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Batch growth kinetics of an indigenous mixed microbial culture utilizing *m*-cresol as the sole carbon source

Pichiah Saravanan[a], K. Pakshirajan[b], Prabirkumar Saha[a,*]

[a] Department of Chemical Engineering, Indian Institute of Technology Guwahati, Assam 781039, India
[b] Department of Biotechnology, Indian Institute of Technology Guwahati, Assam 781039, India

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ABSTRACT

An indigenous mixed microbial culture, isolated from a sewage treatment plant located in Guwahati was used to study biodegradation of *m*-cresol in batch shake flasks. *m*-Cresol concentration in the growth media was varied from 100 mg/L to 900 mg/L. The degradation kinetics was found to follow a three-half-order model at all initial *m*-cresol concentrations with regression values greater than 0.97. A maximum observed specific degradation rate of 0.585 h^{-1} was observed at 200 mg/L *m*-cresol concentration in the medium. In the range of *m*-cresol concentrations used in the study, specific growth rate of the culture and specific degradation rates were observed to follow substrate inhibition kinetics. These two rates were fitted to kinetic models of Edward, Haldane, Luong, Han-Levenspiel, and Yano-Koga that are used to explain substrate inhibition on growth of microbial culture. Out of these models Luong and Han-Levenspiel models fitted the experimental data best with lowest root mean square error values. Biokinetic constants estimated from these two models showed good potential of the indigenous mixed culture in degrading *m*-cresol in wastewaters.

1. Introduction

m-Cresol is a derivative of phenol and is a compound regarded as a priority pollutant by the United States Environmental Protection Agency (USEPA) [1–3]. Industries such as pulp and paper mills, textile mills, coal gasification units, herbicides and fungicides industry, etc., are mainly responsible for discharging *m*-cresol in their aqueous effluents [2,4,5]. *m*-Cresol, a methylated derivative of phenol, leads to serious environmental contamination due to its toxicity towards aquatic biota [1,2]. Mainly due to these reasons, its removal from wastewater before final discharge is required. Among the available treatment methods for degrading *m*-cresol, microbe-based processes seem more promising and economical [1–6]. Although reports on biodegradation of phenolic compounds by pure microbial cultures are known, biodegradation of *m*-cresol using indigenous mixed microbial community is relatively less reported. Aerobic degradation using an indigenous mixed microbial community may be advantageous in complete assimilation (CO_2 and H_2O) of *m*-cresol without producing any toxic residues in the process [2].

Gallego et al. evaluated the biodegradation of 2-chlorophenol, phenol and *m*-cresol as mixed components by using pure and mixed indigenous cultures in aerobic reactors. They observed the biodegradation of *m*-cresol with an initial concentration of 50 mg/L in 27 h by pure bacterial cultures [7]. Recently Yan et al. showed the biodegradation of *m*-cresol in batch shake flask with pure culture of *Candida tropicalis*. They observed that culture could degrade *m*-cresol with a maximum initial concentration of only 280 mg/L within 66 h. In addition, they also studied the intrinsic kinetics of cell growth and substrate degradation with *m*-cresol [1].

However, the reports on biodegradation of high concentration *m*-cresol, employing mixed microbial cultures are limited. Hence the objective of the present work was therefore to study the kinetics of growth and *m*-cresol degradation in batch shake flasks using an indigenous mixed microbial culture. Also, suitable substrate inhibition models, found in the literature were applied to the experimental data to estimate the biokinetic constants with an aim to scale-up the process.

The adopted substrate inhibition models along with their mathematical form have been described below: the earliest model on microbial growth kinetics, the Monods model (1949), relates growth rate of micro-organism to the concentration of a single growth controlling substrate represented by the following

* Corresponding author. Tel.: +91 361 2582257; fax: +91 361 2582257.
E-mail addresses: prabirkumar.saha@gmail.com, p.saha@iitg.ernet.in (P. Saha).

