides, petrochemicals, and paints exhibit deleterious effects on environment upon direct discharge. Performance of conventional biological treatment processes used for such wastewaters is usually unsatisfactory due to toxic effects of chlorophenols on microorganisms. Toxicity of such effluents is usually high and is not controlled along with the other parameters such as COD, nutrients and chlorophenols.

Different physical, chemical and biological methods such as adsorption/ion exchange, chemical oxidation and aerobic/anaerobic biological degradation were used for removal of chlorophenols from industrial wastewater [1–3]. Adsorption and ion exchange methods are used to concentrate the chlorophenols on the solid phase, but not for complete mineralization. Chemical or biological oxidation methods must be used for complete mineralization of chlorophenols usually in combination. Chemical oxidation methods may yield undesirable by products and also are expensive. Biodegradation of chlorophenols is a more specific and relatively inexpensive method which can be realized under aerobic and anaerobic conditions as reported in the literature [2–5]. Most of the investigations on biodegradation of chlorophenols focused on suspended pure culture studies using different bacteria and fungi [6–10]. Biodegradation of chlorophenols was usually accomplished by using a carbohydrate substrate as the primary metabolite and the chlorophenols as the cometabolite [9,10]. Limited number of studies was reported on biological treatment of wastewaters containing chlorophenols [11–15]. 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]. Recent investigations on biodegradation of chlorophenols focused on the use of immobilized cells or biofilm reactors [12–16]. Biofilm reactors are more resistant to high concentrations of chlorophenols because of high biomass concentrations and diffusion barrier within the biofilm for the toxic compounds. Therefore, biofilm systems usually yield higher removal efficiencies for toxic compound as compared to the suspended culture systems [17–19]. The rotating tubes biofilm reactor (RTBR) used in this study is a compact reactor with high biofilm surface area providing good mixing in the liquid phase and effective aeration by direct contact of air and biofilm during rotation.

Toxicity and biodegradability of chlorophenols vary depending on the number and the position of the chlorine groups on the aromatic ring. Biodegradability decreases and toxicity increases with increasing number of chlorine groups [4]. Different biological

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Nomenclature

A	total biofilm surface area of rotating tubes (m^2)
COD_o	chemical oxygen demand in the feed wastewater ($mg\,L^{-1}$)
E_{COD}	percent removal of COD (%)
E_{TCP}	percent removal of trichlorophenol (%)
$E_{Toxicity}$	percent removal of toxicity (%)
L_{TCP}	TCP loading rate ($Q\,TCP_o$, $g\,TCP\,d^{-1}$)
L_{COD}	COD loading rate ($Q\,COD_o$, $g\,COD\,d^{-1}$)
Q	flow rate of wastewater ($m^3\,d^{-1}$)
RTBR	rotating tubes biofilm reactor
TCP_o	trichlorophenol (TCP) concentration in feed wastewater ($mg\,L^{-1}$)
Θ_H	hydraulic residence time, HRT (V/Q, h)

tests were used for toxicity assessment of chemicals and complex effluents [20–23]. One of the newly developed toxicity assessment method is 'Resazurin Assay' which is relatively simple, inexpensive and rapid method for assessment of the toxicity of chemical compounds and water samples [21–23]. 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 [21].

The major objective of this study is to investigate performance of a newly developed biofilm reactor namely the "rotating perforated-tubes biofilm reactor" for biological treatment of 2,4,6-trichlorophenol (TCP) containing synthetic wastewater. COD, TCP and toxicity removals from the synthetic wastewater were investigated under different operating conditions such as the feed COD (1000–4000 $mg\,L^{-1}$), feed TCP (0–400 $mg\,L^{-1}$) and the A/Q ratio (biofilm surface area/ feed flow rate, 23–163 $m^2\,d\,m^{-3}$). A Box–Behnken statistical experiment design method was used to investigate the effects of the operating parameters on percent COD, TCP and toxicity removals. Operating conditions maximizing the COD, TCP and toxicity removal were determined.

