Kinetic modelling for removal of *m*-cresol from wastewater using mixed microbial culture in batch reactor

Sudipta Dey and Somnath Mukherjee

ABSTRACT

An indigenous mixed microbial culture isolated from an effluent treatment section of a coke oven plant has been studied for its *m*-cresol biodegradation capacity under aerobic batch reactor operation. The culture, after acclimatization could biodegrade up to 700 mg/L of *m*-cresol. The *m*-cresol concentration in the present study was at 50 mg/L and then ranged from 100 to 700 mg/L with step up concentration of 100 mg/L. Both biodegradation kinetics and microorganism growth kinetics were studied and kinetic parameters were estimated. The result showed that *m*-cresol was an inhibitory-type substrate and the inhibition effect became predominant after 200 mg/L of initial *m*-cresol. The specific growth rate of microorganisms increased up to 200 mg/L of *m*-cresol as sole carbon source, and then started decreasing. The kinetic data obtained in this study have been fitted to different substrate inhibition models (Haldane, Han-Levenspiel, Edward, Luong, Aiba, Teissier, Yano-Koga). Among all models, Han-Levenspiel and Luong were best fitted for this study (root mean square error = 0.001349). In addition, the variation of observed yield coefficient Yx/s with initial *m*-cresol concentration was investigated. The values of kinetic constants estimated by the models proved that the mixed culture used in the study had good potential for *m*-cresol degradation.

Key words | biodegradation, kinetic models, *m*-cresol, mixed culture, substrate inhibition kinetics

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ABBREVIATIONS

μ	specific growth rate (hr^{-1})
μ_{max}	maximum specific growth rate (hr^{-1})
S	substrate concentration (mg/L)
Ks	half saturation constant (mg/L)
Ki, Ksi	substrate inhibition constant (mg/L)
K_1, K_2, K	positive constant (mg/L)
S_m	critical substrate concentration (mg/L)
q	specific substrate degradation rate (hr^{-1})
q_{max}	maximum specific substrate degradation rate (hr^{-1})
So	initial substrate concentration (mg/L)
X	microorganism concentration (mg/L)
Xo	initial microorganism concentration (mg/L)
Yx/s	microbial growth yield (mg/mg)
T	time (hr)
n, m	empirical constants

INTRODUCTION

Phenolic compounds are common water pollutants from various industrial waste streams, such as polymeric resin producing companies, coal gasification plants, oil refining and coke oven industries, fibreglass units, pharmaceuticals, explosive manufacturers and varnish industries (Juang & Tsai 2006; Yan *et al.* 2006). They are also included in the list of priority organic pollutants of the US Environmental Protection Agency (Yan *et al.* 2006). Among all phenolic compounds, cresols are the major toxic organic pollutants that remain at the top of the list for the inherent difficulties they pose during their degradation. *m*-cresol is an isomeric phenol, with a methyl substituent at the meta position relative to hydroxyl group of phenol. In addition to being highly toxic and a potential carcinogen, cresol causes adverse effects on the central nervous system, lungs, kidneys and

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liver. Therefore wastewater containing *m*-cresol requires careful handling before discharge to receiving water bodies.

Some research has been done on *m*-cresol biodegradation by pure culture (Yan *et al.* 2006; Yao *et al.* 2011). Investigation of the degradation of high concentrations of *m*-cresol using mixed culture is rare. In spite of their toxic properties, *o*-cresol, *m*-cresol and *p*-cresol are utilized by a number of organisms as their sole carbon and energy source, under aerobic conditions, even at very high concentration (Fialova *et al.* 2004). Yan *et al.* (2006) have studied biodegradation of *m*-cresol up to a concentration of 320 mg/L under aerobic conditions in a batch system using *Candida tropicalis*. Their estimates of kinetic parameters using the Haldane model showed that the culture had a high Ks (half saturation constant) value (866 mg/L) indicating low affinity of the organism to degrade *m*-cresol, and a low Ki (substrate inhibition constant) value (4.42 hr^{-1}) indicating less resistance of the organism towards substrate inhibition. Yan *et al.* (2010) also showed that mutated *C. tropicalis* degrades *m*-cresol more rapidly than the wild type strain. The mutated strain has a higher μ_{max} (maximum specific growth rate) value than wild type. Their study also showed that when *m*-cresol was fed to the culture in presence of phenol, then inhibition imposed by *m*-cresol was stronger than that of phenol. Saravaran *et al.* (2008) investigated phenol and *m*-cresol degradation in bi-substrate mode by an indigenous mixed microbial culture, predominantly *Pseudomonas* sp. This culture could degrade up to 600 mg/L of each substrate. The kinetics and interaction studies among the substrates had shown that *m*-cresol was inhibitory to the culture to some extent, but phenol had strong inhibitory effect on *m*-cresol degradation at lower concentration ranges and vice versa.

