Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 20(2008) 1508–1513
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Kinetics of phenol and *m*-cresol biodegradation by an indigenous mixed microbial culture isolated from a sewage treatment plant

Pichiah Saravanan[1], Kannan Pakshirajan[2], Prabirkumar Saha[1,*]

1. Department of Chemical Engineering, Indian Institute of Technology Guwahati, Guwahati, Assam 781039, India. E-mail: abychem@iitg.ernet.in
2. Department of Biotechnology, Indian Institute of Technology Guwahati, Guwahati, Assam 781039, India

Received 18 December 2007; revised 10 March 2008; accepted 12 March 2008

Abstract

An acclimatized mixed microbial culture, predominantly *Pseudomonas* sp., was enriched from a sewage treatment plant, and its potential to simultaneously degrade mixtures of phenol and *m*-cresol was investigated during its growth in batch shake flasks. A 2^2 full factorial design with the two substrates at two different levels and different initial concentration ranges (low and high), was employed to carry out the biodegradation experiments. The substrates phenol and *m*-cresol were completely utilized within 21 h when present at low concentrations of 100 mg/L for each, and at high concentration of 600 mg/L for each, a maximum time of 187 h was observed for their removal. The biodegradation results also showed that the presence of phenol in low concentration range (100–300 mg/L) did not inhibit *m*-cresol biodegradation. Whereas the presence of *m*-cresol inhibited phenol biodegradation by the culture. Moreover, irrespective of the concentrations used, phenol was degraded preferentially and earlier than *m*-cresol. A sum kinetics model was used to describe the variation in the substrate specific degradation rates, which gave a high coefficient of determination value ($R^2 > 0.98$) at the low concentration range of the substrates. From the estimated interaction parameter values obtained from this model, the inhibitory effect of phenol on *m*-cresol degradation by the culture was found to be more pronounced compared to that of *m*-cresol on phenol. This study showed a good potential of the indigenous mixed culture in degrading mixed substrate of phenolics.

Key words: biodegradation; factorial design of experiments; kinetics; *m*-cresol; mixed microbial culture; phenol; sum kinetics model

Introduction

Phenolic compounds, which are regarded as a priority pollutant by USEPA, are used in variety of industries, such as in production of steel, resins, ceramics, fungicides and herbicides (Tsai and Juang, 2006; Juang and Tsai, 2006; Rodriguez *et al*., 2006). Phenol and cresols are, therefore, the most important phenolic compounds in wastewater from these industries (Nuhoglu and Yalcin, 2005; Jiang *et al*., 2006; Kulkarni and Chaudhari, 2006; Pakshirajan *et al*., 2007). Owing to their toxic properties, removal of these phenolics to sufficiently low levels in wastewater is of great importance. The major disadvantages with the available conventional treatments are cost involved in disposal of the final effluent, production of toxic intermediates, and incomplete mineralization of the compounds. In such cases, biological processes using indigenous mixed microbial community seem promising in terms of complete mineralization of phenolics to CO_2 and H_2O without producing any toxic residues (Bai *et al*., 2007). However, inhibition of biodegradation of these phenolic compounds due to presence of two or more such compounds has been reported. Kar *et al*. (1997) observed that phenol and *p*-cresol mutually inhibited their biodegradation by *Arthrobacter*; the inhibition of *p*-cresol to phenol degradation was found stronger than that of *o*-cresol, but *o*-cresol marginally enhanced the phenol degradation. However, Paraskevi and Euripides (2005) reported that addition of *o*-cresol strongly inhibited phenol degradation by the indigenous soil bacterium *Pseudomonad*. Jiang *et al*. (2006) observed that inhibition of phenol biodegradation by *Candida tropicalis* was much stronger due to *m*-cresol than vice versa. They also fitted certain kinetic models to describe the specific growth of the yeast and degradation rates of the two substrates. There are only very few studies in literature describing *m*-cresol degradation in multisubstrate systems involving indigenous microbial culture.