2. Materials and methods

2.1. Experimental system

Fig. 1 depicts a schematic diagram of the RTBR. The experimental system consisted of a feed reservoir, wastewater tank containing rotating tubes, driving motor, shaft and a wastewater pump. The discs containing the tubes were rotated by using a motor and a shaft passing through the central hole on the discs. Rotational speed was 12 rpm ($rev\,min^{-1}$) throughout the experiments. Feed reservoir was placed in a deep refrigerator to keep the temperature below 5 °C in order to avoid any decomposition. The rotating tube system had two sections mounted on the same shaft each having 25 perforated tubes (total of 50 tubes) of length $L = 25$ cm made of PVC. Outer and inner diameter of the tubes were $D_o = 2.1$ cm and $D_i = 1.3$ cm resulting in a total surface area of $A = 1.34\,m^2$. Each tube had twenty holes of 0.5 cm in diameter located diagonally and 1 cm apart on the surfaces which allowed air passage to the inner surface of the tubes. The tubes were located on the outer area of the discs to provide complete contact with the wastewater and wet biofilm surface area during rotation. Organisms grew in form of biofilm on the outer and inner surfaces of the tubes. Total liquid volume in the tank was $V_L = 12$ L. Therefore, the biofilm area per unit wastewater volume in the tank was $a = 111.6\,m^2\,m^{-3}$. Biomass concentration on the tube surfaces in form of biofilm was approximately $50 \pm 1\,g\,dw\,m^{-2}$ and the suspended biomass concentration in the tank was $3 \pm 0.1\,g\,dw\,L^{-1}$. Total amounts of attached and suspended biomass were typically 67 and 36 g, respectively. Wastewater in the tank was gently aerated using fine air-bubble diffusers in order to keep the suspended organisms active. Biofilm organisms were aerated by direct contact of air with the biofilm during rotation of the tubes.

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. The feed COD was varied between 1000 and 4000 $mg\,L^{-1}$ while the feed TCP was between 0 and 400 $mg\,L^{-1}$ as presented in Table 1. COD and TCP concentrations in the feed wastewater were adjusted to desired levels specified by the Box–Behnken experimental design method. COD content of the feed included COD content of TCP (1.34 g COD/g TCP) along with the COD content of diluted molasses (mainly sucrose).

2.3. Organisms

The activated sludge culture used for inoculation was obtained from the Cigli municipal wastewater treatment plant in Izmir, Turkey. The inoculum culture was cultivated for several days in growth media containing diluted molasses, urea, KH_2PO_4, $MgSO_4$ and 50 $mg\,L^{-1}$ TCP on a shaker at 200 rpm and 25 °C.

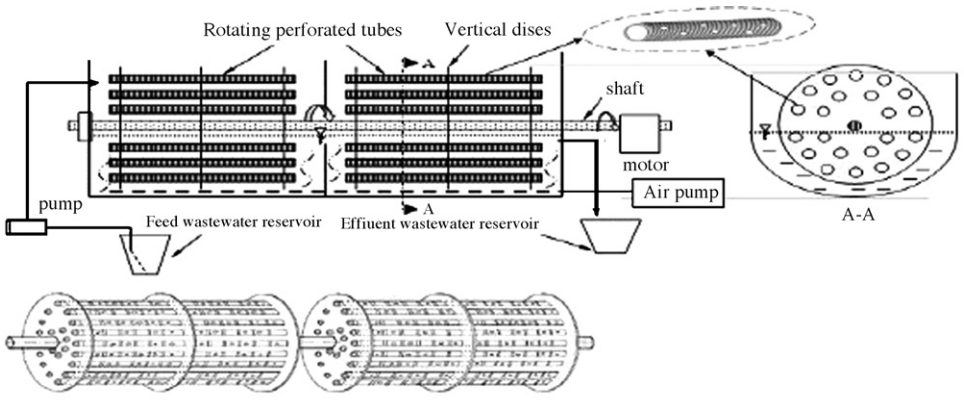

Fig. 1. Schematic diagram of the rotating perforated-tubes biofilm reactor.