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equation[8]:

$$\mu = \frac{\mu_{max} S}{K_s + S} \quad (1)$$

where μ is the specific growth rate (h^{-1}), μ_{max} the maximum specific growth rate (h^{-1}), S the substrate concentration (mg/L) at time t, and K_s is the half saturation coefficient (mg/L). Haldane (1968) proposed the first and most popular model for substrate inhibition kinetics. This model form is given in the following equation [9]:

$$\mu = \frac{\mu_{max} S}{K_s + S + (S^2/k_i)} \quad (2)$$

where K_i is the substrate inhibition constant (mg/L). Due to its significance it was widely adopted by most of the researchers. Yano and Koga proposed an model (1969), based on a theoretical study on the dynamic behavior of single vessel continuous fermentation subject to the growth inhibition at high concentration of rate limiting substrate, e.g., the acetic acid fermentation from ethanol, the gluconic acid fermentation from glucose, the tannase fermentation with tannic acid as the sole carbon source, a bacterium production from pentane, etc. The model form is given in the following equation [10]:

$$\mu = \frac{\mu_{max}}{(K_s/S) + 1 + \sum_{j=1}^{n} (S/K_j)^j} \quad (3)$$

where K is the positive constant. Similarly, Edward (1970) proposed a kinetic model (Eq. (4)), which was the modified form of Haldane model. But he found that his model did not show better result as compared to Haldane model [11].

$$\mu_i = \mu_{max} \frac{S}{S + K_s + (S^2/K_{si})(1 + S/K)} \quad (4)$$

where K_{si} is the substrate inhibition constant (mg/L) and K is the constant. The model proposed by Luong (1987) as represented in Eq. (5), appeared to be useful for representing the kinetics of substrate inhibition. Though the proposed model is of generalized Monod type, but accounts for substrate stimulation at its 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 [12].

$$\mu = \frac{\mu_{max} S}{K_s + S} \left[\frac{1 - S}{S_m}\right]^n \quad (5)$$

Han and Levenspiel (1988) proposed a model (Eq. (6)) to express substrate degradation rate. This model involves a delay function, which has an exponential form and incorporate the critical product or substrate concentration corresponding to the inflection point on the growth [13,14].

$$q = \frac{q_{max} S[1 - (S/S_m)]^n}{K_s + S - [1 - (S/S_m)]^m} \quad (6)$$

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.

In addition to the substrate inhibition model, the kinetics of *m*-cresol degradation by the mixed culture, were applied to growth associated, non-growth-associated kinetic models and three-half-order model which are used to describe degradation of organics by micro-organisms [15–17]. The form of these different types of models and their validity, in relation to initial substrate concentration, S_0, and half-saturation constant, K_s, is given in the following equation:

- Non-growth associated:

$$\text{Zero order}: \quad S = S_0 - k_0 t, \quad S_0 \gg K_s \quad (7)$$

$$\text{First order}: \quad S = S_0 \exp(-k_1 t), \quad S_0 \ll K_s \quad (8)$$

$$\text{Monod with no growth}: \quad K_s \ln \frac{S}{S_0} + S - S_0 = -k_2 t, \quad k_2 = \mu_{max} X_0, \quad S_0 \sim K_s \quad (9)$$

- Growth associated:

$$\text{Logarithmic}: \quad S = S_0 + X_0[1 - \exp(\mu_{max} t)], \quad S_0 \gg K_s \quad (10)$$

$$\text{Logistic}: \quad S = \frac{S_0 + X_0}{1 + (X_0/S_0)[\exp(K(S_0 + X_0)t)]}, \quad K = \frac{\mu_{max}}{K_s}, \quad S_0 \ll K_s \quad (11)$$

$$\text{Monod with growth}: \quad K_s \ln \frac{S}{S_0} = (S_0 + X_0 + K_s) \ln \frac{X}{X_0} - (S_0 + X_0)\mu_{max} t, \quad S_0 \sim K_s \quad (12)$$

- Three-half-order kinetic model:

$$y = \frac{1}{t} \ln \frac{S_0 - (S_0 - S + k_0 t) + k_0 t}{S_0} = -k_1 - \frac{k_2 t}{2}, \quad \text{where } P = S_0 - S + K_0 t \quad (13)$$

where k_0, k_1, k_2 = zero-, first-order and second-order rate constants, S_0 is the substrate concentration (mg/L) at time $t = 0$, and X_0 is the biomass concentration (mg/L) at time $t = 0$.