The present study deals with an indigenous mixed microbial culture that can degrade *m*-cresol as sole carbon and energy source, after acclimatization. The study also aims to find *m*-cresol biodegradation rates and the kinetic constants by fitting the specific growth rate data in different substrate inhibition models and finding the best-fitted model for the present data.

The present study is better than the biodegradation studies done on *m*-cresol so far (Yan *et al.* 2006; Saravaran *et al.* 2009; Yao *et al.* 2011). Since specific single bacteria are seldom available in nature and also difficult to maintain in the field, it is urged that biodegradation study of phenolic compounds should be carried out in presence of mixed populations of bacteria. Yan *et al.* (2006) studied the biodegradation of *m*-cresol for a lower concentration range (0–320 mg/L) than the present study (50–700 mg/L). Although the cultures selected by Saravaran *et al.* (2009) and Yao *et al.* (2011) could biodegrade up to 900 and 1,200 mg/L of *m*-cresol, respectively, the Ks value obtained by both were higher than that obtained in the present study. As a low Ks value indicates high affinity of organism towards substrate, it can be said that the mixed culture used in the present study is potentially very useful for *m*-cresol biodegradation.

The substrate inhibition models chosen along with their mathematical forms are described below. The earliest model on microbial growth kinetics, the Monod model (1949), relates growth rate of microorganisms to the concentration of a single growth-controlling substrate represented by the following equation:

$$\mu = \frac{\mu_{max} S}{Ks + S} \tag{1}$$

where μ is specific growth rate of mixed microbial culture (hr^{-1}) $= (1/X)(dX/dT)$, S is limiting substrate concentration (mg/L), μ_{max} is maximum specific growth rate of the culture (hr^{-1}) and Ks is the half saturation constant (mg/L). Different working groups (Kumar *et al.* 2005; Nuhoglu & Yalcin 2005) have proposed several mathematical models to express culture growth and substrate utilization. Microbial growth can be modelled by the simple Monod equation (Kovar & Egli 1998). However, this equation becomes unsatisfactory for growth in the presence of some inhibitory substances. In such situations, Haldane models are normally used to represent the growth in both lower and higher concentration of inhibitory substance. The Haldane or Andrews model (1968) has the form (Wang & Loh 1999):

$$\mu = \frac{\mu_{max} S}{Ks + S + (S^2/Ki)} \tag{2}$$

where Ki is the substrate inhibition constant (mg/L). Due to its significance it was widely adopted by most researchers.

Aiba *et al.* (1968) proposed a model to express microbial growth rate as given by:

$$\mu = \frac{\mu_{max} S \exp(-S/Ki)}{Ks + S} \tag{3}$$

Yano & Koga (1969) proposed a model based on a theoretical study on the dynamic behaviour of single vessel continuous fermentation, subject to growth inhibition at high concentration of rate-limiting substrate, e.g. acetic acid fermentation from ethanol, gluconic acid fermentation from glucose. The model form is given as:

$$\mu = \frac{\mu_{\max}}{\mathrm{Ks} + S + (S^2/\mathrm{K}_1) + (S^3/\mathrm{K}_2{}^2)} \tag{4}$$