Table 1
List of Box–Behnken statistical design experiments in the order of increasing TCP loading rates

Run	X_1	X_2	X_3	
	COD_o (mg L^{-1})	TCP_o (mg L^{-1})	HRT (h)	A/Q (m^2 d m^{-3})
1	1000	0	20	93
2	2500	0	35	163
3	4000	0	20	93
4	2500	0	5	23
5	1000	200	35	163
6	4000	200	35	163
7	2500	200	20	93
8	2500	200	20	93
9	2500	200	20	93
10	2500	200	20	93
11	2500	200	20	93
12	2500	400	35	163
13	1000	400	20	93
14	4000	400	20	93
15	1000	200	5	23
16	4000	200	5	23
17	2500	400	5	23

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 tubes and was inoculated with 2 L of the inoculum 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 mm was developed on the surfaces of the tubes. Continuous operation was started after biofilm development. Feed wastewater was fed to the reactor with a desired flow rate between 0.34 and 2.4 L h^{-1} resulting in hydraulic residence times (HRT) between 5 and 35 h corresponding to A/Q ratios between 23 and 163 m^2 d 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 (1.34 m^2) throughout the experiments. Temperature and pH were approximately $T = 25 \pm 2$ °C and pH 7.2 ± 0.2 during operation. pH in the feed medium was nearly 6.9 which increased to nearly pH 8 due to ammonia release from urea biodegradation. pH of the reactor media was controlled around 7.2 ± 0.2 by manual addition of dilute sulfuric acid to the reactor several times a day. Biofilm thickness was controlled at approximately 1 mm by removing excess biofilm from the surfaces of the tubes with the aid of knives or brushes when necessary. The aeration rate was adjusted to yield dissolved oxygen (DO) concentration in the wastewater tank to be above 2 mg L^{-1} to avoid DO limitations in liquid phase. Experiments were performed in the order of increasing TCP loading rates ($L_{TCP} = Q TCP_o$) to allow adaptation of the organisms to high concentrations of TCP. Every experiment was conducted until the system reached the steady-state with the same COD and TCP contents in the effluent for the last 3 days. Each experiment lasted about 3 weeks to reach quasi steady-state. The samples collected from the feed and effluent wastewater at the steady-state were analyzed for COD, TCP and percent toxicity 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 TCP contents by using 4-aminoantipyrene colorimetric method developed for determination of phenol and derivatives in form of phenol index as specified in the Standard Methods [24]. COD was determined using the dichromate reflux method according to the Standard Methods [24]. Biomass concentrations (dry weight) from the liquid phase were determined by filtering 10 mL samples through 0.45 μm millipore filter and drying in an oven at 105 °C until constant weight. In determining the biomass concentration on tube surfaces, six tubes were removed from the system, the biomass on the tube surfaces was removed by washing and the concentrations were determined by filtering and drying. The samples were analyzed in triplicates for COD and TCP contents with less than 5% standard deviations from the average.

Resazurin reduction method was used to determine the toxicity of the feed and effluent wastewater [20–23]. The test organisms (washed activated sludge) to be subjected to the toxic feed and effluent wastewater were cultivated on nutrient broth (glucose, yeast extract and peptone) and were used for determination of the toxicity of wastewater samples. The test cultures were transferred every day to the 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, the color of resazurin changes 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–Behnken statistical experiment design

Box–Behnken statistical experiment design method was used to determine the effects of operating parameters such as A/Q ratio, feed COD and TCP concentrations on percent COD, TCP and toxicity removals. The Box–Behnken experiment design method is an independent, rotatable quadratic design with no embedded factorial or fractional factorial points and requires fewer runs than the other statistical experiment design methods, e.g., 15 runs for a 3-factor experimental design. Three important operating parameters; feed COD_o (X_1) and TCP_o (X_2) concentrations and A/Q ratio (X_3) were considered as independent variables. Feed COD concentration (X_1) was varied between 1000 and 4000 mg L^{-1} while the feed TCP concentration (X_2) was between 0 and 400 mg L^{-1} and the A/Q ratio (X_3) was between 23 and 163 m^2 d m^{-3} resulting in HRT values between 5 and 35 h.