In the present work, statistically valid 2^2 full factorial design of experiments was successfully employed to carry out experimental investigations to study the kinetics of simultaneous degradation of phenol and *m*-cresol by an indigenous mixed microbial culture, predominantly *Pseudomonas* sp. of bacteria, isolated from a sewage treatment plant in Guwahati, India, with the objective of developing a suitable biological reactor system employing the microorganisms in treating phenolics containing wastewater.

Compared to conventional one factor at a time experiments, statistical based factorial design of experiments give more meaningful information on the effects, main

* Corresponding author. E-mail: p.saha@iitg.ernet.in.

and interaction, of the factor involved in a given study (Montgomery, 2004). Moreover, the added advantage of reduction in the number of experiments to be performed employing such techniques proves more attractive for systematic investigations.

1 Materials and methods

1.1 Chemicals and reagents

Phenol, *m*-cresol, glucose, and inorganic salts used in preparing microbial growth media in the study were of analytical grade, and were purchased from Merck, India. The Hi 25^{TM} Enterobacteriaceae, Hi mobility^{TM} Biochemical kit and the staining agents for microbial characterization were purchased from Himedia Laboratories, India.

1.2 Microorganism and culture conditions

An indigenous mixed microbial culture, potent to degrade phenolic compounds, was isolated and enriched from a sewage treatment plant located in Guwahati, India as reported by Nuhoglu and Yalcin (2005). The predominant bacterial species in the mixed microbial culture was later identified to be *Pseudomonas* sp. The culture was initially grown in a 250-ml Erlenmeyer flask containing 100 ml of mineral salt medium (MSM) having the composition (mg/L) of $(NH_4)_2{\cdot}SO_4$ 230, $CaCl_2$ 8.0, $FeCl_3$ 1.0, $MnSO_4{\cdot}H_2O$ 100, $MgSO_4{\cdot}7H_2O$ 100, K_2HPO_4 500, KH_2PO_4 250, and glucose 2 g/L and pH 7.0 under agitation condition (150 r/min). The culture was then acclimatized over a period of one month to grow in MSM containing *m*-cresol as the sole carbon source up to a concentration of 1,000 mg/L. The detailed acclimatization phase of the culture to degrade *m*-cresol is shown in Fig.1.

1.3 Batch biodegradation experiments

All batch biodegradation experiments in this study were conducted in 250 ml Erlenmeyer flasks containing 100 ml of sterile MSM with different initial concentrations of both phenol and *m*-cresol. Before using the acclimatized culture in the biodegradation experiments, the culture was subjected to two transfers in 100 ml of fresh MSM containing *m*-cresol and grown at 27°C in an incubator shaker maintained at 150 r/min.

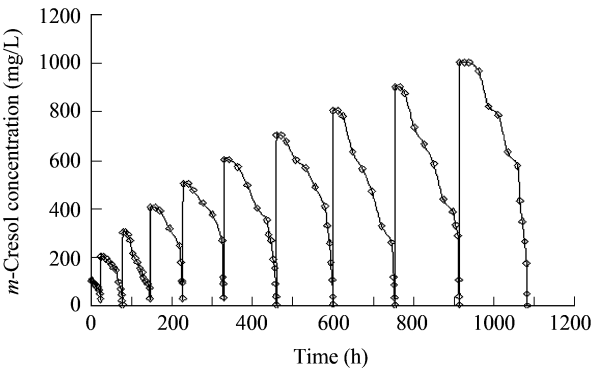

Fig. 1 *m*-Cresol degradation profile followed during the acclimatization period.