where K_1, K_2 are positive constants. Similarly, Edward (1970) proposed a kinetic model (Equation (5)), which was a modified form of the Haldane model. But he found that his model did not perform better than the Haldane model:

$$\mu = \mu_{\max} \frac{S(1 + (S/\mathrm{K}))}{S + \mathrm{Ks} + (S^2/\mathrm{Ksi})} \tag{5}$$

where Ksi is the substrate inhibition constant (mg/L) and K is the constant. The Teissier model to predict substrate inhibition at higher substrate concentration (Edward 1970) is given as:

$$\mu = \mu_{\max}[\exp(-S/\mathrm{Ki}) - \exp(-S/\mathrm{Ks})] \tag{6}$$

The model proposed by Luong (1987), as represented in Equation (7), appeared to be useful for representing the kinetics of substrate inhibition. Although the proposed model is of the generalized Monod type, it accounts for substrate stimulation at both low and high concentrations. The model has the capability to predict the values of S_m, the maximum substrate concentration, above which the growth is completely inhibited:

$$\mu = \frac{\mu_{\max} S}{S + \mathrm{Ks}} \left[1 - \frac{S}{S_m}\right]^n \tag{7}$$

Han & Levenspiel (1988) proposed a model (Equation (8)) to express substrate degradation rate. This model involves a delay function, which has an exponential form and incorporates the critical product or substrate concentration corresponding to the inflection point on the growth curve:

$$q = \frac{q_{\max} S[1 - (S/S_m)]^n}{S + \mathrm{Ks} - [1 - (S/S_m)]^m} \tag{8}$$

where q is the specific substrate degradation rate (h^{-1}), q_{max} the maximum specific substrate degradation rate (h^{-1}), S_m the critical inhibitor concentration (mg/L) above which the reactions stops, and m and n are the empirical constants.

MATERIALS AND METHODS

Microorganisms and culture acclimatization conditions

The mixed microbial sludge was collected from a coke oven effluent treatment plant in Durgapur, West Bengal, India. The existing effluent treatment plant is operated on the principle of a suspended growth biological reactor facilitated with an extended aeration system. Effluents from coke ovens contain high to moderate concentrations of different phenolic compounds including *m*-cresol. So, the collected sludge was assumed to contain some organisms that could degrade phenolic compounds and thus chosen for the present study. The indigenous mixed microbial culture was first acclimatized to *m*-cresol, so that, microbes could produce *m*-cresol degrading enzymes in laboratory conditions. For acclimatization of sludge with *m*-cresol, first the culture was grown at very low concentration of *m*-cresol (5 mg/L) in a 250 mL conical flask containing 100 mL of mineral salt (MS) medium with 100 mg/L of glucose and 100 mg/L of beef extract, under continuous stirring (110 rpm). The composition (mg/L) of MS medium is: $(NH_4)_2SO_4$ 230, $CaCl_2$ 7.5, $FeCl_3$ 1.0, $MnSO_4.H_2O$ 100, $MgSO_4.7H_2O$ 100, K_2HPO_4 500, KH_2PO_4 250 (pH 7.0 ± 0.2). Then glucose and beef extract concentration were gradually decreased by 20 mg/L in every batch and supplemented by increased concentration of *m*-cresol. Batch process was used for sludge acclimatization. After 3 months of acclimatization, the sludge was changed to MS medium with *m*-cresol as sole carbon and energy source up to a concentration of 700 mg/L.

Analytical procedure

m-cresol concentration was analytically estimated by high performance liquid chromatography (HPLC) (Shimadzu) equipped with an ultraviolet-visible (UV-VIS) detector and C18 column. The mobile phase used was an acetonitrile and water mixture (60:40). The flow rate of the eluent was

set to 1 mL/min and the detection wavelength was 275 nm. The biomass growth in the sample was monitored by measuring its absorbance at 600 nm wavelength using a UV-VIS spectrophotometer (Shimadzu). Then biomass concentration was calculated from a standard graph plotted as dry cell mass of microbial culture vs. optical density measured at 600 nm (Saravaran *et al.* 2008). All the chemicals and other reagents were purchased from Merck®, India.

