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Kinetics of phenol degradation and growth of predominant *Pseudomonas* species in a simple batch stirred tank reactor

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A culture predominantly consisting of the *Pseudomonas* species was isolated from a sewage treatment plant and was utilized to study phenol degradation and culture growth kinetics. The culture was capable of completely degrading phenol at a maximum initial concentration up to 400 mg/l in a simple batch stirred tank reactor. The maximum time taken to degrade phenol at this concentration level was 194 h. Kinetics of phenol degradation at various initial concentrations in the media largely exhibited zero order rate with a maximum value of the coefficient of determination (R^2) = 0.99 at 400 mg/l of phenol. Deterministic models such as those of Haldane, Han-Levenspiel, Luong, Edward, Yano and Koga, Tseng and Wayman were employed to fit the data of substrate inhibition on the kinetics of culture growth and phenol degradation. All models tested, except the Tseng and Wayman model fitted well the experimental data.

Keywords: Batch stirred tank reactor; biodegradation; phenol; substrate inhibition models; *Pseudomonas* sp.

1. INTRODUCTION

Synthetic organic chemicals like phenols and its derivatives lead to severe environmental contamination due to their toxicity towards aquatic biota. In particular, various types of industries such as pulp and paper mills, herbicides and fungicides production, etc., discharge phenols in their aqueous effluents [1,2]. The growing need of controlling the discharge of wastewater containing phenols and other organics into the environment has led to the search for better wastewater treatment methods.

Among the available methods for treating phenols in wastewater before its discharge into the environment, microbe based degradation of phenols seems more promising. Aerobic degradation of phenol using pure microbial cultures has been studied extensively; for example, *Pseudomonas putida* has been widely studied for its phenol biodegradation potential [3-5]. Aerobic degradation with a mixed culture may be advantageous in complete degradation of phenols without leaving any hazardous residues in the processed medium. Moreover, investigating the degradation kinetics of phenol in a suitable reactor system using mixed microbial culture further leads to its better applicability in a given wastewater treatment facility.

In order to evaluate the potential of a mixed microbial culture, isolated from a sewage treatment plant, in phenol degradation, batch experiments were performed in a simple stirred tank reactor with an objective to investigate the culture growth and degradation kinetics in the system and its modeling.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Phenol was of analytical grade and was purchased from Merck®, India. Glucose and inorganic salts used in preparing microbial growth media were of reagent grade, obtained from Sisco Research Laboratories, India.

2.2 Microorganism and culture conditions

A mixed microbial culture, capable of phenol degradation, was isolated and enriched from a sewage treatment plant located in Guwahati, India. The isolation procedure, as reported by Nuhoglu and Yalcin [6], was adopted in this study. The

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culture was identified as a mixture of *Pseudomonas* species according to the biochemical tests; scanning electron microscopy also confirmed the results. It was initially grown in a 250 ml Erlenmeyer flask containing 100 ml of mineral salt medium (MSM) of the following composition: $(NH_4)_2SO_4$ 230 mg/l, $CaCl_2$ 7.5 mg/l, $FeCl_3$ 1.0 mg/l, $MnSO_4 \cdot H_2O$ 100 mg/l, $MgSO_4 \cdot 7H_2O$ 100 mg/l, K_2HPO_4 500 mg/l, KH_2PO_4 250 mg/l and glucose 2 g/L at pH 7.0 under agitation (150 rpm). The culture was then acclimatized for a period of one month to grow in MSM containing phenol as the sole carbon source up to a concentration of 800 mg/l.

2.3 Experimental setup

The experimental setup used in this phenol biodegradation study consisted of a 5-liter glass vessel fitted with an impeller driven by a DC motor. Ports for sampling, liquid addition and withdrawal (phenol in MSM) through peristaltic pump, and thermometer for monitoring the temperature inside the vessel were provided in the setup. A schematic of the setup is illustrated in Fig. 1.