In our previous study (Saravanan *et al*., 2007), it was found that *m*-cresol presented as the sole carbon source in the medium inhibited the growth of the culture when concentrations higher than 200 mg/L; similarly, phenol inhibited the culture growth when higher than 300 mg/L. Based on these results of the single substrate degradation study, two different concentration ranges for phenol and *m*-cresol were chosen in this present multisubstrate degradation study: (I) low concentration range (100 to 300 mg/L phenol, 100 to 200 mg/L *m*-cresol) and (II) high concentration range (300 to 600 mg/L phenol, 200 to 600 mg/L *m*-cresol). These two concentration ranges of the compounds also represent their effects on the culture specific degradation rate, where the rate enhanced in lower range and inhibited in higher range. For choosing the combination level of these two substrates, a factorial design of experiments with the two substrates as the factors at two different levels was planned. Table 1 shows the design matrix employed in the study. Three center point replicates in the design were included to check experimental error. In total, seven combinations (experimental runs) of *m*-cresol and phenol initial concentrations were investigated for studying their biodegradation and the culture growth in the low and high initial concentration ranges adopted in this study.

During the experiments, a sample volume of exactly 1 ml was withdrawn at regular time intervals until complete degradation of the substrate(s). After analysis for cell density, the samples were centrifuged at 10,000 ×*g* for 3 min. The resulting supernatant was analyzed for residual phenol and *m*-cresol concentrations. Control experiments without the culture were also performed, and the initial concentrations of the substrates were found to remain unchanged, indicating that abiotic loss of the substrates in the study was negligible.

1.4 Analytical methods

Cell density in the samples was estimated with Diode array spectrophotometer (Spekol 1200, Analytik Jena, Germany) by measuring its absorbance (OD) at 600 nm wavelength. Then, OD_{600} was converted to dry cell weight by a calibration curve, which was obtained by plotting dry weight of biomass per millilitre vs. OD_{600}. High performance liquid chromatography (UV 200, Perkin Elmer, USA) was employed to quantify phenol and *m*-cresol concentrations in the biomass free samples. The analysis

Table 1 2^2 full factorial design with three center point replicates employed in the biodegradation study for both the low and high initial concentration ranges

Experimental run no.	Factor and level	
	Phenol	*m*-Cresol
1	–1	–1
2	–1	+1
3	+1	–1
4	+1	+1
5	0	0
6	0	0
7	0	0

–1: low level; +1: high level; 0: center point or middle level of the factors.

was performed with C18 column (150 mm × 4.6 mm × 5 μm; chromotopak) with acetonitrile/water (60/40, *V/V*) as the mobile phase at a flow rate of 1 ml/min, with a UV detector set at 275 nm. The retention period for phenol was 2.75 min, and for *m*-cresol, it was 3.25 min.

1.5 Biochemical characterization and scanning eletron microscopy for microbial species identification

In order to characterize the isolates, morphology, staining, biochemical tests were carried out using the Hi 25^{TM} Enterobacteriaceae, Hi mobility^{TM} Biochemical kit. The staining and characterization tests were carried out as per procedure detailed in the kits. For observation of the microbial cells with scanning electron microscopy (SEM), freshly grown cells in MSM were centrifuged, dried and coated with gold film in a sputter coater, and finally, the cell morphology was recorded in LEO 1430VP SEM instrument at a magnification of 4,350 at 15 kV.

2 Results and discussion

2.1 Biochemical and SEM analysis of the mixed culture

Under light microscope, the indigenous mixed microbial culture was observed to be uniformly small Gram-negative rods of varying lengths. The SEM image also confirmed the same finding, which is shown in Fig.2. Biochemical tests were carried out for different bacterial species and it was found that mixed culture consists of predominantly *Pseudomonas* sp. Further confirmatory tests like urease, catalase, oxidase, IMViC (indole production; methyl-red: Vogeas Proskauers; citrate utilization) and Gram-staining tests also confirmed (Radehaus and Schmidt, 1992). This *Pseudomonas* sp. predominantly present in the acclimatized mixed culture is responsible for phenolics degradation in the study. Table 2 presents the detailed results of the tests on the mixed microbial culture for its biochemical characterization.