Batch biodegradation study

m-cresol biodegradation experiments using the mixed microbial culture were carried out in a 3 L capacity bioreactor with air supplied by a mini air compressor for necessary aeration. Compressed air was fed to the reactor through an air filter at 2.5 L/min. In every batch the total volume of synthetic wastewater was 1 L. Experiments were performed in a batch reactor containing MS medium with *m*-cresol as sole carbon and energy source. An inoculum of 60 mL was added to the bioreactor for each set of experiments by direct transfer, under aseptic conditions, of freshly *m*-cresol-acclimatized culture to MS medium with *m*-cresol at different concentrations. Initial *m*-cresol concentration in the MS medium for the biodegradation kinetics study was: 50, 100, 200, 300, 400, 500, 600 and 700 mg/L. Samples were withdrawn at predetermined time intervals, after which, the biomass concentration and the residual *m*-cresol concentrations were analysed. For each initial concentration of *m*-cresol, experiments were carried out in triplicate under identical conditions and the average values are reported. All the experiments were done until the concentration of residual *m*-cresol in the reactor was found to reach equilibrium concentration at the specific time. For each reaction batch, specific growth rates of the culture have been calculated and fitted in several substrate inhibition models, as described in the Introduction.

RESULTS AND DISCUSSION

Effect of initial *m*-cresol concentration on its own biodegradation

Figure 1 shows the time course profile of *m*-cresol biodegradation by the mixed culture. It was seen that the mixed culture could degrade up to 700 mg/L *m*-cresol completely in almost 73 hr. The time taken by the mixed culture to degrade *m*-cresol depended on its initial concentration (Figure 1). It was found that the biodegradation rate increased with the increase in *m*-cresol concentration up to 500 mg/L, but then started decreasing. A maximum rate ($dS/d\theta$) of 10 mg/(L.hr) was obtained at initial *m*-cresol concentration of 500 mg/L. At concentration of 600 mg/L, the degradation rate was 9.677 mg/(L.hr). The rate was less than 9.677 mg/(L.hr) for 50 and 700 mg/L.

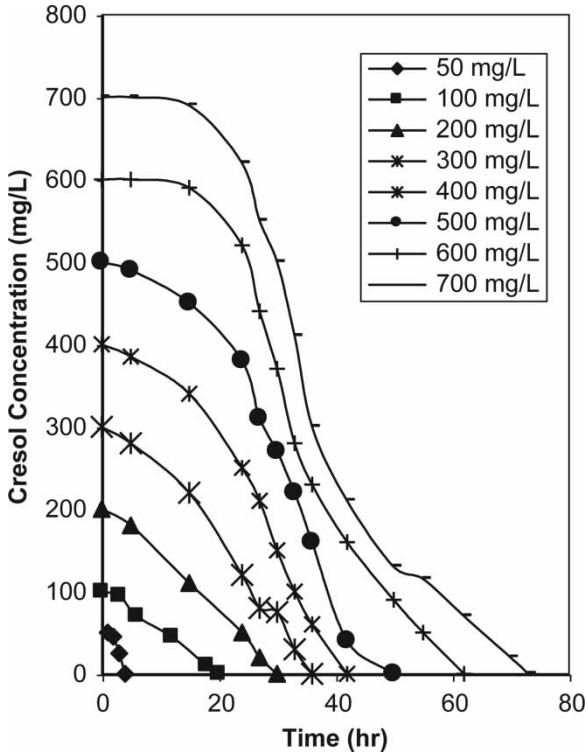

Figure 1 | *m*-cresol biodegradation profile with time.