2. Materials and methods

2.1. Chemicals and reagents

m-Cresol used in the study was of an analytical grade, glucose and inorganic salts used in preparing microbial growth media were of reagent grade. All the chemicals and other reagents were purchased from Merck®, India.

2.2. Micro-organism and culture conditions

An indigenous mixed microbial culture, potent to degrade phenolic compounds including *m*-cresol, was isolated and enriched from a sewage treatment plant located in Guwahati, India. The isolation procedure as reported by Nuhoglu and Yalcin was adopted in this study [18]. The culture was initially grown in 250 mL Erlenmeyer flask containing 100 mL of mineral salt medium (MSM) having the composition (mg/L): $(NH_4)_2SO_4$ 230 g/L, $CaCl_2$ 8.0 g/L, $FeCl_3$ 1.0 g/L, $MnSO_4 \cdot H_2O$ 100 g/L, $MgSO_4 \cdot 7H_2O$ 100 g/L, K_2HPO_4 500 g/L, KH_2PO_4 250 g/L and glucose 2 g/L and pH 7.0 under agitation condition (150 rpm). The culture was then acclimatized, over a period of 1 month, to grow in MSM containing *m*-cresol as the sole carbon source up to a concentration of 1000 mg/L. The detailed acclimatization phase of the culture to degrade *m*-cresol is presented in Fig. 1.

2.3. Batch biodegradation study

All biodegradation experiments using the indigenous mixed microbial culture, acclimatized to grow using *m*-cresol as the sole

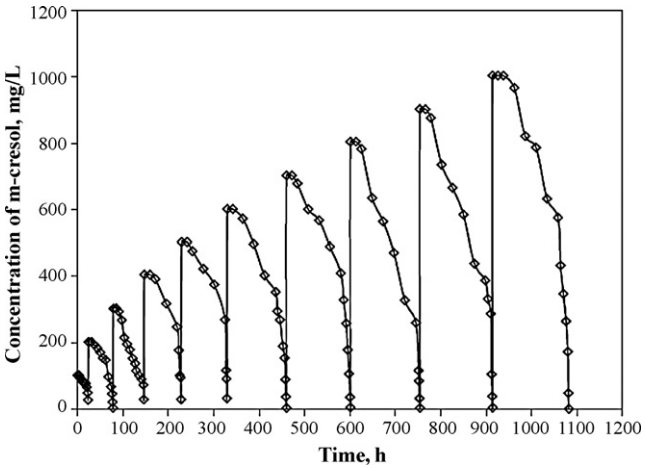

Fig. 1. *m*-Cresol degradation profile followed during the acclimatization period.

carbon source, were performed in 250 mL Erlenmeyer flask containing 100 mL of MSM containing *m*-cresol at concentration ranging from 100 mg/L to 900 mg/L. For each concentration, duplicate flasks were set up and incubated in an orbital shaker maintained at 27° C and 150 rpm. 1% of working volume (100 mL) was added as inoculum to each biodegradation flasks. This was accomplished by transferring directly (under aseptic conditions), freshly acclimatized culture in liquid medium broth to MSM containing *m*-cresol at different concentrations. Samples were withdrawn at regular time interval, analyzed for biomass concentration and subsequently centrifuged (10,000 × *g* for 3 min) and the resulting supernatant was analyzed for residual *m*-cresol concentration. The results obtained from the same set of duplicate experiments were averaged and are reported. The standard deviation of obtained experimental data was in the range of 0.3–3.5.

2.4. Software used

Regression analysis was performed with the data analysis tool pack of Microsoft Excel®. The model equations were solved using nonlinear regression method using MATLAB® 7.0.

3. Analytical methods

m-Cresol content in biomass free samples was determined quantitatively using a PerkinElmer high-performance liquid chromatograph equipped with a UV–vis detector and C18 column. The dimensions of the column used were 220 mm × 4.6 mm and the particle size of the packing was 5 μm. The eluent used was acetonitrile:water (80:20) mixture and its flow rate was kept constant at 1 mL/min. The detection wavelength was 275 nm. Biomass concentration in the samples was monitored by measuring its absorbance at 600 nm wavelength using a diode array spectrophotometer (Spekol 1200, Analytik Jena, Germany). OD_{600} of the culture was then converted to dry cell weight with the help of a calibration curve, obtained by plotting dry weight of biomass per milliliter vs. OD_{600}.