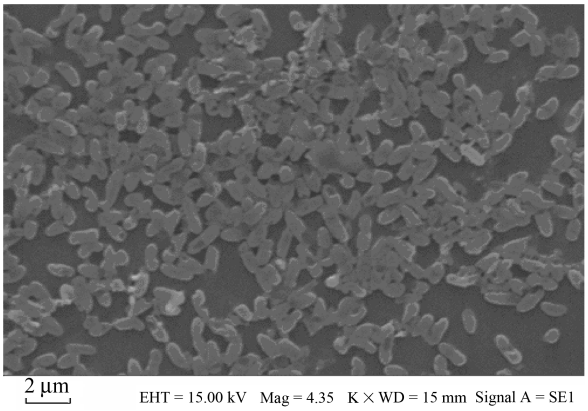

Fig. 2 SEM image of the indigenous mixed microbial culture showing predominant presence of rod shaped bacteria *Pseudomonas* sp.

2.2 Simultaneous degradation of phenol and *m*-cresol in the multisubstrate system

The biodegradation patterns due to the mixed microbial culture for low and high phenol and *m*-cresol initial concentrations, are presented in Figs.3a and 3b, respectively. It could be observed that for a given concentration of the substrate, the culture took longer duration for complete utilization of *m*-cresol compared to phenol. Also, while phenol, in either low or high initial concentration range, did not show any lag phase in its degradation, lag phase in *m*-cresol degradation was found to be significant, especially in its high concentration range. It should be noted here that each experimental run in the study always contained both the substrates. However, the concentration of *m*-cresol was less compared to phenol.

Table 2 Results of the various biochemical tests to identify the predominant microbial species in the acclimatized indigenous mixed culture

Test	Result	Test	Result
Gram stain	–	Motility	+
ONPG	+	Catalase	+
H_2S	–	Voges-Proskauer	–
Citrate utilization	–	Urea	–
Indole	–	Methyl red	–
Esculin	+	Nitrate	–

–: negative result; +: positive result.

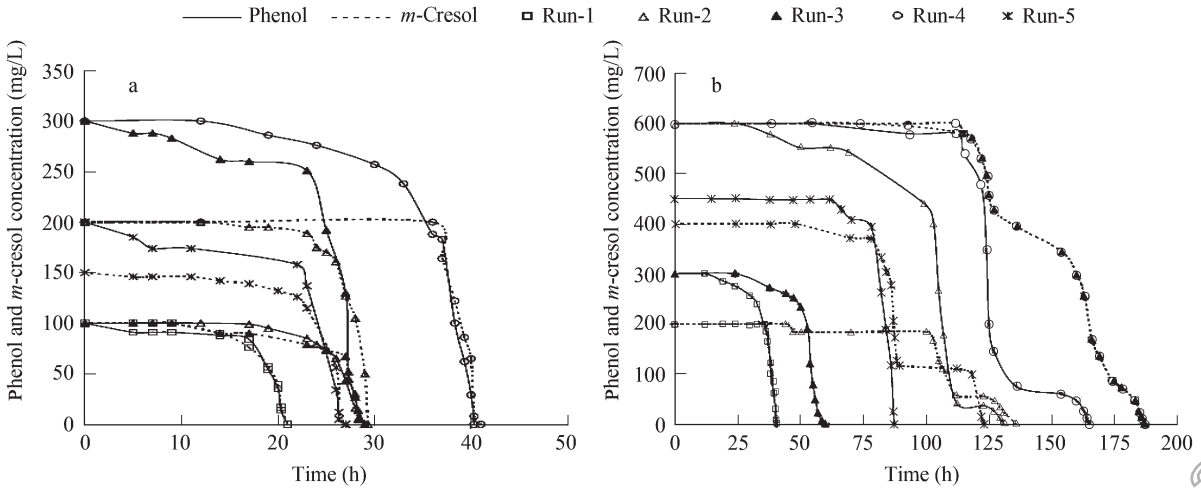

Fig. 3 Phenol and *m*-cresol biodegradation patterns shown by the culture in the low (a) and high (b) initial concentration ranges of the substrates.