Response functions describing variations of dependent variables (percent COD, TCP and toxicity removals) with the independent variables (X_i) can be written as follows:

$$Y = b_o + \sum \underbrace{b_i * X_i}_{Linear} + \sum \underbrace{b_{ij} * X_i * X_j}_{interaction} + \sum \underbrace{b_{ii} * X_i^2}_{squared} \quad (1)$$

where Y is the predicted response, b_0 is offset term, b_i is the linear effect while b_{ii} and b_{ij} are the square and the interaction effects, respectively.

Experimental data points used in Box–Behnken statistical design are presented in Table 1. The response function coefficients were determined by correlating the experimental data with the response functions using the Stat-Ease Design Expert 7.0 computer program. The response functions for COD, 2,4,6-TCP and toxicity removals were approximated by the standard quadratic polynomial equation as presented below.

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

3. Results and discussion

Experimental data was used for determination of the response function coefficients for each independent variable by iteration. Dif-

Table 2
Predicted response function coefficients for percent COD, TCP and toxicity removals

	b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_{11}	b_{22}	b_{33}
Y_{COD} R^2 = 0.98	63.79	0.01830	−0.02717	0.09576	-3.8×10^{-06}	−0.00004	−0.00120	-5×10^{-06}	4.54×10^{-05}	0.00048
Y_{TCP} R^2 = 0.98	23.05	0.04631	−0.43537	0.81789	-9.3×10^{-06}	0.00029	−0.00451	1.67×10^{-05}	1.67×10^{-05}	0.00154
$Y_{Toxicity}$ R^2 = 0.97	12.95	0.04758	−0.35497	0.93465	-9.4×10^{-06}	0.00020	−0.00479	2.58×10^{-05}	1.43×10^{-05}	0.00125

Table 3
Comparison of the predicted and experimental percent COD, TCP and toxicity removals

Run	Experimental E_{COD} (%)	Predicted E_{COD} (%)	Experimental E_{TCP} (%)	Predicted E_{TCP} (%)	Experimental $E_{Toxicity}$ (%)	Predicted $E_{Toxicity}$ (%)
1	79	81	NA	NA	100	99
2	90	88	NA	NA	100	101
3	91	91	NA	NA	100	102
4	90	90	NA	NA	100	98
5	77	77	64	62	54	55
6	92	94	95	86	75	72
7	93	94	93	95	71	83
8	93	94	97	95	87	83
9	95	94	99	95	98	83
10	95	94	95	95	80	83
11	95	94	93	95	80	83
12	97	97	95	100	89	91
13	80	80	49	45	38	36
14	86	84	80	82	58	59
15	77	75	10	19	9	12
16	73	73	35	37	23	23
17	70	72	25	21	3	2

NA: not applicable since TCP was zero for those experiments.

ferent response functions were used to correlate the experimental data and the most suitable one was determined by using the analysis of variance (ANOVA) program. ANOVA tests for all response functions indicated that the quadratic model provided the best fit to the experimental data with the lowest standard deviation, the highest correlation coefficient and the lowest p-value. The estimated coefficients of the response functions are presented in Table 2. Positive b_1 and b_3 values indicate positive effects of increases in the feed COD and A/Q ratio on percent COD, TCP and toxicity removals; and negative b_2 values indicate adverse effects of increasing TCP concentrations on response functions. The predicted values of percent COD, TCP and toxicity removals from the response functions with the estimated coefficients are compared with the experimental results in Table 3. Response function predictions were in good agreement with the experimental data with R^2 values larger than 0.97.