4. Results and discussion

In our previous study [19] raw water from a sewage treatment plant located in Guwahati, India, was used in isolation of an indigenous mixed microbial culture capable of degrading phenol and its derivatives. The present study aims to investigate the potential of this indigenous mixed culture in degrading *m*-cresol in shake flasks as batch reactor with a view to scale-up the process.

4.1. Effect of initial concentration on m-cresol biodegradation

Fig. 2 shows the time profile of *m*-cresol degradation by the indigenous mixed culture. It is clear from the profile that the time taken by the mixed culture to degrade *m*-cresol was dependent upon its initial concentration. For example, for degrading 100 mg/L of *m*-cresol the culture took about 14 h but for 900 mg/L it took about 136 h for complete degradation. However, a different relation between *m*-cresol degradation rate and its initial concentrations was observed (data not shown). A maximum degradation rate could be achieved at 300 mg/L of *m*-cresol; concentrations below and above 300 mg/L gave lesser degradation rates, indicating a strong influence of *m*-cresol concentration on its degradation rate [20].

4.2. Modeling the degradation kinetics of m-cresol biodegradation

The kinetics of *m*-cresol degradation by the mixed culture was applied to growth-associated, non-growth-associated models and three-half-order kinetic model. The form of these different types of models is given in Eqs. (7)–(13). Among the models tested, only non-deterministic three-half-order kinetic model could fit the data well with coefficient of determination (R^2) value greater than 0.97. With the other models, only very low coefficient of determination values (less than 0.8) were obtained revealing poorer performances of these models in comparison with the three-half-order kinetic model. More over, the three-half-order model could be considered a more refined model in comparison with others owing to the fact that it integrates both the kinetics of substrate degradation and growth of the culture [16]. The other models, however, do not consider both these aspects simultaneously and hence perform poorly. A better fitness value of this model to the experimental data also

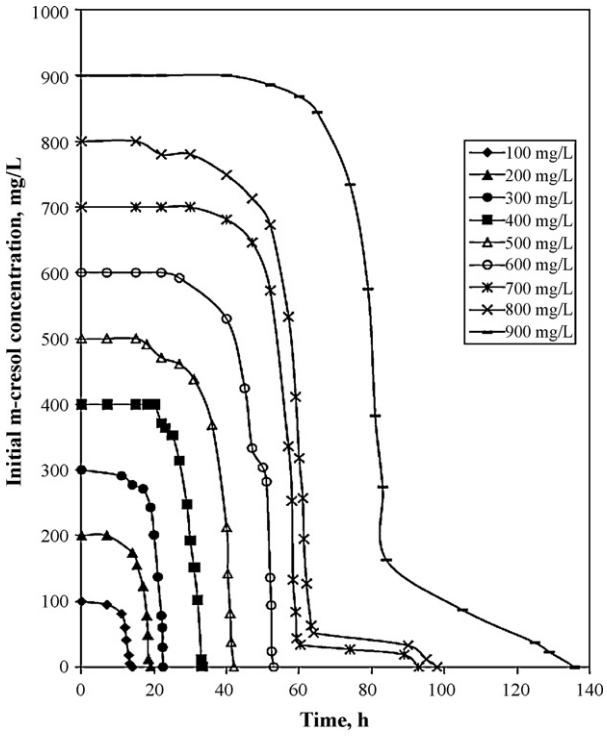

Fig. 2. Time profile of *m*-cresol degradation by the mixed culture.