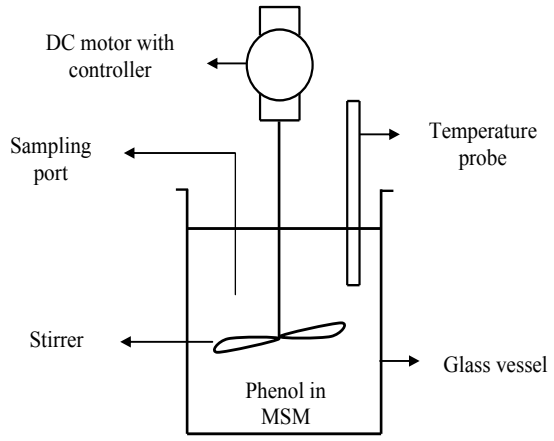

Fig. 1. Schematic of the simple batch stirred tank reactor.

2.4 Batch biodegradation study

Four batches of experiments were conducted with initial phenol concentration varying from 100 mg/l to 400 mg/l and a working volume of 4 liters of MSM using Milli-Q Elix® water. These concentration levels were chosen based on the total time taken by the culture for complete removal/degradation of phenol in the media. The experiments were carried out under batch mode at a constant temperature of 29º C ± 2º C with continuous stirring at 150 rpm. Samples were withdrawn at regular time intervals (approximately 6 hours). Samples were thereby centrifuged (10 000×g for 3 min) and analyzed for residual phenol concentration (Biofuge Pico, Rota No.3328, Heraeus). Each experiment was repeated until the residual phenol concentration in the media was reduced to nearly 0 mg/l. For each concentration duplicate experiments were performed under the same conditions and average values of each experiment were reported.

2.5 Analytical methods

Samples were centrifuged at 10 000×g for 3 min to separate the biomass. The phenol content in the biomass samples was determined quantitatively on a Perkin Elmer High Performance Liquid Chromatograph (HPLC) with a UV-Visible detector and C18 column. The dimensions of the column used were 250×4.6 mm and the particle size of the packing was 5 μm. The eluent used was a mixture of acetonitrile:water (80:20). The flow rate of the eluent was kept at 1 ml/min and the detection wavelength was 275 nm. The retention time for phenol was 3.05 min. Cell concentrations in the samples were measured as optical density at 600 nm (OD_{600}) wavelength using a Diode Array Spectrophotometer (Spekol 1200, Analytik Jena, Germany) and correlated to biomass concentration.

3. RESULTS AND DISCUSSION

In order to establish the potential of the culture in higher-scale phenol degradation, the kinetics of phenol degradation and growth of the culture in a simple batch stirred tank reactor of higher volume were investigated.

3.1 Biomass growth and phenol degradation at different initial phenol concentrations

Fig. 2(a-d) shows the time profile of biomass growth (OD_{600}) and phenol degradation by the culture. It is seen that the time taken by the culture to degrade phenol was dependent on the initial phenol concentration in the media. The culture could, however, degrade well up to a maximum concentration of 400 mg/l phenol in the media, which took a maximum time of about 194 hours. Comparing this result with our earlier batch degradation study [7] conducted in small-volume shake flasks (100 ml MSM), the time taken by the culture for complete degradation in batch stirred tank reactor was found to be much higher. The quantity of biomass (OD_{600}) produced was also less compared to the previous study. It should be mentioned here that in order to do a fair comparison

between the two modes of study (shake flasks and batch reactor), no attempt was made to control the main environmental parameter, the pH of the media, though it was continuously monitored. Although the same amount of inoculum (in %) and temperature were