Variations of percent COD removal with A/Q ratio at different feed COD contents are depicted in Fig. 2 at constant feed TCP of 200 mg L^{-1}. Percent COD removal increased with increasing A/Q ratio due to high biofilm surface area or high active biomass concentrations at high A/Q ratios. At low feed COD contents such as 1000 and 2000 mg L^{-1} percent COD removal was maximum at an A/Q ratio of nearly 110 m^2 d m^{-3}. Further increases in the A/Q ratio reduced percent COD removal due to insufficient COD loading to support biofilm organisms at low flow rates or high A/Q ratios. The optimum A/Q ratio shifted to larger values for high feed COD contents yielding the optimum A/Q value of nearly 140 m^2 d m^{-3} for the fed COD of 3000 and 4000 mg L^{-1}. High COD loadings required larger A/Q ratios or larger biofilm areas for maximum COD removal. At constant A/Q ratio percent COD removal increased with increasing feed COD up to 3000 mg L^{-1} indicating COD limitations at low feed COD levels. COD removal decreased for the feed COD of 4000 mg L^{-1} due to adverse effects of excess COD loading at high feed COD levels. When the feed TCP was 200 mg L^{-1}, the maximum percent COD removal (97%) was obtained with the feed COD of nearly 3000 mg L^{-1} and A/Q ratio of 133 m^2 d m^{-3}.

Fig. 3 depicts variation of percent COD removal with the feed TCP content at different feed COD's and a constant A/Q ratio of 93 m^2 d m^{-3}. Percent COD removal decreased with increasing feed TCP due to toxic effects of TCP contents on the microorganisms. This decrease was more pronounced at high feed COD contents.

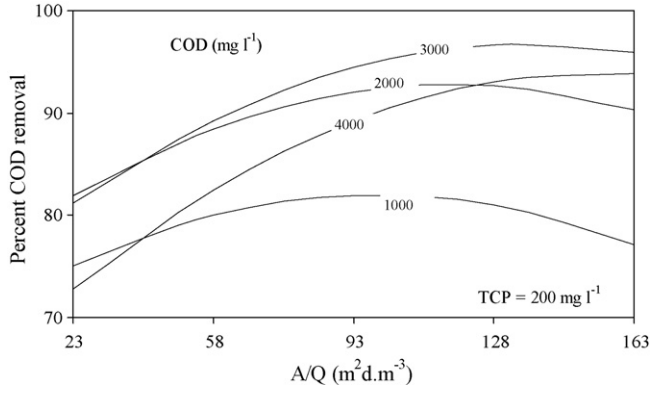

Fig. 2. Variation of percent COD removal with A/Q ratio at different feed COD and constant TCP concentration of 200 mg L^{-1}.

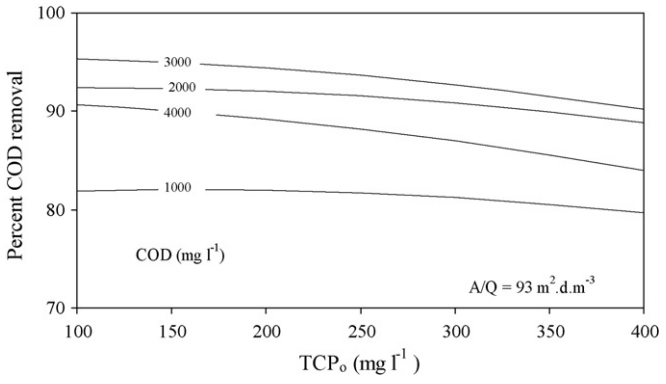

Fig. 3. Variation of percent COD removal with the feed TCP at different feed COD contents and a constant A/Q ratio of 93 m^2 d m^{-3}.

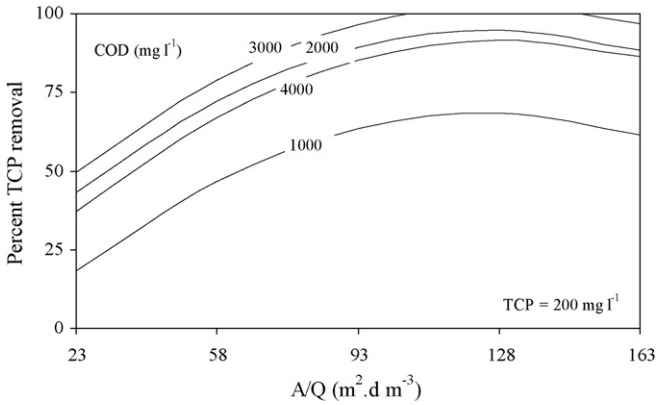

Fig. 4. Variation of percent TCP removal with A/Q ratio at different feed COD and constant TCP concentration of 200 mg L^{-1}.