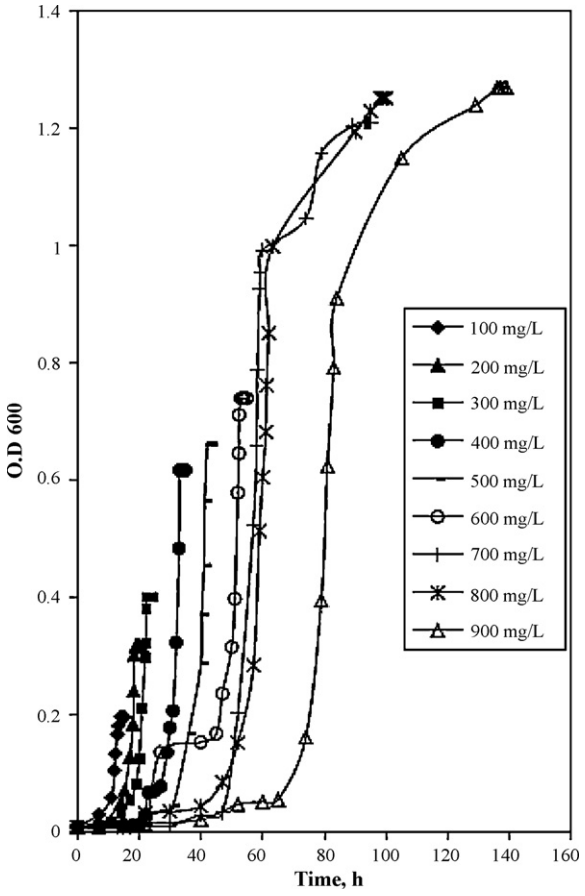

Fig. 3. Time profiles of biomass output (OD_{600}) at different initial *m*-cresol concentrations.

shows that *m*-cresol degradation in the study is growth-associated phenomenon, where the substrate is utilized for the culture growth. From the time profile of *m*-cresol degradation by the culture the whole degradation process for each concentration could be divided into two-phases: an initial lag phase and an active degradation phase (Fig. 2). Based on this observation, it is highly expective of a three-half-order model containing zero-, first- and second-order rate to fit such kinetics to a greater degree as compared to the other models [21].

4.3. Effect of initial m-cresol concentration on the growth of the culture

Compared to time taken by the culture to degrade *m*-cresol, at its various initial concentrations, the culture growth also followed a similar pattern. This is illustrated in Fig. 3 where biomass growth (OD_{600}) of the culture is plotted against time for various *m*-cresol concentrations in the media. It could be seen from Figs. 3 and 4 that *m*-cresol concentration between 100 mg/L and 300 mg/L did not show any repression on the biomass output but at its concentration greater than 300 mg/L, a lag phase in the growth was evident. The lag phase observed in its utilization (degradation) and therefore the culture growth could be easily attributed to the highly toxic nature of the compound, above certain level; in this case 300 mg/L. However, at concentrations below 300 mg/L, no such lag phase cold be observed (Figs. 2 and 3).

Moreover, as the *m*-cresol concentration was increased in the media the culture took more time to show complete utilization (14 h at 100 mg/L vs. 136 h at 900 mg/L). In order to establish the effect of *m*-cresol concentration on growth of this mixed culture, specific growth rates of the culture at different *m*-cresol concentrations were calculated as per the following relationship:

$$\mu = \frac{1}{X}\frac{dX}{dt} \tag{14}$$

where X is biomass concentration (mg/L) at time, t (h) and μ is the specific growth rate (h^{-1}) [8,22]. A relationship between the two parameters viz., μ and *m*-cresol concentration, showed that μ increased only for the first two concentrations: 100 mg/L and 200 mg/L; μ declined progressively with further increase in *m*-cresol concentrations above 200 mg/L. This type of behavior is clearly indicative of substrate inhibition on the growth of a culture that has been shown by the other authors as well [1,2].

Further, specific substrate degradation rate (μ, h^{-1}) was calculated according to the following equation that indicates specific substrate degradation rate:

$$q = -\frac{1}{X}\frac{dS}{dt} \tag{15}$$

where X and S are the biomass and *m*-cresol concentrations in mg/L at time t, in h [20]. Fig. 4 is a plot between the two rates calculated at different initial *m*-cresol concentrations in the media, which clearly indicates that both these rates corresponded with each other, implying that the culture is very well capable of utilizing *m*-cresol from the media. However, both the rates declined after an initial rise revealing substrate inhibition characteristics in the system [1,2]. In order to predict the patterns of *m*-cresol degradation and culture growth in the system, kinetics of these two phenomena were analyzed by fitting the data to substrate inhibition models.