Effect of *m*-cresol concentration on the growth of the culture

Higher *m*-cresol concentrations had an inhibitory effect on microbial growth. The growth profile of the culture at different initial *m*-cresol concentrations is shown in Figure 2. It was observed that *m*-cresol concentration below 200 mg/L showed almost no inhibitory effect, as the lag phase of growth was very short. For concentrations higher than 200 mg/L, the lag phase was longer. This resulted in a longer degradation time for higher initial concentration (Figure 1). At initial concentrations

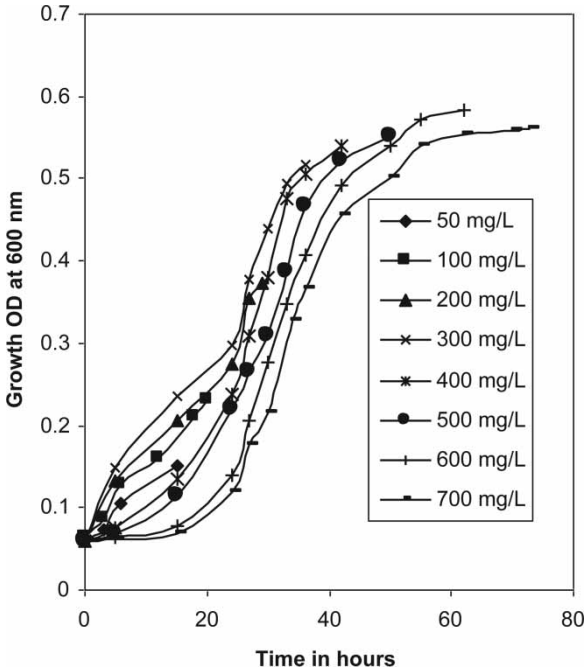

Figure 2 | Microbial growth profile with time in presence of different initial *m*-cresol concentration.

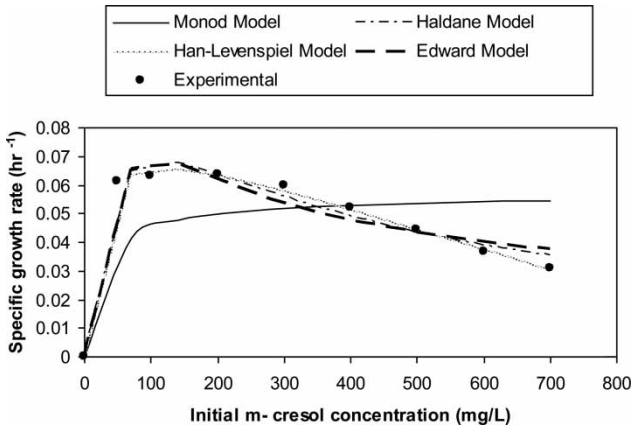

Figure 3 | Comparison of experimental and Monod, Haldane, Han-Levenspiel and Edward model predicted specific growth rates of the culture.

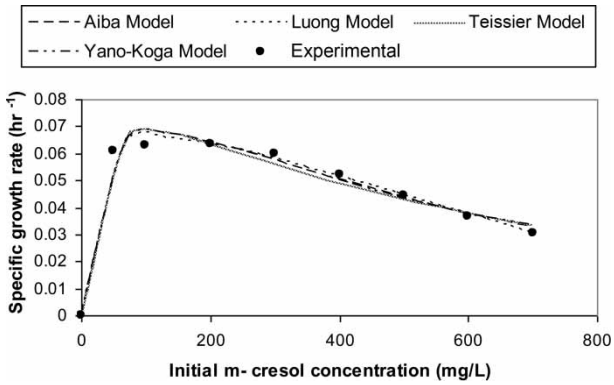

Figure 4 | Comparison of experimental and Luong, Aiba, Teissier and Yano-Koga model predicted specific growth rates of the culture.

above 200 mg/L, there was distinct substrate inhibition. Yan *et al.* (2010) showed substrate inhibition in mutated *C. tropicalis* culture with initial *m*-cresol concentration above 50 mg/L. Wang *et al.* (2009) also reported substrate inhibition above 50 mg/L of *m*-cresol as seen by the decrease in the specific growth rate of *Candida albicans* PDY-07 under anaerobic conditions. It can be concluded that the mixed microbial culture used in the present study had greater resistance to substrate inhibition by *m*-cresol compared to results reported in the literature for *m*-cresol biodegradation by pure culture. The mixed culture had greater potential to degrade *m*-cresol of higher concentration more efficiently than the pure cultures. In the present study, the specific growth rate of the culture increased (highest $\mu = 0.06345\ \mathrm{hr}^{-1}$) up to initial *m*-cresol concentration of 200 mg/L (Figures 3 and 4). For initial concentration higher than 200 mg/L, specific growth rate decreases and became lowest at 700 mg/L ($\mu = 0.0306\ \mathrm{hr}^{-1}$) of *m*-cresol.