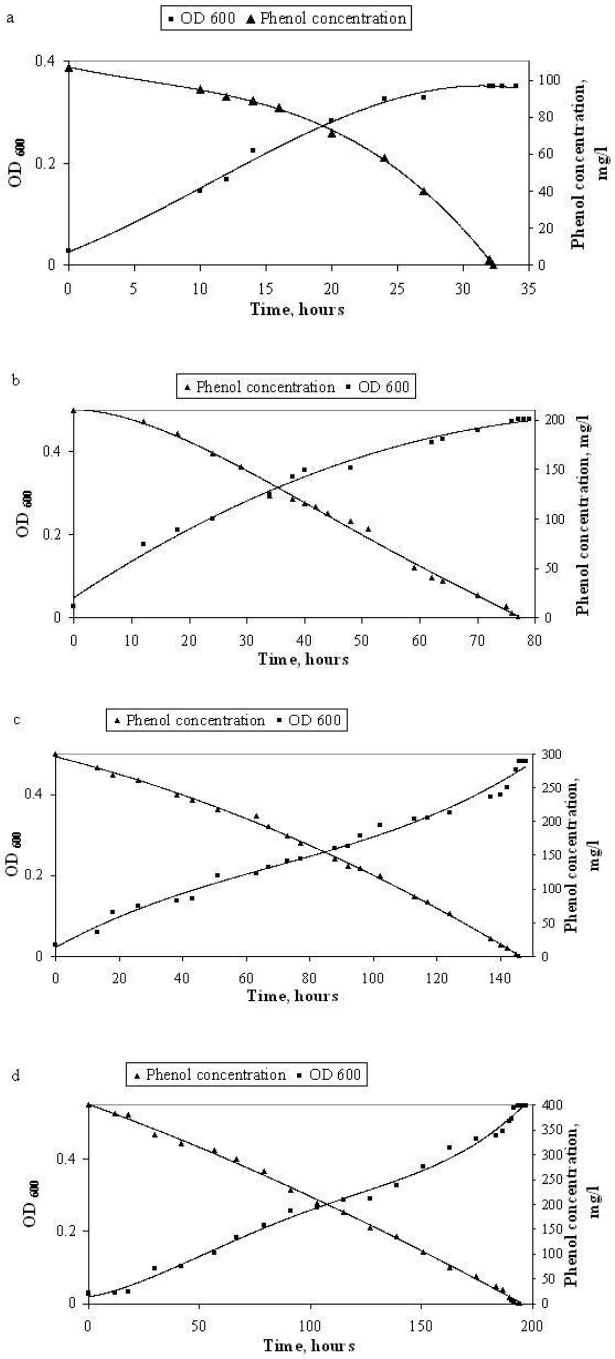

Fig. 2. Time profiles of biomass output (OD_{600}) and phenol degradation at different initial phenol concentrations: (a) 100 mg/l, (b) 200 mg/l, (c) 300 mg/l and (d) 400 mg/l.

maintained in the two studies, the time taken in this scaled up study was considerably higher than in the previous shake flasks study. This large difference in time could have been probably avoided by improving contact between the substrate and the microbes and/or by proper aeration of the system [5]. But this aspect needs further investigations to confirm.

Phenol has a significant inhibitory effect on the growth of microorganisms at its higher initial concentration [4, 5]. Therefore, acclimatization of the mixed culture, isolated from the sewage treatment plant, was carried out by allowing it to grow in presence of phenol as the sole carbon source. Maximum concentration of phenol, used for the above purpose, was 800 mg/l. Initially glucose was fed during acclimatization stage to boost up the initial growth of biomass; however the culture was grown only in presence of phenol in the later stage. From Fig. 2 (a-d) showing biomass concentration (OD_{600}) of the mixed culture at different initial concentrations of phenol, it was observed that phenol concentrations between 100 and 300 mg/l have no inhibitory effect on the microorganisms, as indicated by the nearly lacking lag phase. However, the maximum absorbance of the culture obtained at these phenol concentrations was found to be lower, as compared to that grown at a phenol concentration of 400 mg/l. Moreover, within this concentration range (100 - 300 mg/l), a stationary phase was reached and the time taken for this occurrence depended upon the initial concentration of phenol in the media. For example, at an initial phenol concentration of 300 mg/l the time taken by the culture to reach stationary growth phase was higher than that at lower initial concentrations of phenol. As regards the culture growth at 400 mg/l of phenol, the inhibition was found to be distinct and the culture took much longer time for its growth (maximum 194 h). However, the amount of biomass (OD_{600}) produced at this concentration was high, indicating that the mixed culture was capable of utilizing phenol very efficiently. From the findings on the biomass (OD_{600}) output at different times for various initial phenol concentrations, it is quite reasonable to state that both phenol and biomass amounts corresponded nearly well with each other indicating that phenol was well utilized in the process [4, 5].

