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Bioremediation of wastewaters containing various phenolic compounds by phenol-acclimated activated sludge

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ABSTRACT

This study was conducted to investigate the potential of activated sludge which had been acclimated to phenol in biodegrading different concentrations of various phenolic compounds in the batch reactors. The phenol-acclimated activated sludge was capable to completely remove the initial phenol concentration of 280 mg/L, which was twice the concentration it had been acclimated. In the presence of *o*-cresol and *m*-cresol at the initial concentration of 280 mg/L each in the separated batch reactors, more than 95% of cresols could be removed by the phenol-acclimated activated sludge. However, the phenol-acclimated activated sludge was only able to achieve 38% of 4-chlorophenol removal efficiency at this initial concentration. The presence of 2-chlorophenol and 3-chlorophenol in the separated batch reactors at initial concentrations of, as low as, 35 mg/L was adequate to inhibit the phenol-acclimated activated sludge from removing them. The kinetic data for the phenolic compounds and their respective chemical oxygen demand (COD) removal were well fitted to the pseudo-first-order formulation. The pseudo-first-order rate constants of each phenolic compound and their respective COD removal demonstrated that the toxicity exerted by each phenolic compound which inhibited its own degradation followed 2-chlorophenol > 3-chlorophenol > 4-chlorophenol > *o*-cresol > *m*-cresol > phenol.

Keywords: Activated sludge acclimated to phenol; Degradation of phenolic compounds; Removal efficiency; Pseudo-first-order rate constant

1. Introduction

Phenolic compounds are found in the wastewaters discharged from many industrial processes, including pharmaceuticals, pesticides and herbicides, petroleum and oil refining, pulp and paper plants, solvents, paints, and wood preservative industries [1–5]. The presence of phenolic compounds in wastewaters are significantly harmful to the environment because of their high toxicity, recalcitrance, and bioaccumulation, and suspected to be carcinogenic and cause mutagenesis in living organisms [6,7]. Thus, the removal of the phenolic compounds to sufficiently low level in wastewaters is of great importance.

The removal of phenolic compounds from wastewaters can be commonly attained either by physical or chemical methods such as activated carbon adsorption, solvent extraction, and chemical oxidation [1,7–11]. However, adsorption and solvent extraction only transfer the phenolic compounds from one phase to another, which require further treatment, whereas,

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chemical oxidation always lead to incomplete degradation [1]. On the other hand, the biological treatment process of phenolic compounds has been proven to be more cost-effective, practical, and versatile approach as it leads to low possibility of by-product formation. The biological remediation which incorporates microorganisms in different kinds of batch and continuous processes is being extensively studied to treat wastewaters containing phenolic compounds [4,12–14]. Sarfaraz et al. [1] had showed that the removal efficiency of phenol exceeds 80% in the influent concentration of 1,050 mg/L when the granular denitrifying sludge was used in the sequencing batch reactor (SBR). In addition, Atuanya et al. [12] demonstrated that 2,4-dichlorophenol was degraded to the extent of 52 and 78% in the up-flow anaerobic sludge blanket and aerobic suspended growth reactors, respectively at organic loading rates of 0.18 kg/m^3/day and hydraulic retention time of 26.4 h. Nevertheless, most of the researchers only studied the application of activated sludge in removing the phenolic compounds where the activated sludge had been acclimated with the respective compound early. Thus, the presence of the mixture of phenolic compounds in the industrial wastewaters warrant further investigation of the capability of single phenolic compound-acclimated activated sludge to treat these wastewaters.

In most of the studies, the use of acclimated activated sludge can enhance the degradation ability of the target compounds [15,16]. Vazquez-Rodriguez et al. [17] revealed that the phenol-acclimated activated sludge did not exhibit any lag phase and the activated sludge only showed a residual growth after the phenol exhaustion. However, the presence of different substituted groups or same substituted group, but at different positions in the aromatic ring, would markedly inhibit these phenolic compounds degradation by phenol-acclimated activated sludge at different intensities. Since the accessible information of using a single phenolic compound-acclimated activated sludge to treat wastewaters containing various phenolic compounds are still limited, which hinder the design and application in the wastewater treatment plants; the main objective of this study is to evaluate the removal efficiencies of various phenolic compounds, namely phenol, *o*-cresol, *m*-cresol, 2-chlorophenol (2-CP), 3-chlorophenol (3-CP), and 4-chlorophenol (4-CP) and their respective chemical oxygen demand (COD) by phenol-acclimated activated sludge. In addition, the biodegradation kinetics of each phenolic compound at different concentrations are also determined in order to comprehend the time course of the degradation which is practically important to estimate the time required for achieving complete degradation during the treatment process.