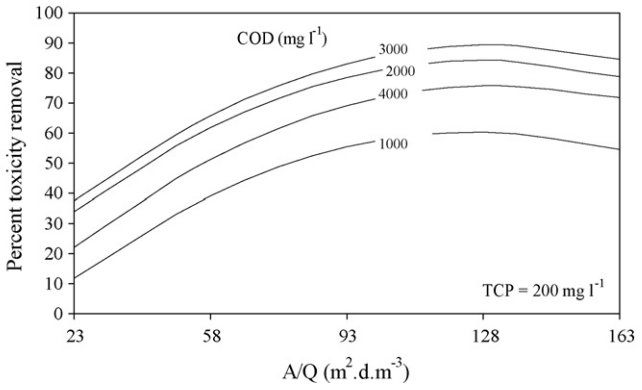

Fig. 6. Variation of percent toxicity removal with the A/Q ratio at different feed COD and constant feed TCP content of 200 mg L^{-1}.

At low feed COD concentrations (<2000 mg L^{-1}) COD removal was not affected from the feed TCP probably due to effective biodegradation of TCP at low feed COD levels in the absence of sufficient COD to support the microorganisms. However, at high feed COD's the adverse effects of TCP was more pronounced at high TCP levels due to preferable use of sucrose present in molasses instead of TCP biodegradation. At a constant feed TCP, COD removal increased with increasing feed COD up to 3000 mg L^{-1} and then decreased due adverse effects of high COD loadings. The optimal feed COD was 3000 mg L^{-1} for all feed TCP contents when A/Q was 93 m^2 d m^{-3}.

Variation of percent TCP removal with A/Q ratio at different feed COD's are depicted in Fig. 4 at a constant feed TCP of 200 mg L^{-1}. TCP removal increased with A/Q ratio due to high biomass concentrations at high A/Q ratios since biomass concentration is proportional to the biofilm surface area (A). A/Q ratio of 120 m^2 d m^{-3} was sufficient for maximum TCP removal at all feed COD contents tested. At constant A/Q ratio (constant biofilm surface area) percent TCP removal increased with increasing feed COD up to 3000 mg L^{-1} due to COD limitations at low COD loadings. TCP removal decreased with further increases in the feed COD to 4000 mg L^{-1}. Probably the biofilm organisms did not degrade TCP at high COD loadings and preferably degraded sucrose in molasses which resulted in lower percent TCP removals at high COD loadings. The optimal feed COD and A/Q ratio were approximately 3000 mg L^{-1} and 130 m^2 d m^{-3}, respectively, for complete removal of TCP when the feed TCP was 200 mg L^{-1}.

Variations of percent TCP removal with the feed TCP at different feed COD contents and constant A/Q ratio of 93 m^2 d m^{-3} are depicted in Fig. 5. Percent TCP removal decreased with increasing feed TCP due to toxic effects of high TCP concentrations. Adverse

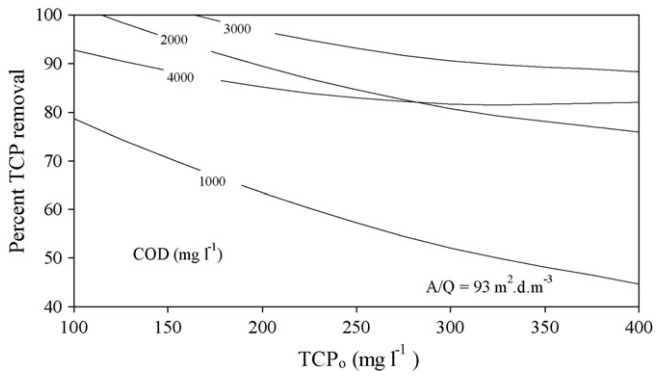

Fig. 5. Variation of percent TCP removal with the feed TCP at different feed COD contents and a constant A/Q ratio of 93 m^2 d m^{-3}.