Variation of yield coefficient with initial *m*-cresol concentration

The batch experiment data for biomass growth and *m*-cresol degradation at different initial *m*-cresol concentrations were used to calculate the yield coefficient Yx/s. At one value of initial *m*-cresol concentration (X – Xo) divided by (So – S) for the entire batch period gives the value of yield coefficient. Figure 5 shows the variation of yield coefficient with initial *m*-cresol concentration. The yield coefficient decreased with the increase in initial *m*-cresol concentration. The yield coefficient varied marginally when initial *m*-cresol concentration was below 300 mg/L, but decreased drastically above this concentration. This occurred because there was no or very little inhibition by substrate on the growth of the culture below 300 mg/L of initial *m*-cresol. But as the initial substrate concentration increased, the substrate inhibition played a major resistive role on the growth of the culture and thus sharply decreased the value of the yield coefficient. Similar results of decreasing Yx/s with

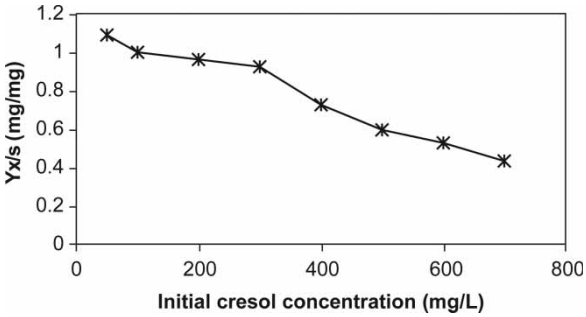

Figure 5 | Variation of yield coefficient with initial *m*-cresol concentration.

increase in substrate concentration in the inhibitory region have been reported previously (Singh *et al.* 2008).

Exploration of best-fit kinetic model for *m*-cresol biodegradation

Figure 1 shows that the pattern of *m*-cresol removal throughout the respective biodegradation period is similar for each initial *m*-cresol concentration. Figures 3 and 4 show the comparative plots of experimental specific growth rates ($\mu = (1/X)(dX/dT)$) and the rates predicted by the models as given by Equations (1)–(8) and solved by MATLAB© 7.1. The trend of experimental specific growth rates with initial *m*-cresol concentration shows that μ increases as the *m*-cresol concentration increases, rises to a peak value and finally decreases. The value of maximum specific growth rate was found to be equal to 0.06345 hr^{-1} and it is achieved at initial *m*-cresol concentration of 200 mg/L (Figures 3 and 4). In the present study, the growth kinetic (substrate inhibition) models (except Monod) were fitted well to the experimental data. The Han-Levenspiel and Luong models fitted reasonably well as determined by the root mean square error (RMSE = 0.001349) calculated between experimental and the model predicted specific growth rate values. The biokinetic constants of growth of the culture obtained from these models along with RMSE between experimental and predicted rate values are shown in Table 1. The Han-Levenspiel and Luong models predicts marginal differences in both Ks and μ_{max} values, but do not differ in RMSE calculated between experimental and model predicted specific growth rates. These models also predicts the critical substrate concentration (S_m) value, at which specific growth rate fall to zero (1,111 and 1,109 mg/L, respectively). Here the S_m value from both models agreed well with the experimental results. The Ks value predicted by the Han-Levenspiel (18.13 mg/L) and Luong models (16.97 mg/L) are very low. A low value of Ks indicates high affinity of the culture towards the substrate indicating possible high rate of *m*-cresol degradation by this culture. Another study by Saravaran *et al.* (2009) on biodegradation of *m*-cresol as sole carbon source showed that mixed microbial culture could biodegrade up to 900 mg/L. The Luong and Han-Levenspiel models were fitted well for their study. But compared to the present study, the Ks value found by them for the Luong (94.50 mg/L) and Han-Levenspiel (77.75 mg/L) models were higher. From the Ks point of view, the culture used in the present study is a potential mixed culture to degrade *m*-cresol at its high concentration. The difference in the models predicted kinetic constant values for the present experiment is, perhaps, due to the fact that the two models were originally developed for systems containing a different microorganism and substrate. Table 2 shows the comparison of biokinetic