2. Materials and methods

2.1. Culturing phenol-acclimated activated sludge

The phenol-acclimated activated sludge used in this study was initially collected from a local municipal sewage treatment plant in Penang, Malaysia. It was then cultured in a SBR with a base mix of the following composition (in mg/L): peptone (32), sucrose (109), CH_3COONa (56), $(NH_4)_2SO_4$ (405), KH_2PO_4 (35), K_2HPO_4 (180), $MgSO_4$ (49), $NaHCO_3$ (354), $FeCl_3.6H_2O$ (10), and $CaCl_2$ (42). The SBR was operated with the sequencing periods of instantaneous Fill, 0 h; React, 12 h; Settle, 1.5 h; Draw, 1 h; and Idle, 9.5 h for a cycle of 24 h. The total working volume was 12.8 L with the exchange volume of 9 L in each cycle. The sludge age was maintained for 40 days by wasting an appropriate amount of mixed liquor at the end of the React phase. Once the activated sludge was acclimated to the base mix, phenol with the initial concentration of 140 mg/L during the start of the React period (200 mg/L of phenol concentration in the 9 L of base mix) was maintained for a period of five months. Upon reaching the quasi-steady-state (defined as a fairly constant reaction time for the complete mineralization of phenol in the SBR), the mixed liquor volatile suspended solids (MLVSS) concentration and sludge volume index (SVI) value reached 4,500 ± 100 mg/L and 80 ± 7 mL/g, respectively. The MLVSS concentration was determined by subtracting the total weight of dried solids at 105˚C with the total weight left after ignition at 550˚C and divided by the sample volume [18]. The SVI was determined by dividing the value of 30-min settled sludge volume with its suspended solids concentration [18].

2.2. Batch experiment set up

All the biodegradation studies of various phenolic compounds by phenol-acclimated activated sludge obtained from the SBR during the quasi-steady state were carried out in the beaker with a total working volume of 2 L. A base mix volume of 1.4 L spiked with various phenolic compounds to obtain the different initial concentrations during the start of the reaction period was initially prepared in the separated beakers (Table 1). Then, the phenol acclimated activated sludge was then added into each of the beakers to attain the initial MLVSS concentration of 800 ± 50 mg/L with aeration and agitation started immediately. The pH and DO concentration were maintained in the range of

Table 1
Different initial concentrations of each phenolic compound during the start of the reaction period in the separated batch reactors

Phenolic compound	Initial concentration (mg/L)
Phenol	35, 70, 140, 210, 280
o-Cresol	18, 35, 70, 140, 280
m-Cresol	18, 35, 70, 140, 280
2-CP	18, 35, 70, 105, 140
3-CP	18, 35, 53, 70, 88
4-CP	18, 35, 70, 140, 280

7.5 ± 0.3 and 6.0 ± 0.5 mg/L, respectively throughout the reaction period. The samples were collected at regular time intervals to determine the concentrations of phenolic compounds and COD following standard methods [18] until the phenolic compounds were degraded to constant concentration in the separated batch reactors. Each of the phenolic compound's concentrations was analyzed via 4-aminoantipyrine method, which is described for phenol concentration determination in the standard methods [18] by using spectrophotometer model HALO VIS-10 Visible at λ_{max} of 500 nm. The concentration of COD was determined via closed reflux titrimetric methods in which the digested sample with standard potassium dichromate solution using specially made block heater with temperature manually controlled at 150 ± 2°C was titrated with standard ferrous ammonium sulfate titrant. Each batch experiment was repeated at least two times for every concentration of phenolic compounds studied. Abiotic tests were also carried out by using the autoclaved phenol-acclimated activated sludge for all the phenolic compounds concentrations studied under the same conditions to determine the loss of phenolic compounds via volatilization and adsorption which was found to be less than 4% for all the batch experiments performed.

