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Kinetic Modelling of Phenol Biodegradation by Mixed Microbial Culture in Static Batch Mode

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Abstract: In the present study, mixed microbial culture isolated from the sludge of effluent treatment plant of a refinery was tested for its phenol biodegradation potential under static batch condition. The result showed that, after acclimatization, the culture could biodegrade upto 750 mg L^{-1} of phenol. 100% phenol degradation was achieved for the various concentrations studied. Kinetic study showed that specific growth rate of microorganisms and specific substrate degradation rate increased up to 300 mg L^{-1} of initial phenol concentration and then started decreasing. The biodegradation kinetics was fitted to different substrate inhibition models by using optimization software tool (solver) in Microsoft office 2007. Among all models, Aiba model (μ_{max} = 0.3187 h^{-1}, K_I = 400, R^2 = 0.915) and Edward Model (μ_{max} = 0.0011 h^{-1}, K_I = 210 mg L^{-1}, R^2 = 0.942) were fitted the best. Growth kinetics was also fitted well to the classical Haldane model. The values of inhibition constant, K_I from Yano model indicated that this culture may well degrade phenol beyond 750 mg L^{-1}.

Key words: Phenol, mixed culture, batch mode, growth kinetics, inhibition model.

Introduction

Phenol and phenolic compounds are used in various industries such as resin manufacturing, petroleum refineries, petrochemical, textile, dying, phenolic resin manufacturing, glass fibre and varnish industries (Hsien and Lin, 2005; Juang and Tsai, 2006). These compounds are found commonly in industrial effluents and surface water. Phenolics are placed in the priority pollutant list as declared by the U.S. Environment Protection Agency (Yan et al., 2006). These compounds are highly toxic to aquatic life and plants and gives bad odour upon chlorination even at low concentration of 5 mg L^{-1}. WHO has prescribed 0.5 mg L^{-1} as the permissible limit in drinking water. Therefore it is necessary to reduce phenol concentration in order to preserve the quality of water. Conventional techniques like adsorption and coagulation are expensive and produce toxic chemical sludge whereas biodegradation, which is gaining a lot of interest now-a-days, uses microbial cultures to convert phenol to CO_2 and H_2O without leaving any residual toxic compounds (Bai et al., 2007). Most of the studies reported on phenol biodegradation have used pure cultures in batch scale. As a specific single bacterium is never found in nature, it is difficult to isolate and maintain pure cultures for real life applications. Recently mixed cultures have shown potential to degrade phenol (Banerjee and Ghoshal, 2010; Pradhan et al., 2012); however, there is plenty of scope to do further research on mixed cultures.

Microbial degradation of phenol was tested long years ago by Yang and Humphrey (1975) in static as well as continuous batch mode where they could degrade up to 1-2 mg L^{-1} of phenol concentration. Wang and Loh (1999) and Monteiro et al. (2000) investigated kinetics by pure culture of *pseudomonas putida* and tested in batch reactor. Till now lot of work has been done on pure microbes for concentrations ranging up to greater than 1000 mg L^{-1}. Some work on mixed culture was reported in the literature

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(Kumar et al., 2005; Sarvanan et al., 2008) but they are not adequate. Moreover, there is very little study on biodegradation of phenol by mixed microbes in the static batch mode (Yang and Humphrey, 1975) which might be useful in designing the batch reactor or packed bed reactor which maintains the same conditions as that of static batch or where diffusion mass transfer is dominant over convection.

The main objective of present study is to find out the biodegradation kinetics of phenol by using it as the soul carbon source in static batch condition. For this study, mixed microbial culture was isolated from the sludge of an oil refinery located in Guwahati, India and the growth kinetics is tested for Monod, Haldane, Yano, Aiba, Han and Levenspiel, Webb and Edward models.

Kinetic Models

The principles of microbial growth kinetics, which is the relationship between specific growth rate and substrate concentration, were developed mainly between 1940 and 1970. A variety of substrate utilization and inhibition models have been used to describe the dynamics of microbial growth on phenol. The substrate inhibition models along with their mathematical forms have been described below.

The earliest model on microbial growth kinetics is the Monod model (Monod, 1949). It relates the growth rate of microorganism to the concentration of a single growth controlling substrate represented by the following equation:

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

where μ is specific growth rate of mixed microbial culture (h^{-1}) = $\frac{1}{X}\frac{dX}{dt}$, S is limiting substrate concentration (mg/L), μ_{max} is maximum specific growth rate of the culture (h^{-1}) and K_S is half saturation constant (mg/L).

Microbial growth can be modelled by simple Monod equation (Kovar and Egli, 1998). However this equation became unpopular for growth in presence of some inhibitory substance. In such situation, Haldane model is normally used to represent the growth in both lower and higher concentration of inhibitory substance. Haldane model has the form (Wang and Loh, 1999) as:

$$\mu = \frac{\mu_{max} S}{K_s + S + \frac{S^2}{K_I}} \tag{2}$$

where K_I is the substrate inhibition constant (mg/L). Due to its significance, this model was widely adopted by most of the researchers.