effects of feed TCP were more pronounced at low feed COD concentrations due to low active biomass concentrations at low COD loadings. As the feed COD increased the adverse effects of TCP became less pronounced due to high active biomass concentrations at high COD loadings. TCP removal also increased with increasing feed COD up to 3000 mg L^{-1} at a constant feed TCP content. Further increases in the feed COD above 3000 mg L^{-1} resulted in decreases in TCP removal probably because of preferable use of sucrose present in molasses over TCP at high COD loadings. Feed COD should be around 3000 mg L^{-1} in order to maximize TCP removal (>90%) for all feed TCP contents for an A/Q value of 93 m^2 d m^{-3}.

Toxicity removals from the wastewater depicted similar behavior to the TCP removals since the major toxic compound in the medium was TCP or its degradation products. Variations of percent toxicity removal with A/Q ratio at different feed COD's and constant TCP of 200 mg L^{-1} is depicted in Fig. 6 which shows the same trends as in Fig. 4 (TCP removal). Percent toxicity removal increased with increasing A/Q ratio due to high concentrations of active biofilm organisms at high A/Q ratios since biomass concentration is proportional to the biofilm surface area (A). A/Q ratio of 120 m^2 d m^{-3} was sufficient for maximum toxicity removal at all feed COD contents tested. At constant A/Q ratio (constant biofilm surface area) percent toxicity removal increased with increasing feed COD up to 3000 mg L^{-1} due to COD limitations at low COD loadings. Toxicity removal decreased with further increases in the feed COD to 4000 mg L^{-1}, due to preferable utilization of sucrose present in molasses instead of TCP yielding high TCP and toxicity levels. The optimal feed COD and A/Q ratio were approximately 3000 mg L^{-1} and 130 m^2 d m^{-3}, respectively for complete removal of toxicity when the feed TCP was 200 mg L^{-1}. Percent toxicity removals were lower than TCP removals probably due to some toxic intermediate formation during TCP biodegradation.

Fig. 7 depicts variations of percent toxicity removal with the feed TCP at different feed COD contents and a constant A/Q ratio of 93 m^2 d m^{-3}. The curves in Fig. 7 depict the similar trends as in Fig. 5 (TCP removal). Toxicity removal decreased with increasing feed TCP due to toxic effects of high TCP concentrations on the microorganisms. Reductions in percent toxicity removal with increases in the feed TCP were more pronounced at low feed COD contents due to low active biomass concentration at low COD loadings. Adverse effects of high feed TCP contents were reduced by increasing the feed COD content yielding high active biomass concentrations. Percent toxicity removal at a constant feed TCP increased with increasing feed COD up to 3000 mg L^{-1} due to COD limitations at low COD loadings. Further increases in the feed COD yielded lower toxicity removals probably due to utilization of sucrose in molasses

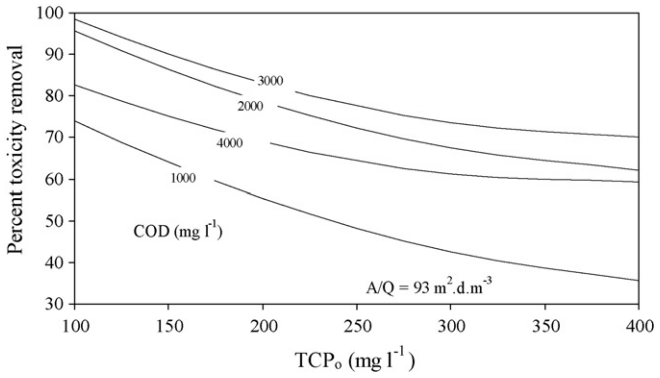

Fig. 7. Variation of percent toxicity removal with the feed TCP at different feed COD contents and a constant A/Q ratio of 93 m^2 d m^{-3}.

instead of TCP at high COD loadings yielding high TCP and toxicity levels in the effluent. The system should be operated with a fed COD of 3000 mg L^{-1} at all feed TCP contents to maximize toxicity removal when A/Q ratio was 93 m^2 d m^{-3}.