2.3. Kinetic model for the removal of phenolic compounds and COD

The time course data of phenolic compounds and their respective COD concentrations from each of the batch experiment studies (Section 2.2) were fitted to the pseudo-first-order model as shown in Eq. (1):

$$[S]_t = [S]_0 \exp(-k_S t) \quad (1)$$

where $[S]_t$ = Concentration of phenolic compounds or COD at time, t; $[S]_0$ = Initial concentration of phenolic compounds or COD; k_S = Pseudo-first-order rate constant of phenolic compounds or COD removal and t = Time course.

The values of k_S of phenolic compounds or COD removal were then compared in order to determine the sequence of the toxicity and inhibition effect exerted by each of the phenolic compounds on phenol-acclimated activated sludge.

3. Results and discussion

3.1. Removal of phenolic compounds

The time courses of phenolic compounds removal, namely phenol, *o*-cresol, *m*-cresol, 2-CP, 3-CP, and 4-CP at different initial concentrations and their respective COD removal efficiencies by the phenol-acclimated activated sludge are shown in Figs. 1 and 2, respectively. Phenol was completely degraded in all the batch studies with the initial phenol concentration ranging from 35 to 280 mg/L (Table 1). This was further substantiated by the COD removal efficiency of approximately 100%, which explained no accumulation of intermediates during the degradation process. In comparison with the study performed by Banerjee and Ghoshal [19], only about 40% of COD was removed at the phenol concentration of 100 mg/L after 24 h of reaction period with pure culture of either *Bacillus cereus* MTCC 9817 strain AKG1 or *Bacillus cereus* MTCC 9818 strain AKG2. Thus, this verified that the presence of mixed culture of phenol-acclimated activated sludge was able to degrade higher concentrations of phenol which probably due to the interaction among all the species present in the flocs. Kim et al. [20] documented that the degradation of phenol and 4-chlorophenol by *Pseudomonas testosteroni* CPW301 was seriously inhibited by 2,4,6-trichlorophenol. However, a mixed culture of *Pseudomonas testosteroni* CPW301 and *Pseudomonas solanacearum* TCP114 (which could only degrade 2,4,6-trichlorophenol) could treat phenol, 4-chlorophenol, and 2,4,6-trichlorophenol completely and overcome the inhibition of substrates to the other micro-organisms. Although the phenol-acclimated activated sludge in this study was being acclimated to only 140 mg/L of initial phenol concentration in the SBR, it was capable to completely degrade twice the initial phenol concentration which it had been acclimated. In the study with phenol-free activated sludge (unacclimated activated sludge), more than 420 min was required in order to remove the initial phenol concentration of only 10 mg/L. Moreover, the check with this concentration of other phenolic compounds studied showed that the unacclimated activated sludge required even more time than phenol to remove them, which generated a negligible rate of