Yano S. Koga (1969) proposed the following model to express microbial degradation, where K is a positive constant.

$$\eta = \frac{\eta_{max} S}{K_s + S + \left(\frac{S^2}{K_I}\right)\left[1 + \left(\frac{S}{K}\right)\right]} \tag{3}$$

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

$$\eta = \frac{\eta_{max} S}{K_s + S} \exp\left(\frac{-S}{K_I}\right) \tag{4}$$

J.L. Webb (1963) proposed the modified form of Haldane model as given by equation (5)

$$\mu = \mu_{max} \frac{S\left(1 + \frac{S}{K}\right)}{S + K_s + \left(\frac{S^2}{K_I}\right)} \tag{5}$$

It was found that this model did not show any significant improvisation to Haldane model. Edward (Edward, 1970) proposed another model to predict substrate inhibition at higher substrate concentration as given by the equation (6).

$$\mu = \mu_{max} S\left[\exp\left(\frac{-S}{K_I}\right) - \exp\left(\frac{-S}{K_s}\right)\right] \tag{6}$$

Han and Levenspiel (1988) proposed a model to express substrate degradation rate. The corresponding form of this equation is:

$$\mu = \frac{\mu_{max} S[1 - S/S_m]^n}{S + K_s - [1 - S/S_m]^m} \tag{7}$$

where S_m is critical inhibitor concentration (mg/L).

Materials and Methods

Chemicals and Culture Medium

The microorganisms were grown in mineral salt medium (MSM) containing (mg/L)**:** $(NH_4)_2SO_4$ 325, K_2HPO_4 2627, KH_2PO_4 1436, $MgSO_4$ 65, $MnSO_4$ 8.45, $CaCl_2$ 9.0, $FeCl_3$ 2.0 and glucose 2 g L^{-1} was used for growth of microorganisms at 180 rpm and 37°C. The culture was

then grown with phenol as a sole carbon source. Medium was first autoclaved at 121°C for 15 min before use. Solid media was prepared by adding 20 g L^{-1} agar to the medium. Phenol and all inorganic salts used were of analytical grade and purchased from Merck, India.

Microorganisms and Culture Conditions

The mixed microbial culture capable to degrade phenol was isolated from the sludge of a refinery wastewater plant located in Guwahati, India. All experimental work in this study was done in 250 mL Erlenmeyer flask containing 100 mL of sterilized carbon-free MSM and phenol. The mixed culture was acclimatized for one month so as to make microbes compatible to take phenol at different concentrations under agitated condition up to 750 mg L^{-1}. Acclimatized culture was then used to study the kinetics of phenol biodegradation in static batch condition. Experiment was performed by transferring the phenol acclimatized culture to the MSM at phenol concentration up to 750 mg L^{-1}.

Batch Biodegradation Study

Biodegradation of phenol by mixed culture was studied in static batch mode condition. Microbial culture obtained after acclimatization was used to study the kinetics of phenol biodegradation at different initial concentrations of phenol while using it as the sole carbon source. Inoculation of acclimatized mixed culture was performed by addition of 1% of acclimatized mixed culture volume (100 mL) in 250 mL Erlenmeyer flask containing 100 mL of MSM and phenol concentration starting from 100 mg L^{-1} to 750 mg L^{-1} (140, 300, 400, 500, 600 and 750 mg L^{-1}). After addition of phenol, MSM flasks were closed with cotton plug and incubated inside a BOD incubator maintained at 37 °C. Thereafter samples were analyzed for biomass growth at regular time interval and at the same time centrifuged at 10,000 × g for 5 min and the supernatant were analyzed for phenol concentration. Degradation of phenol and biomass growth was monitored for kinetic study.

Modelling the Decay Kinetics of Phenol Biodegradation

Specific growth rate (μ) was calculated at different initial phenol concentrations and values were tested with various model equations from (1) to (7). Corresponding equations were solved by using solver tool in Microsoft Excel 2007.

Analytical Procedure

Growth of biomass was measured by absorbance at 600 nm using a high precision UV-VIS spectrophotometer (PerkinElmer, Model Lamda 35). Estimation of phenol was done by direct photometric method where reaction takes place between phenolic compounds and 4-aminoantipyrine at pH 7.9 ± 0.1 in the presence of potassium ferricyanide. As a result coloured antipyrine dye is formed which was kept under aqueous condition and absorbance was measured at 500 nm in UV-VIS spectrophotometer (APHA and AWHA, 1994).

For SEM analysis, 10 mL of the sample was centrifuged and 20 μL of the supernatant was treated with 2.5% glutaraldehyde and placed on the sample holder. The sample was then washed with different concentrations of ethanol to remove the unbound samples. The sample was dried overnight at 37 °C. The sample was coated with gold to build-up the electric charge. The coated sample was analysed at 10× magnification for the morphology study using a scanning electron microscope (LEO 1430vp, Oxford).

Results and Discussions

Identification of the Mixed Microbial Consortium

SEM images of the mixed microbial culture showed spherical shape (Figure 1) of the organism. The organism was characterized to be gram positive. The SEM images showed the organisms present in a bead-like chain which is the characteristic feature of the *Streptococci* sp.