Table 4 summarizes the optimum feed COD and A/Q ratios maximizing COD, TCP and toxicity removals for different feed TCP concentrations. For the feed TCP of 100 mg L^{-1}, feed COD of 1950 mg L^{-1} and A/Q ratio of 104 m^2 d m^{-3} are required for maximum COD (92%), TCP (100%) and toxicity (96%) removals. The feed COD and A/Q ratio of 2985 mg L^{-1} and 165 m^2 d m^{-3} are required for the feed TCP of 400 mg L^{-1} in order to maximize COD (99%), TCP (100%) and toxicity (93%) removals. High feed TCP contents required high A/Q ratio and high feed COD contents for high removal efficiencies.

Literature studies report less than 65% TCP removals for the feed TCP contents lower than 100 mg L^{-1} in aerobic treatment of TCP containing wastewaters [25–27]. Combined anaerobic–aerobic treatment systems were reported to result in higher TCP removals due to anaerobic degradation of TCP [2,3,26]. However, the feed TCP contents in those studies were much lower than that of our study. As compared to the literature studies on biological treatment of TCP containing wastewaters, our study with the RTBR resulted in more than 95% COD, TCP and toxicity removals from synthetic wastewater containing TCP concentrations up to 400 mg L^{-1} with an A/Q ratio of 165 m^2 d m^{-3}. As compared to our previous study on TCP removal using a rotating brush biofilm reactor (RBBR) [28], this study with RTBR yielded higher COD and TCP removals for the same A/Q ratio and the feed COD contents probably due to high biomass concentrations per unit surface area of the support media. For the feed TCP of 400 mg L^{-1}, a feed COD of 3000 mg L^{-1} and A/Q ratio of 256 m^2 d m^{-3} were required for maximum COD (96%), TCP (100%) and toxicity (100%) removals in RBBR [28] as compared to feed COD and A/Q requirements of 3000 mg L^{-1} and 165 m^2 d m^{-3} with the RTBR in this study. Lower surface area requirements for the same degree of removal in this study is probably due to thicker biofilm formation on the rotating tube surfaces as compared to the brush surfaces.

Table 4
Optimum operating conditions for different feed TCP concentrations as predicted from the response functions

TCP (mg L^{-1})	COD (mg L^{-1})	A/Q (m^2 d m^{-3})	E_{TCP} (%)	E_{COD} (%)	E_{TOX} (%)
100	1950	104	100	92	96
200	2936	133	100	97	90
300	2884	147	100	97	87
400	2985	165	100	99	93

4. Conclusions

A newly developed rotating perforated-tubes biofilm reactor was used for biological treatment of 2,4,6-trichlorophenol containing synthetic wastewater. Effects of major operating variables such as the feed COD, TCP and also A/Q ratio on percent COD, TCP and toxicity removals were investigated by using a Box–Behnken statistical experiment design approach. Different response functions were correlated with the experimental data using the ANOVA test and a quadratic polynomial equation was found to be the most suitable one with the highest correlation coefficients. Percent COD removals decreased with increasing feed TCP concentration due to toxic effects of TCP on the organisms, but increased with increasing A/Q ratio due to high concentrations of biofilm organisms at high A/Q ratios. Percent TCP and toxicity removals also increased with increasing A/Q ratio and decreasing feed TCP concentrations. Operation at high A/Q ratios above 120 m^2 d m^{-3} resulted in high biomass concentrations and eliminated TCP and toxicity from the effluent. High A/Q ratios (>120 m^2 d m^{-3}) and feed COD concentrations (3000 mg L^{-1}) must be used in order to obtain high removal efficiencies at high feed TCP contents (>200 mg L^{-1}). For the feed TCP content of 400 mg L^{-1}, the optimal operating conditions maximizing COD (99%), TCP (100%) and toxicity (93%) removals were COD_o of 3000 mg L^{-1} and A/Q ratio of nearly 165 m^2 d m^{-3}. Percent toxicity removals were always less than TCP removals indicating presence of other toxic compounds or formation of some toxic intermediates during TCP biodegradation.

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