4.4. Modeling the kinetics of the culture growth on m-cresol degradation

Since both the rate of growth (μ) and *m*-cresol degradation (q) were subjected to substrate inhibition, due to *m*-cresol, the varia-

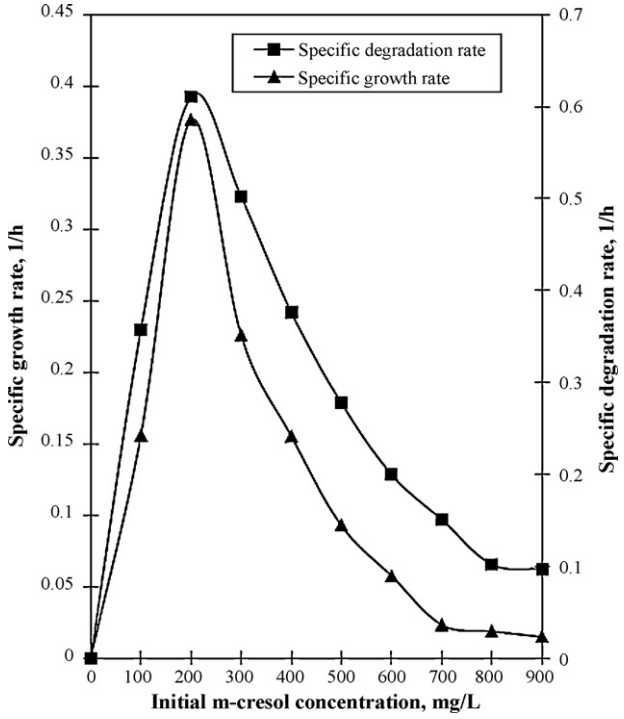

Fig. 4. Comparison of specific growth and specific degradation rates at different initial *m*-cresol concentrations.

Table 1
Specific growth rate kinetic parameters obtained for different models during biodegradation of *m*-cresol by the indigenous mixed culture in a batch shake flask

Model	μ_{max} (h^{-1})	K_s (mg/L)	K_{si}/K_i (mg/L)	S_m (mg/L)	n	m	K	Root mean square error
Monod	0.5155	117.71	–	–	–	–	–	–
Edward	0.5180	71.40	200.00	–	–	–	10.00	0.0168
Haldane	0.6819	79.14	204.42	–	–	–	–	0.0352
Luong	0.6430	94.50	–	900.00	0.85	–	–	0.0159
Han-Levenspiel	0.5561	77.75	–	900.00	1.00	1.00	–	0.0167
Yano-Koga	0.5961	63.42	–	–	1.00	–	250.00	0.0375

tion of these two rates with respect to the *m*-cresol concentrations were modeled using suitable deterministic models, reported in the literature [8–13].

The model equations were solved using nonlinear regression method using MATLAB® 7.0 and were first applied directly on the experimental data on specific growth rate of the culture at different *m*-cresol concentrations. All the practical constraints were considered while solving these equations. The variation of specific substrate degradation rate at all concentrations of *m*-cresol was also modeled using the same set of equations. However, for applying the models to the data on specific degradation rate the terms, μ and μ_{max}, in the original equations were replaced with q and q_{max} representing specific substrate degradation and maximum specific substrate degradation rates, respectively.

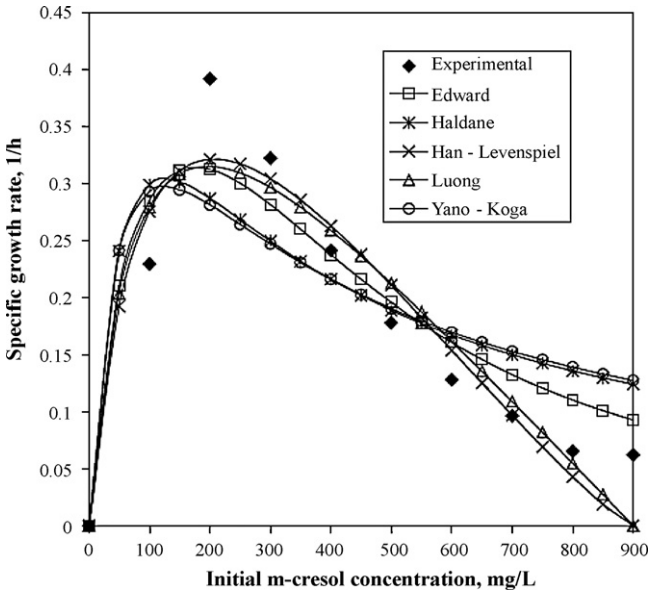

Fig. 5. Experimental and predicted specific growth rate of the culture at different *m*-cresol concentrations.