3.2 Kinetics of culture growth and phenol degradation

To test the kinetics of phenol degradation by the mixed culture, the time profile of degradation, at all initial concentrations of phenol, was applied to growth associated and non-growth associated kinetic models, which are used to describe degradation of organics by microorganisms [8]. The

different types of models and their validity, in relation to initial substrate concentration, S_0, and half-saturation constant, K_s, are given in Table 1. Among the six different models tested, only the non-deterministic zero-order kinetic model could fit the data well with coefficient of determination (R^2) values greater than 0.97 at/above 200 mg/l initial phenol concentrations. However, for initial phenol concentration of 100 mg/l, the zero-order model fit

Table. 1. Kinetic models tested with the data on phenol degradation

	Model	Mathematical form	Valid for
Non-growth associated	Zero order	$S = S_0 - k_0 t$	$S_0 >> K_S$
	First order	$S = S_0 \exp(-k_1 t)$	$S_0 << K_S$
	Monod with no growth	$K_s \ln\frac{S}{S_0} + S - S_0 = -k_2 t$ $(k_2 = \mu_{max} X_0)$	$S_0 \sim K_S$
Growth associated	Logarithmic	$S = S_0 + X_0\left[1 - \exp(\mu_{max} t)\right]$	$S_0 >> K_S$
	Logistic	$S = \frac{S_0 + X_0}{1 + \frac{X_0}{S_0}[\exp(K(S_0 + X_0)t)]}$	$S_0 << K_S$
	Monod with growth	$K_s \ln\frac{S}{S_0} = (S_0 + X_0 + K_s)\ln\frac{X}{X_0} - (S_0 + X_0)\mu_{max} t$	$S_0 \sim K_S$

was slightly poorer as determined by its R^2 value of 0.79, which could be explained by the fact that the zero-order kinetic model is best valid for values of $S_0 >> K_S$; where K_S in this study, as presented later, was found to be ~40 mg/l.

All the other models gave a poor fit with R^2 values less than 0.5 at all initial concentrations of phenol. Although by fitting the zero-order kinetic model the culture proved complete degradation of phenol at/above these concentrations, a relationship between the rate of culture growth and phenol degradation could not be found. Therefore, in order to relate the pattern of phenol degradation with the culture growth in the system, the kinetics of these two phenomena were analyzed. This was achieved by calculating the specific growth rate (μ, h^{-1}) and specific substrate degradation rate (q, h^{-1}) from the biomass output and phenol degradation profiles, respectively, according to the following relationships:

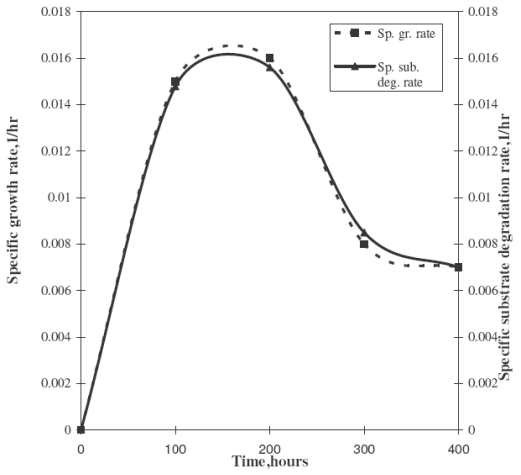

Fig. 3. Comparison of specific growth and specific substrate utilization rates at different initial phenol concentrations.