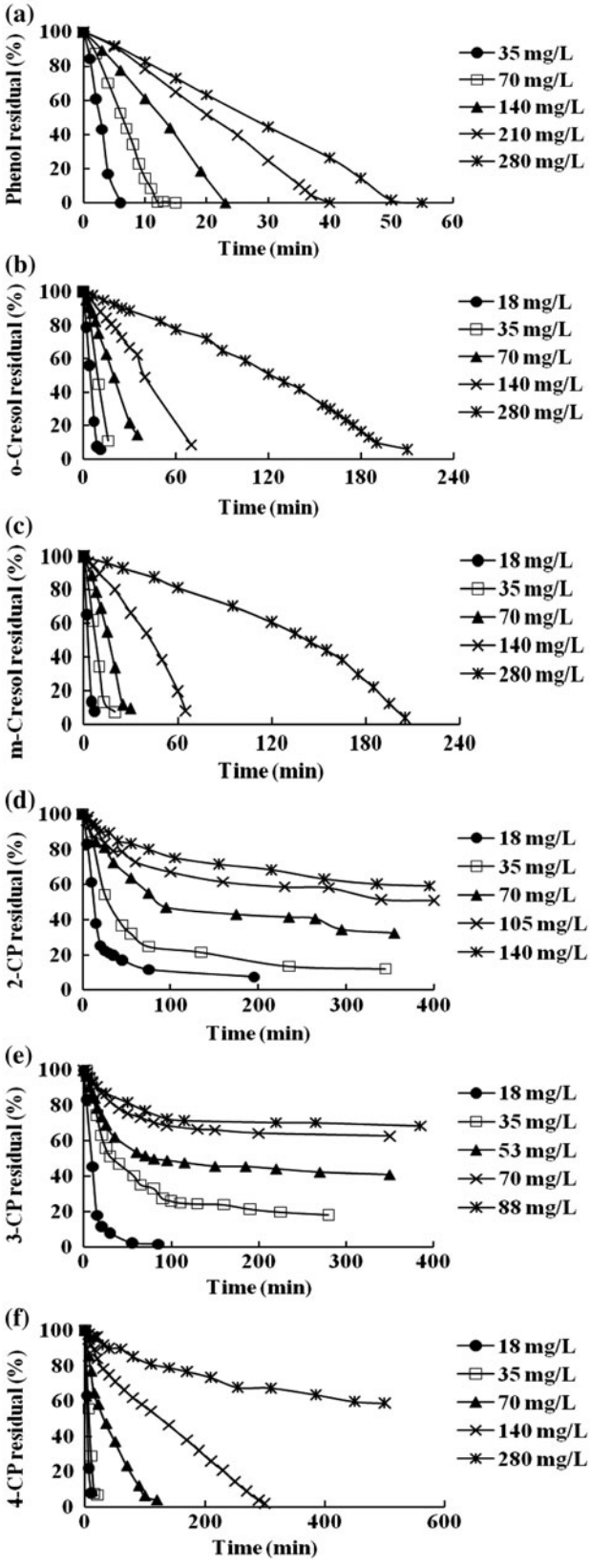

Fig. 1. Time courses of phenolic compounds removal; (a) phenol, (b) *o*-cresol, (c) *m*- cresol, (d) 2-CP, (e) 3-CP and (f) 4-CP.

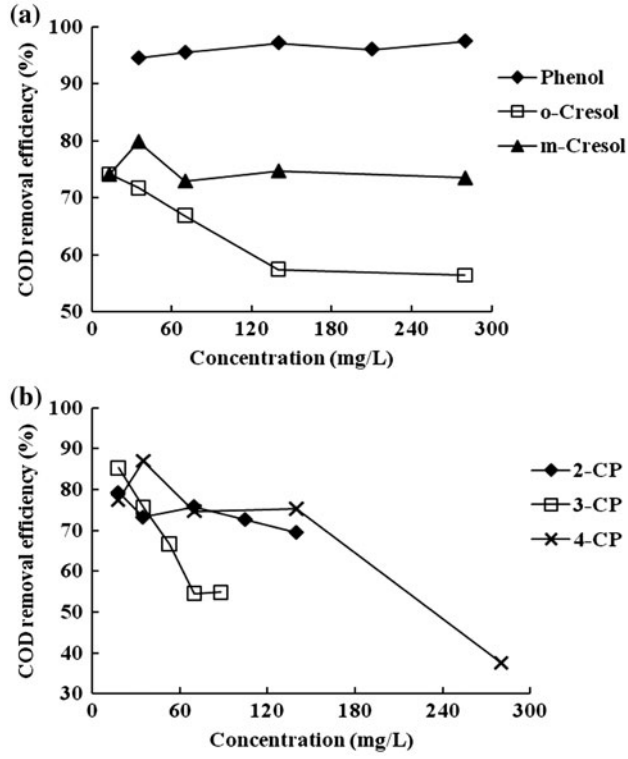

Fig. 2. COD removal efficiency at different initial concentrations; (a) phenol, *o*-cresol and *m*-cresol and (b) 2-CP, 3-CP, and 4-CP.

removal. This substantiated the importance of acclimation process before the activated sludge could be used to degrade the organic compounds where it was acclimated early. Nevertheless, the presence of the increasing phenol concentrations would require longer time period in order to achieve complete degradation by the phenol-acclimated activated sludge. These results were confirmed by the finding of Saravanan et al. [21] which also showed that longer time was consumed when degrading higher concentration of phenol by the mixed culture.