Compared to single substrate (phenol/*m*-cresol) degradation, the combination of phenol and *m*-cresol as substrates exhibited different degradation patterns of the substrate in the study by Saravanan *et al*. (2007, 2008). Preferential uptake of phenol over *m*-cresol for given concentrations of the substrates, indicate that phenol compared to *m*-cresol is a much simpler carbon source, and therefore can be easily utilized by the mixed culture. This preferential uptake of phenol was observed mainly in the high concentration range of the substrates, but at lower concentration range of the substrates, the total time taken by the culture for simultaneous degradation of phenol and *m*-cresol were nearly the same. Similar to this present study, Bai *et al*. (2007) also observed that phenol was preferentially degraded over *m*-cresol, and *m*-cresol uptake was only towards depletion of phenol in the media. Jiang *et al*. (2006) using the yeast *Candida tropicalis* and dealing with mixed substrate biodegradation reported that the presence of phenol at low concentrations (0–1,000 mg/L) enhanced the rate of *m*-cresol biodegradation with an observed maximum biodegradation velocity at an initial phenol concentration of 80 mg/L. A similar effect on *m*-cresol degradation rate was also observed for initial concentrations of 100 mg/L phenol and 200 mg/L *m*-cresol.

2.3 Biomass yield and specific degradation rate of the substrates

The experimental data on the substrate degradation at various combinations of initial concentrations of phenol and *m*-cresol were utilized for calculating the culture biomass yield and specific degradation rates of the substrates according to the following equations:

$$Y_{X/S} = \frac{X_M - X_0}{S_0 - S_M} \quad (1)$$

$$q = -\frac{1}{x}\frac{dS}{dt} \quad (2)$$

In the above expression for specific degradation rate (q) of substrate S (phenol or *m*-cresol), X (mg/L) is the biomass concentrations at time t (h), and in the expression for biomass yield ($Y_{X/S}$), X_M and X_0 are the respective maximum and initial dry cell concentrations corresponding to the total substrate (phenol + *m*-cresol) concentrations for initial (S_0) and final (S_M). The calculated total biomass yield values at the both concentrations ranges are presented in Table 3. It could be seen that high values of yield coefficients were obtained in the study, especially at lower concentrations of the substrate combinations. However, irrespective of the combinations of initial *m*-cresol and phenol concentrations, the biomass yield decreased with an increase of the *m*-cresol concentration. Abuhamed *et al*. (2004) observed a maximum biomass yield value of 0.75 due to benzene at 700 mg/L initial concentrations on the growth of a *Pseudomonas* putida strain. In the present study, the highest yield value of 2.17 was obtained at low concentrations 100 mg/L phenol and 100 mg/L *m*-cresol; the least value of 1.44 was obtained at high concentrations of 300 mg/L phenol and 400 mg/L *m*-cresol. A higher biomass yield value obtained in the present study could be attributed to the mixed microbial culture and the substrates. It could be observed clearly from the obtained biomass yield values that the substrate inhibition of the culture occurred at high concentrations. Irrespective of the concentration levels of the substrates, the yield value was found to saturate at high concentration combinations, which is above 300 mg/L of both the substrates the yield remained almost the same. Table 4 presents the calculated individual q values for both phenol and *m*-cresol obtained in this multi substrate degradation study, which shows that in high concentration range combinations of the substrates, phenol specific degradation rates were found to be always higher than those of *m*-cresol, except when both the substrates were present in their low levels (–1). Between the two concentration ranges, low concentration range of the substrates yielded more specific degradation rates of the substrates than the high concentration range. However, within a given concentration range, especially in the low range, the q values (of both phenol and *m*-cresol) varied largely depending upon the combination levels in each experiment. Despite this observation, it was found that experimental error in the study was highly negligible owing to similar results obtained for the q values of phenol and *m*-cresol when three trials were run at their center point levels (0).

Table 3 Total biomass yield coefficient calculated in the study at low and high concentration ranges of the substrates (unit: g/g)

Phenol (mg/L)	*m*-Cresol concentration (mg/L)		
	100	150	200
100	2.17	2.16	2.05
200	2.16	1.76	1.89
300	1.7	1.8	1.9
Phenol (mg/L)	*m*-Cresol concentration (mg/L)		
	200	400	600
300	1.9	1.44	1.6
450	1.5	1.6	1.6
600	1.6	1.58	1.6

In order to get the role played by interaction between the two substrates on their individual specific uptake rates, the experimental data were fitted to a sum kinetic model proposed by Yoon *et al*. (1977).