Specific growth rate:

$$\mu = \frac{1}{x}\frac{dx}{dt} \qquad (1)$$

Specific substrate degradation rate:

$$q = -\frac{1}{x}\frac{ds}{dt}, \qquad (2)$$

where x and s are the biomass and phenol concentrations in mg/l at a time, t in hours.

Table. 2. Models for substrate inhibition on growth rate of microorganisms

Model	Mathematical form	Reference
Haldane	$\mu = \frac{\mu_{\max} S}{K_s + S + \frac{S^2}{K_i}}$	Wang and Loh, [17]
Yano-Koga	$\mu = \frac{\mu_m}{(K_s / S) + 1 + \sum_{j=1}^{n} (S / K_j)^j}$	Yano and Koga [18]
Edward	$\mu_i = \mu_m \frac{S}{S + K_s + (S^2 / K_{si})(1 + S / K)}$	Mulchandani and Luong, [12]
Tseng-Wayman	$\mu = \mu_m \frac{S}{S + K_s}, S < S^*$	Tseng and Wayman [16]
Luong	$\mu = \frac{\mu_m S}{K_s + S}\left[1 - \frac{S}{S_m}\right]^n$	Luong [10]
Han-Levenspiel	$q = \frac{q_{\max} S\left[1 - \frac{S}{S_m}\right]^n}{K_s + S - \left[1 - \frac{s}{s_m}\right]^m}$	Han and Levenspiel [5]

Fig.3 illustrates a plot between the two rates calculated at different initial phenol concentrations in the media. This figure clearly indicates that both these rates correlate well with each other. However, the two rates declined after an initial rise thus revealing substrate inhibition characteristics in the system [9].

3.3 Modeling the kinetics of culture growth and phenol degradation

Because the rates of culture growth (μ) and phenol degradation (q) indicated substrate inhibition characteristics due to phenol, the variations of these two rates with respect to the phenol concentrations were modeled using suitable deterministic models, based on substrate inhibition on growth of microbial cultures, reported in the literature. These model equations are listed in Table 2 and a brief summary of each of these models is mentioned, as follows.

The earliest model on microbial growth kinetics, the Monod model, relates growth rate of microorganism to the concentration of a single growth controlling substrate as $\mu = f(s)$ via two parameters, maximum specific growth rate (μ_{max}) and half saturation constant (K_s), represented by the equation:

$$\mu = \frac{\mu_{\max} S}{K_S + S} \quad (3)$$

Since growth is a result of catabolic and anabolic enzymatic activities, these processes, i.e., substrate degradation or growth–associated product formation, can also be quantitatively described on the basis of Monod growth model [10]. But this model fails to explain substrate inhibition on either growth of microorganisms or substrate degradation. Haldane, cf. ref. [17], proposed the first and most popular model for substrate inhibition kinetics. This model was utilized by most of the researchers for growth inhibiting substrates like phenols and phenolics. Meric *et al.* [11] and Yano&Koga [12] models are based on a theoretical study on the dynamic behavior 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, tannase fermentation with tannic acid as the sole carbon source, bacterium production from pentane, etc. The model resembles the Monod kinetic model with a slight modification [12].

Edward, cf. ref. [12] proposed a kinetic model, which is a modification of the Haldane model. After extensive evaluation he found that this model didn't show better results when compared to Haldane model and moreover, the value of the parameter K was very large [13].

Tseng&Wayman [14] in their model described substrate inhibition kinetics by accounting for the fact that below a threshold substrate concentration, S*, there is no growth inhibition. However, for concentrations above S*, microbial growth decreased linearly with respect to the concentration (S - S*) [13]. However, the discontinuous nature of the model is a major drawback. Luong [15] model, which appeared to be useful for representing the kinetics of substrate inhibition, 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 [15]. Mulchandani&Luong [13] stated that description of the microbial growth by the Haldane model did not prove that a similar mechanism is operative in the microbial growth. They particularly stressed on the importance of critical and maximum substrate concentrations [13]. Han&Levenspiel [16] proposed a model 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 [16].