Table 1 | List of kinetic coefficients predicted by different substrate inhibition models

Model	μ_{max} (hr^{-1})	Ks (mg/L)	Ki	Ksi	K	S_m	*n*	*m*	RMSE
Haldane	0.1204	44.07	296.8	–	–	–	–	–	0.003659
Han-Levenspiel	0.0843	18.13	–	–	–	1,109	1	1	0.001349
Luong	0.08411	16.97	–	–	–	1,111	1	–	0.001349
Edward	0.197	98.23		80.56	800	–	–	–	0.005204
Aiba	0.1001	28.49	644.8	–	–	–	–	–	0.002332
Teissier	0.08282	34.03	774.3	–	–	–	–	–	0.003337
Yano and Koga	0.09394	26.09	–	–	K1 = 784.8 K2 = 727.5	–	–	–	0.002694

Table 2 | Summary of growth kinetics parameter for biodegradation of cresols obtained in different studies

						Haldane model		
Sl No	Authors	Microbial strains	Type of cresol	System	Concentration range (mg/L)	μ_{max} (hr^{-1})	Ks (mg/L)	Ki (mg/L)
1	Maeda *et al.* (2005)	Mixed culture	*o*-Cresol	Slurry bioreactor	30–600	0.368	92.4	125.2
2	Acuna-Arguelles *et al.* (2003)	Mixed culture	*p*-Cresol	Series batch system	Multisubstrate each 100	1.0044	75.6	680
3	Yan *et al.* (2006)	*Candida tropicalis*	*m*-Cresol	Batch system	0–320	2.78	866	4.42
4	Huchinson & Robinson (1988)	*Pseudomonas putida*	*p*-Cresol	Bubble column ferementor	0–200	0.304	–	–
5	R. Singh *et al.* (2008)	*Gliomastix indicus* MTCC 3869	*p*-Cresol	Batch reactor	10–700	0.8009	42.37	43.28
6	Yao *et al.* (2011)	*Lysinibacillus cresolivoans*	*m*-Cresol	Batch system	0–1,200	0.89	426.25	51.26
7	Saravanan *et al.* (2009)	Mixed culture	*m*-Cresol	Batch system	0–900	0.6819	79.14	204.42
8	Present study	Mixed culture	*m*-Cresol	Batch reactor	50–700	0.1204	44.07	296.8

constants as obtained by different investigators as well as in the present study.

CONCLUSIONS

The kinetics of *m*-cresol degradation were studied under aerobic conditions in a batch reactor using an indigenous mixed microbial culture, isolated from an effluent treatment section of a coke oven plant. The culture could grow and biodegrade *m*-cresol up to 700 mg/L. However, *m*-cresol exhibited inhibition to growth rate above 200 mg/L of its initial concentration. Specific growth rates of the culture under different initial *m*-cresol concentration from 50 to 700 mg/L have been calculated. By fitting specific growth rates on suitable substrate inhibition models, biokinetics constant that are necessary to understand the kinetics of biodegradation process were evaluated by MATLAB© 7.1 software. RMSEs between the experimental specific growth rates and the model predicted values have been calculated for different substrate inhibition models. It is observed that the models that best fit the present study are the Han-Levenspiel and Luong models which have the lowest RMSE value of 0.001349 and predict reasonable kinetic coefficient values. Therefore, the mixed culture used in the present work is a potential culture that can be used for *m*-cresol biodegradation under aerobic conditions in real life wastewater treatment.

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