The degradation of *o*-cresol and *m*-cresol by phenol-acclimated activated sludge are shown in Fig. 1(b) and (c), respectively. The removal efficiencies of both *o*-cresol and *m*-cresol were always higher than 95% for all the initial concentrations studied. However, the time period required to remove both *o*-cresol and *m*-cresol, especially at the higher concentrations was longer as compared with the phenol degradation. This could be observed at the initial concentration of 280 mg/L with phenol degradation that required virtually 50 min, whereas both *o*-cresol and *m*-cresol required nearly 210 min each, respectively. Additionally, at this initial concentration, the COD removal efficiencies for both *o*-cresol and *m*-cresol were only

56% and 73%, respectively against approximately 100% for the phenol degradation. The plausible reason was the activated sludge used in this study was only acclimated to phenol. Therefore, the presence of cresols, particularly at higher concentrations would inhibit their own degradation by phenol-acclimated activated sludge due to the surplus formation of intermediates. This was consistent with the results obtained by Lee et al. [16] which showed that the degradation rate of cresols by phenol-acclimated aerobic granules decreased with the increasing cresols concentrations.

Finally, the degradation of monochlorophenols, namely 2-CP, 3-CP, and 4-CP by phenol acclimated activated sludge are demonstrated in Fig. 1(d)–(f), respectively. It was observed that 2-CP and 3-CP exerted the highest inhibitory effect on their own degradation and COD removal by phenol-acclimated activated sludge compared with the other phenolic compounds. The degradation of 2-CP and 3-CP decreased from 91 to 40% and 96 to 27%, respectively when their initial concentrations were increased from only 18 to 140 and 18 to 88 mg/L, respectively. Furthermore, the COD removal efficiencies of 2-CP and 3-CP reached only 70 and 55%, respectively when their initial concentrations were 140 and 88 mg/L, respectively. The lower COD removal efficiencies of 2-CP and 3-CP were mainly due to incomplete degradation by phenol-acclimated activated sludge. Farrell and Quilty [22] had documented that the degradation of 2-CP and 3-CP via meta-cleavage would produce a toxic metabolite which inactivated the catechol-2,3-dioxygenase enzyme and prevented further degradation of 2-CP and 3-CP. Moreover, the accumulation of brown colour polymers as reported by Farrell and Quilty [22] was also found in this study with 2-CP and 3-CP, which possibly indicated that catechol-2,3-dioxygenase enzyme was being inhibited in the batch reactors. The time courses of 4-CP degradation show that the phenol-acclimated activated sludge was able to remove more than 92% of 4-CP up to the initial concentration of 140 mg/L. However, when the initial concentration of 4-CP was increased to 280 mg/L, the 4-CP removal decreased drastically to only 38% with the respective COD removal efficiency of 37%. These results authenticated that the inhibition of 4-CP towards the phenol-acclimated activated sludge was only occurring at the initial concentration of more than 140 mg/L.

3.2. Kinetics study

The kinetics of removal of each phenolic compound and their respective COD by phenol-acclimated activated sludge during the time course studies were investigated. The k_S values of phenolic compounds and COD removal are listed in the Table 2 together with the respective R^2 always greater than 0.9 indicating that the pseudo-first-order model provided a reasonable correlation.

It could be observed that when the initial concentrations of each phenolic compound were increased, the pseudo-first-order rate constants of phenolic compounds removal ($k_{\text{phenolic compound}}$) and their respective pseudo-first-order rate constants of COD removal (k_{COD}) would decrease. This signified that the strength of phenolic compounds toxicity and the inhibitory effect towards their own degradation by phenol-acclimated activated sludge were greater at higher concentrations. Among the phenolic compounds studied, the presence of phenol in the base mix generated the highest k_{phenol} and $k_{\text{COD, phenol}}$ values in comparison with cresols and monochlorophenols at the same initial phenolic compounds concentrations. Again, this proved that the activated sludge which had been acclimated to phenol in the SBR is more capable to degrade phenol than other phenolic compounds.