2.4 Sum kinetics model fitting of experimental specific degradation rate

Sum kinetics model, proposed by Yoon *et al*. (1977) was slightly modified to fit the experimental data on specific degradation rates of the substrates, wherein the specific growth rate μ in the original equation was replaced with specific degradation rate q, as represented in Eq.(3). This model was utilized to evaluate and estimate the relative interaction effects on the individual degradation rates.

$$q = \frac{q_{max,1} S_{1L}}{K_{s,1} + S_{1L} + \frac{S_{1L}^2}{K_{1i}} + I_{2,1} S_{2L}} + \frac{q_{max,2} S_{2L}}{K_{s,2} + S_{2L} + \frac{S_{2L}^2}{K_{2i}} + I_{1,2} S_{1L}} \quad (3)$$

Table 4 Calculated specific degradation rates of phenol and *m*-cresol obtained in low and high initial concentration ranges of the substrates

Experimental run no.	Initial concentration level		Phenol specific degradation rate (h^{-1})		*m*-Cresol specific degradation rate (h^{-1})	
	Phenol	*m*-Cresol	Low range	High range	Low range	High range
1	–1	–1	0.114	0.0786	0.099	0.095
2	+1	–1	0.208	0.0093	0.194	0.0057
3	–1	+1	0.342	0.043	0.218	0.0087
4	+1	+1	0.079	0.0085	0.095	0.0081
5	0	0	0.09	0.0776	0.27	0.0082
6	0	0	0.0908	0.077	0.273	0.00821
7	0	0	0.091	0.0777	0.275	0.00823

where, the interaction parameter $I_{i,j}$ indicates the degree to which substrate i affects the biodegradation of substrate j; a large value of the parameter indicates a strong inhibition on the substrate uptake by the microorganism (Yoon *et al*., 1977). The other kinetic parameters q_{max}, K_s, K_i in the equation are the same as those for any single substrate system, which are namely maximum specific degradation rate, half-saturated and inhibition constants due to substrate i in the medium. A non-linear regression technique involving constraints for positive integer values of the parameters was employed in solving the model equation in MATLAB 7. Very high determination coefficient (R^2) values of 0.99 and 0.98 were obtained by fitting the model equation to the experimental q values of phenol and *m*-cresol respectively obtained using different combinations of the two substrates in their low concentration range. The following interaction parameters (I_{ij}) values were thus obtained by solving the model equations:

$$\begin{aligned} I_{\text{phenol 100 mg/L, } m\text{-cresol}} &= -1.37 \quad \text{at } m\text{-cresol 100 mg/L} \\ &= 1.322 \quad \text{at } m\text{-cresol 200 mg/L} \end{aligned} \tag{4}$$

$$\begin{aligned} I_{\text{ } m\text{-cresol 100 mg/L, phenol}} &= 0.103 \quad \text{at phenol 100 mg/L} \\ &= -0.36 \quad \text{at phenol 200 mg/L} \end{aligned} \tag{5}$$