The substrate inhibitions model equations were solved by the nonlinear regression method using MATLAB® 7.0 and were applied directly on the experimental data on specific growth rate of the mixed culture at different phenol concentrations. For applying the models to predict the specific substrate degradation rates, the term μ was replaced with q and then solved, as before. The fitness of these models in predicting the specific growth and specific substrate degradation rates is depicted in Figs. 4 and 5 respectively. From the figures, it could be seen that except the Tseng - Wayman model, all other models fit the data very well. The biokinetic constants of growth of the culture obtained from these models along with Root Mean Square (RMS) error between experimental and

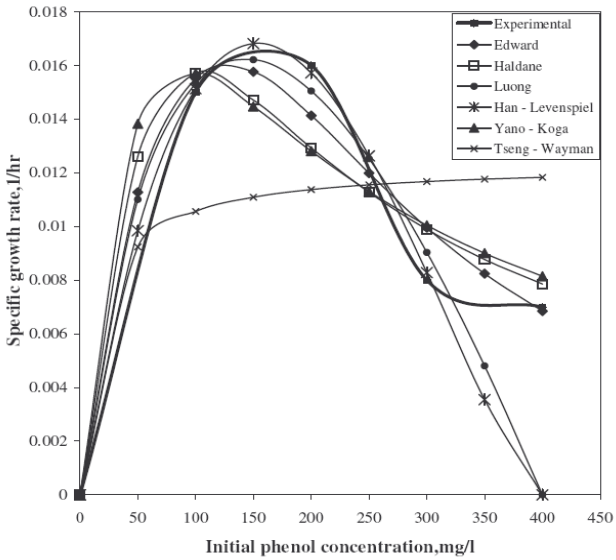

Fig. 4. Experimental and predicted specific growth rate of the culture according to the different models.

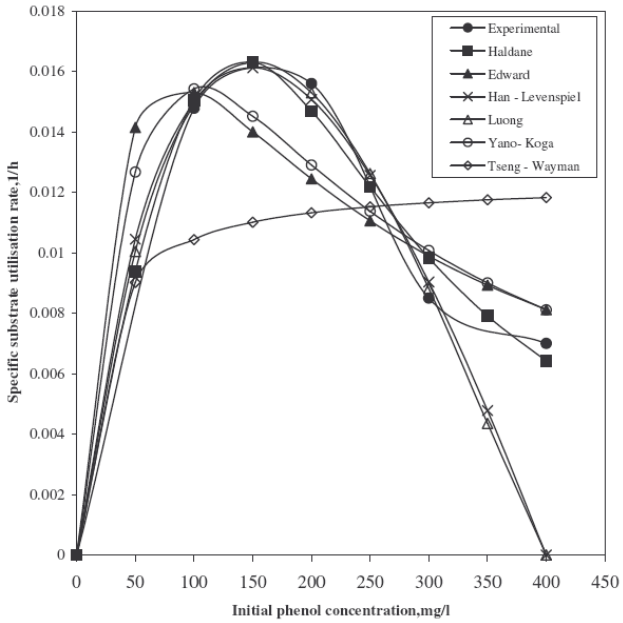

Fig. 5. Experimental and predicted specific substrate degradation rate obtained by applying different substrate inhibition models.

predicted values are shown in Table 3. These values of the constants obtained from the different models, but with the same set of experimental data, agree quite closely with each other except in case of the Tseng – Wayman model, which did not fit properly (RMS error $>70\times10^{-5}$). Edward, Haldane and Yano-Koga models predicted the substrate inhibition

Table. 3. Biokinetic constants estimated by fitting the various substrate inhibition models on the data on growth rate of the culture