In the presence of *o*-cresol and *m*-cresol, the $k_{o\text{-cresol}}$ and $k_{m\text{-cresol}}$ values (Table 2) decreased substantially from 239 to 12×10^{-3} and 341 to 5×10^{-3}/min, respectively when their initial concentrations were increased from 18 to 280 mg/L. Similar trends could be seen for $k_{\text{COD, }o\text{-cresol}}$ and $k_{\text{COD, }m\text{-cresol}}$ values which decreased from 79 to 3×10^{-3} and 138 to 5×10^{-3}/min, respectively at the same initial concentration increased. At higher initial concentrations, especially more than 140 mg/L of *o*-cresol and *m*-cresol, the $k_{o\text{-cresol}}$ and $k_{m\text{-cresol}}$ and $k_{\text{COD, }o\text{-cresol}}$ and $k_{\text{COD, }m\text{-cresol}}$ were not significantly different. However, the $k_{m\text{-cresol}}$ and $k_{\text{COD, }m\text{-cresol}}$ were always higher than $k_{o\text{-cresol}}$ and $k_{\text{COD, }o\text{-cresol}}$, respectively at the initial concentrations lower than 140 mg/L confirming that *o*-cresol was more toxic and exerting higher inhibitory effect towards its own degradation by phenol-acclimated activated sludge than *m*-cresol.

The batch studies with monochlorophenols demonstrated that the $k_{\text{phenolic compound}}$ and k_{COD} were the lowest as compared with phenol and cresols (Table 2). The $k_{4\text{-CP}}$ values were always higher than $k_{2\text{-CP}}$ and $k_{3\text{-CP}}$ at the same phenolic compound initial concentrations which again proved that 4-CP was more capable to be degraded by phenol-acclimated activated sludge. These results were further supported by the higher $k_{\text{COD, 4-CP}}$ values than $k_{\text{COD, 2-CP}}$ and $k_{\text{COD, 3-CP}}$. Between 2-CP and 3-CP, at the initial phenolic compounds higher than 70 mg/L, the $k_{2\text{-CP}}$ and $k_{3\text{-CP}}$ and $k_{\text{COD, 2-CP}}$ and $k_{\text{COD, 3-CP}}$ were not significantly different. Nevertheless, at the initial phenolic

Table 2
Pseudo-first-order rate constants of phenolic compounds removal ($k_{\text{phenolic compound}}$) and their respective pseudo-first-order rate constant of COD removal (k_{COD}) at different initial phenolic compounds concentrations

Phenolic compounds (mg/L)	$k_{\text{phenolic compound}} \times 10^3$ (1/min)	R^2	$k_{\text{COD}} \times 10^3$ (1/min)	R^2
Phenol				
35	280 ± 7	0.981	245 ± 5	0.978
70	136 ± 39	0.977	138 ± 10	0.991
140	59 ± 1	0.979	102 ± 9	0.978
210	36 ± 3	0.976	38 ± 1	0.997
280	24 ± 5	0.981	25 ± 6	0.998
o-Cresol				
18	239 ± 14	0.948	79 ± 5	0.959
35	134 ± 6	0.920	60 ± 5	0.983
70	44 ± 4	0.972	31 ± 10	0.951
140	31 ± 7	0.933	10 ± 1	0.943
280	12 ± 2	0.917	3 ± 1	0.972
m-Cresol				
18	341 ± 69	0.969	138 ± 29	0.979
35	154 ± 35	0.927	68 ± 1	0.972
70	79 ± 3	0.932	37 ± 2	0.971
140	29 ± 10	0.932	16 ± 1	0.988
280	5 ± 1	0.971	5 ± 1	0.973
2-CP				
18	70 ± 6	0.973	74 ± 6	0.975
35	19 ± 2	0.978	42 ± 12	0.975
70	6 ± 0	0.904	28 ± 0	0.977
105	1 ± 0	0.979	27 ± 3	0.949
140	1 ± 0	0.988	1 ± 0	0.923
3-CP				
18	106 ± 9	0.964	81 ± 10	0.979
35	24 ± 0	0.978	63 ± 3	0.943
53	15 ± 1	0.992	45 ± 5	0.959
70	7 ± 2	0.988	41 ± 11	0.905
88	3 ± 0	0.962	28 ± 6	0.981
4-CP				
18	260 ± 6	0.970	97 ± 12	0.991
35	129 ± 29	0.967	95 ± 38	0.971
70	23 ± 5	0.981	45 ± 2	0.966
140	6 ± 0	0.978	10 ± 8	0.938
280	1 ± 0	0.967	5 ± 2	0.952