The interaction parameter $I_{\text{phenol, } m\text{-cresol}}$ represents the effect of phenol on *m*-cresol degradation, the interaction parameter ($I_{m\text{-cresol, phenol}}$) represents the effect of *m*-cresol on phenol degradation by the culture. In general the negtive sign in the interaction parameter represents the intensity of the inhibition, which is strong. Hence from the obtained interaction values of these parameters, it could be said that *m*-cresol at 100 mg/L exhibits stronger inhibition on phenol degradation, compared to its effect at 200 mg/L. But for *m*-cresol, its effect on phenol degradation was stronger at 200 mg/L. In literature, a maximum interaction parameter value of 5.16 has been reported for the effect of toluene on benzene degradation by a *Pseudomonas putida* strain (Abuhamed *et al*., 2004). Considering the fact that phenol being a simpler and easily assimilable substrate, a large value of its inhibitory effect on *m*-cresol degradation by the indigenous mixed culture followed in the present study is not unlikely. It was observed that the sum kinetic model failed or did not fit the degradation kinetics of the substrates in their high concentration range combination. This finding could also be justified with interactions between the two substrates on phenol specific degradation rate, in both low (Fig.4a) and high (Fig.4b) concentration ranges. In general, interaction plots illustrate the significance of interactions of any two factors involved in a study. Such a plot involves dependent variable represented on the ordinate and one of the factors on the abscissa. The remaining factor is plotted for high values of both factors. Hence two lines are obtained whose deviation from being parallel is related to the degree of interaction (Montgomery, 2004). Fig.4a shows highly significant interactions, where the lines are completely unparallel and cross each other. In other words, the effect of one factor depends on the level of the other. Whereas in the high concentration range combinations of the substrates, the two lines in Fig.4b is relatively parallel to each other, which confirms that there exist least interaction between the substrates at higher concentrations on phenol degradation rate (Pakshirajan *et al*., 2007). Similar observations were found for the interaction between the two substrates on *m*-cresol degradation rates.

Based on the significant results obtained in this study, a

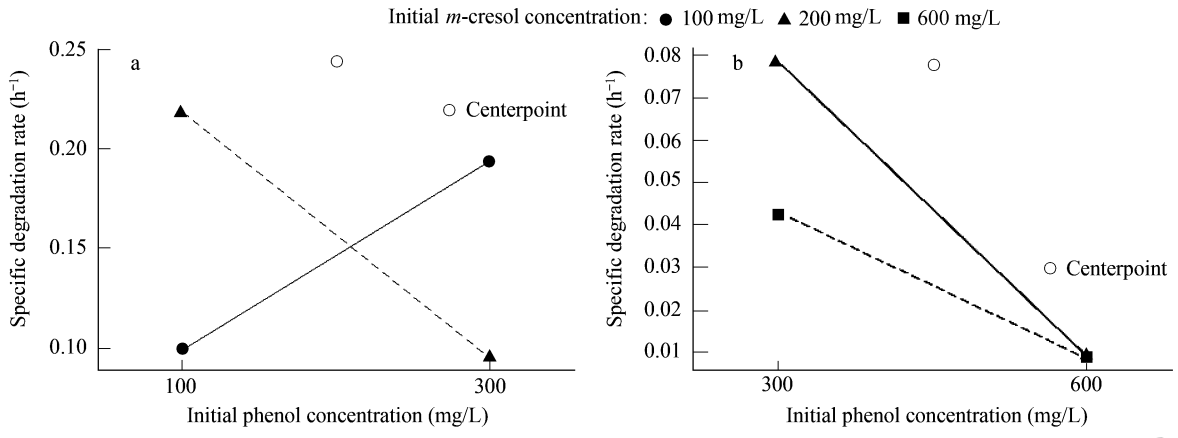

Fig. 4 Interaction effect between phenol and *m*-cresol in low (a) and high (b) concentration ranges combination on phenol specific degradation rate.

specially fabricated laboratory scale Internal Loop Airlift Bioreactor (ILALR) is currently being employed in the Department of Chemical Engineering, IIT Guwahati to study phenolics degradation by the indigenous mixed microbial culture, whose results will be reported in a separate paper elsewhere.

3 Conclusions

An acclimatized indigenous mixed microbial culture that was predominantly *Pseudomonas* sp. and was obtained from a sewage treatment plant, showed complete removal of phenol and *m*-cresol to a maximum concentration of 600 mg/L each. Phenol degradation was, quick and normally preceded *m*-cresol degradation. Total biomass yields and specific degradation rates of the substrates revealed that the *m*-cresol and phenol combination with a certain high concentration inhibited the culture growth, and that the biomass yield was high also. Parameter values for interaction between the two substrates, obtained by fitting a sum kinetic model to the experimental degradation rates of the substrate phenol showed that strong inhibitory effect on *m*-cresol degradation lower concentration ranges viceversa. Kinetic analysis of the results and the estimated sum kinetics model parameters helped in good interpretation role of the individual substrate in the multisubstrate system

References

Abuhamed T, Bayraktar E, Mehmetoglu T, Mehmetoglu U, 2004. Kinetics model for growth of *Pseudomonas putida* F1during benzene, toluene and phenol biodegradation. *Pro Biochem*, 38(8): 983–988.