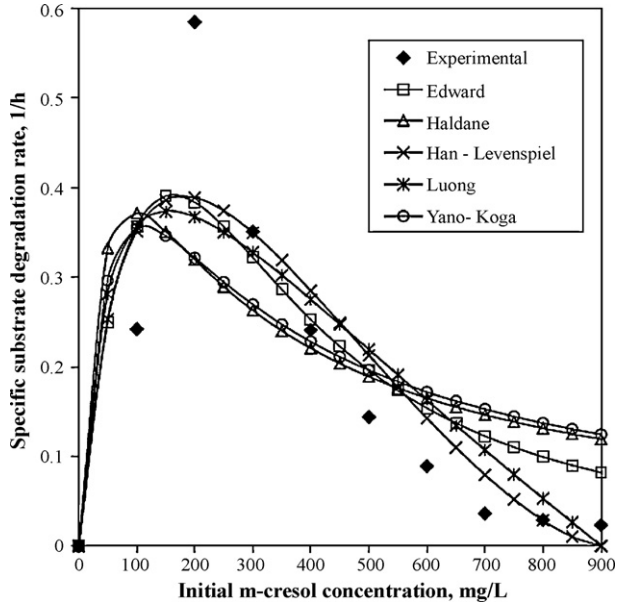

Fig. 6. Experimental and predicted specific degradation rate of the culture at different *m*-cresol concentrations.

Among the several models fitted to explain the experimental data on specific growth and degradation rates in the system, not only Luong model, but also Han-Levenspiel model proved to be better fits as determined by their root mean square (RMS) errors between the experimental and model predicted values of specific growth and degradation rates. This could be attributed based on the models themselves, which are considered more refined from the standpoint of development of these models. The fitness of these models in predicting the two rates is depicted in Figs. 5 and 6, respectively. The biokinetic constants of growth of the culture obtained from these models along with root mean square error between experimental and predicted rate values are shown in Table 1. The table also reports the parameters, K_s and μ_{max}, estimated using the linearised form of Monods model. Edward, Haldane and Yano-Koga models predicted the substrate inhibition constant (K_i) value, above which the specific growth and substrate degradation rates decline, more accurately and corresponded with the experimentally obtained value of 200 mg/L. Luong and Han-Levenspiel models also predicted critical substrate concentration (S_m) value, at which critical growth rate fall to zero, at ~900 mg/L, but this value was observed to be different from that obtained in the experiments. With respect to substrate degradation rates shown by the culture similar values were obtained (data not shown) which again confirmed substrate inhibition behavior in the system and that the *m*-cresol degradation was efficient in relation with the growth of the culture [1,2]. It is to be mentioned here that while attempting to compare the model parameter values obtained in the study, no other relevant studies, with only *m*-cresol and mixed culture carried out by other authors were found in literature [1,2].

To facilitate *m*-cresol biodegradation process by the indigenous mixed culture in real wastewater treatment system, which may contain many other contaminants, investigations on multisubstrate degradation by the culture in semicontinuous and continuous process reactors have been recently initiated by the authors.

5. Conclusions

Kinetics of *m*-cresol degradation was studied using an indigenous mixed microbial culture, isolated from a sewage treatment plant, in batch shake flasks. A non-deterministic and three-half-order kinetic model was found to describe the entire degradation profile, at all different *m*-cresol concentrations, quite accurately. The calculated specific growth and specific degradation rates corresponded well with each other through-out the studied concentration range indicating that the culture could grow and degrade *m*-cresol very efficiently. However, *m*-cresol exhibited inhibition

on the two rates above 200 mg/L concentration in the media. By fitting the experimentally obtained values of these two rates, at different concentrations, to suitable substrate inhibition models found in the literature, biokinetic constants that are necessary to evaluate a degradation process were obtained. This study revealed the potential of an indigenous mixed microbial culture in treating wastewaters containing highly recalcitrant compound such as *m*-cresol.

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