compounds concentrations lesser than 70 mg/L, the $k_{3\text{-CP}}$ and $k_{\text{COD, 3-CP}}$ were always higher than $k_{2\text{-CP}}$ and $k_{\text{COD, 2-CP}}$, respectively verifying that 2-CP was more toxic and exerts higher inhibitory effect towards its own degradation by phenol-acclimated activated sludge than 3-CP.

Among all phenolic compounds experimented with the phenol-acclimated activated sludge, it could be concluded that the toxicity exerted by each phenolic compound and the inhibition towards their own degradation followed 2-CP > 3-CP > 4-CP > *o*-cresol > *m*-cresol > phenol based on $k_{\text{phenolic compound}}$ and k_{COD} values obtained. This could be plausibly explained by the differences in the substituted groups and positions in the aromatic ring which would contribute to the differences in toxicity intensities and inhibitory effect levels towards their own degradation by phenol-acclimated activated sludge. The presence of chlorine-substituted group would deactivate the aromatic ring due to the electron-withdrawing characteristic of chlorine group [23]. Thus, monochlorophenols would be less reactive than phenol and cresols and resist their own degradation by phenol-acclimated activated sludge. These observations concurred with the results obtained by Andreozzi et al. [24] that reported that the introduction of chlorine atom in the aromatic ring would cause reduction in the biodegradation rate, which was due to the electron-withdrawing characteristic of chlorine group. Furthermore, the differences in the substituted positions of the same substituted group in the aromatic ring would produce different intermediates during the degradation process by phenol-acclimated activated sludge which would inhibit the mineralization of the phenolic compounds at different levels. Masunaga et al. [25] found that the biodegradation of *o*-cresol by phenol-acclimated activated sludge was transformed to three dihydroxytoluenes, namely 3-methylcatechol, 4-methylresorcinol, and methylhydroquinone. However, Hopper and Taylor [26] have shown that *m*-cresol was metabolized to 3-hydroxybenzoate followed by hydroxylation to gentisate. In the degradation of monochlorophenols, Farrell and Quilty [22] had unveiled that the degradation of 2-CP and 3-CP by the mixed culture would produce 3-chlorocatechol which form toxic intermediates in the cells when further metabolized leading to their own inhibition of degradation, whereas 4-CP was degraded to 5-chloro-2-hydroxymuconic semialdehyde before being completely degraded.

4. Conclusion

The activated sludge acclimated to only 140 mg/L of phenol was capable to completely degrade twice of this initial phenol concentration in the batch reactor which was further verified by the approximately 100% of COD removal efficiency. In the presence of cresols, the phenol-acclimated activated sludge was able to achieve more than 95% of *o*-cresol and *m*-cresol removal efficiencies each at the initial concentration of 280 mg/L. However, only 38% of 4-CP was removed at this initial concentration although more than 92% of

4-CP was removed at the initial concentrations ranging from 18 to 140 mg/L. The phenol-acclimated activated sludge was unable to completely remove 2-CP and 3-CP presented in the batch reactors even though at the initial concentrations lower than 35 mg/L. Based on the data obtained from pseudo-first-order rate constants of each phenolic compound and their respective COD removal, the toxicity exerted by each phenolic compound and the inhibition towards their own degradation by phenol-acclimated activated sludge followed 2-CP > 3-CP > 4-CP > *o*-cresol > *m*-cresol > phenol.

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