Bai J, Wen J P, Li H M, Jiang Y, 2007. Kinetic modeling of growth and biodegradation of phenol and *m*-cresol using *Alcaligenes faecalis*. *Pro Biochem*, 42(4): 510–517.

Jiang Y, Wen J P, Bai J, Wang D Q, Hu Z D, 2006. Phenol biodegradation by the yeast *Candida tropicalis* in the presence of *m*-cresol. *Biochem Eng J*, 29(3): 227–234.

Juang R S, Tsai S Y, 2006. Growth kinetics of *Pseudomonas putida* in the biodegradation of single and mixed phenol and sodium salicylate. *Biochem Eng J*, 3(1): 133–140.

Kar S, Swaminathan T, Baradarajan A, 1997. Biodegradation of phenol and cresol isomer mixtures by *Arthrobacter*. *World J Microbiol Biotech*, 13(6): 659–663.

Kenneth F R, Douglas C M, Julia, D B R, 2000. Biodegradation kinetics of benzene, toluene, and phenol as single and mixed substrates for *Pseudomonas putida* F1. *Biotech Bioeng*, 69(4): 385–400.

Kulkarni M, Chaudhari A, 2006. Biodegradation of *p*-nitro phenol by *P. Putida*. *Biores Technol*, 97(8): 982–988.

Montgomery D C, 2004. Design and Analysis of Experiments. New York: John Wiley & Sons.

Nuhoglu N, Yalcin B, 2005. Modeling of phenol removal in a batch reactor. *Pro Biochem*, 40(3-4): 1233–1239.

Pakshirajan K, Chugh D, Saravanan P, 2007. Feasibility of *m*-cresol degradation using an indigenous mixed microbial culture with glucose as co-substrate. *Clean Technol Environ Policy*, 10(3): 303–308.

Paraskevi N P, Euripides G S, 2005. Effect of temperature and additional carbon sources on phenol degradation by indigenous soil *Pseudomonad*. *Biodegradation*, 16(5): 403–413.

Radehaus P M, Schmidt S K, 1992. Characterization of a novel *Pseudomonas* sp. that mineralizes high concentrations of pentachlorophenol. *App Environ Microbiol*, 59(9): 2879–2885.

Rodriguez G V, Youssef C B, Vilanova J W, 2006. Two-step modeling of the biodegradation of phenol by an acclimated activated sludge. *Chem Eng J*, 117(3): 245–252.

Saravanan P, Pakshirajan K, Saha P, 2008. Growth kinetics of an indigenous mixed microbial consortium during phenol degradation in a batch reactor. *Biores Technol*, 99(1): 205–209.

Saravanan P, Pakshirajan K, Saha P, 2007. Kinetics of growth and multi substrate degradation by an indigenous mixed microbial culture isolated from a wastewater treatment plant in Guwahati, India. Proceedings of the 2nd IWA - ASPIRE Asia-Pacific Regional Group Conference & Exhibition, Water and Sanitation in the Asia-Pacific Region: Opportunities, Challenges and Technology. Perth, Australia.

Okpokwasili G C, Nweke C O, 2005. Microbial growth and substrate utilization kinetics. *Afr J Biotechnol*, 5(4): 305–317.

Tsai S Y, Juang R S, 2006. Biodegradation of phenol and sodium salicylate mixtures by suspended *Pseudomonas putida* CCRC 14365. *J Hazard Mater*, 138(1): 125–132.

Yoon H, Klinzing G, Blanch H W, 1977. Competition for mixed substrates by microbial populations. *Biotech Bioeng*, 19(8): 1193–1210.