Model	μ_{max} (h^{-1})	K_s(mg dm^{-1})	K_i(mg dm^{-1})	S_m(mg dm^{-1})	n	m	K	RMS error ($\times 10^{-5}$)
Edward	0.0290	63.77	100.00	-	-	-	6	1.15
Haldane	0.0324	40.57	140.65	-	-	-		1.86
Luong	0.0238	46.67	-	400	2.1	-		5.24
Han-Levenspiel	0.0257	40.55	-	400	0.6	1		5.62
Yano - Koga	0.0358	47.78	-	-	1	-	125	1.79
Tseng - Wayman	0.0123	16.74	-	-	-	-		77.70

constant (K_i) value, above which the specific growth and substrate degradation rates decline, more accurately and correlated well with the experimentally obtained value of 200 mg/l. Luong and Han - Levenspiel models also predicted the critical substrate concentration (S_m), at which critical growth rates fall to zero, to be ~ 400 mg/l, but this value was observed to be different from that obtained in the experiments. Similar observations on the specific substrate degradation rate were found to be valid for its biokinetic constants estimated from the models by applying the data on specific degradation rates, as well. Overall, the different models, despite their differences in background of origin, could well predict the behaviour of the system and the values of biokinetic constants obtained using these models revealed a fairly high potential of the microorganism in degrading phenol in a simple batch stirred tank reactor such as that used in this study.

4. CONCLUSIONS

The kinetics of phenol degradation was studied using a mixed microbial culture, isolated from a sewage treatment plant, in a simple stirred tank reactor operated under batch mode. The kinetics of phenol degradation was best explained by a non growth associated zero-order model with a maximum coefficient of determination value of >0.99 at 400 mg/l initial concentration of phenol in the media. The culture specific growth and phenol specific degradation rates correlated well with each other at all initial phenol concentrations. In the concentration range studied, phenol was found to exhibit substrate inhibition characteristics on these two rates, at 200 mg/l. The values of the biokinetic constants estimated from the models showed a good potential of the mixed microbial culture in treating phenol containing wastewaters using simple stirred tank reactors operated under batch mode.

NOTATIONS

k_0, k_1	zero, first order rate constants
K	positive constant in Yano – Koga
K	constant in Edward model
K_s	half saturation coefficient (mg dm^{-1})
K_i, K_{si}	substrate inhibition constants (mg dm^{-1})
m, n	empirical constants
q	specific substrate degradation rate (h^{-1})
q_{max}	maximum specific substrate degradation rate (h^{-1})
S	substrate concentration (mg dm^{-1}) at time t
S^*	substrate concentration (mg dm^{-1}) at which specific growth rate is maximum
S_0	substrate concentration (mg dm^{-3}) at time t=0
S_m	critical inhibitor concentration (mg dm^{-1}) above which the reactions stops
t	time (h)
x	biomass concentration (mg dm^{-1}) at time t
X_0	biomass concentration (mg dm^{-1}) at t=0
μ	specific growth rate (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})

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КИНЕТИКА НА РАЗЛАГАНЕ НА ФЕНОЛ И РАСТЕЖ НА ПРЕОБЛАДАВАЩИ ВИДОВЕ PSEUDOMONAS В ОБИКНОВЕН РЕАКТОР С ПЕРИОДИЧНО РАЗБЪРКВАНЕ

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(Резюме)

Култура, съдържаща предимно видове *Pseudomonas,* изолирана от завод за третиране на канални води, се използува за изследване на кинетиката на разлагане на фенол и растеж на културата. Културата е способна да разложи фенол с максимална начална концентрация до 400 mg/l в обикновен реакторен съд с периодично разбъркване. Максималното време, необходимо за разлагане на фенол с такова концентрационно ниво, беше 194 h. Кинетиката на разлагане на фенола при различна начална концентрация в средата показва предимно скорост от нулев порядък с максимална стойност на коефициента на определеност (R^2) = 0.99 при 400 mg/l фенол. Детерминистични модели като тези на Халдейн, Хан-Левеншпиел, Луонг, Едуърд, Яно и Кога, Ценг и Уейман са използвани за описание на данните за инхибиране от субстрата в зависимост от кинетиката на растеж на културата и разлагане на фенола. Всички изпробвани модели описват добре експерименталните данни с изключение на модела на Ценг и Уейман.