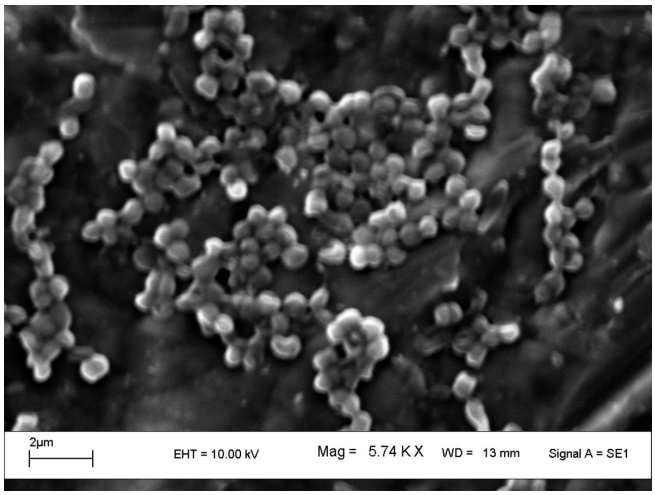

Figure 1: Scanning-electron micrograph of the mixed culture.

Acclimatization of the Microorganism

Acclimatization of the microorganism was done by gradual increase of phenol concentration by 25 mg L^{-1} successively to every new batch. To check the viability of the culture, dilution plating was performed

(Bandyopadhyay et al., 1998). The acclimatization phase was continued for a maximum phenol concentration of 750 mg L^{-1} under static batch condition. The complete acclimatization phase of the mixed culture responsible for phenol degradation is shown in Figure 2.

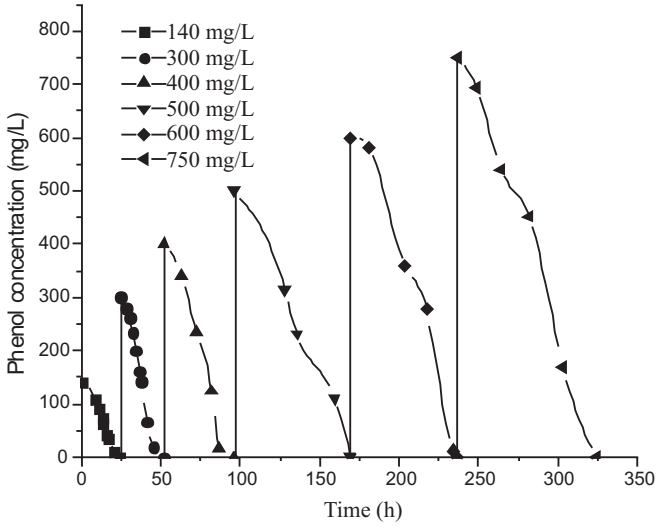

Figure 2: Phenol acclimatization profile at 37 °C and pH 7.0.

Effect of Initial Concentration of Phenol on Its Biodegradation

Profile of phenol degradation with time is shown in Figure 3. From the figure it can be seen that time taken by mixed microbes to completely biodegrade the phenol at 750 mg L^{-1} under static batch condition was 87 h. It was clear from the profile that the time taken by the mixed culture to degrade phenol was dependent on its initial concentration (Pradhan et al., 2012). It was observed that maximum degradation rate of phenol was increased up to 300 mg L^{-1}; thereafter it started decreasing as also reported to be 376.44 mg L^{-1} by Bajaj et al. (2009).

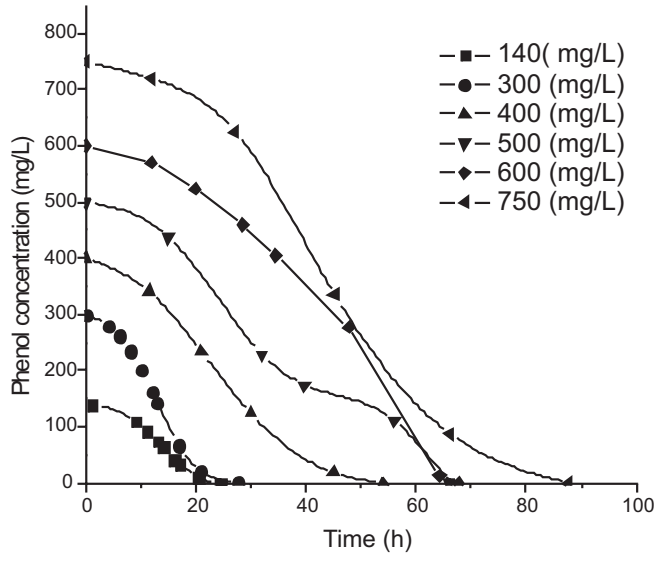

Figure 3: Phenol biodegradation profile with time at 37 °C and pH 7.0.

Effect of Initial Concentration of Phenol on the Growth of Culture

At higher initial concentration of phenol, growth inhibition takes place which has been reported in various literatures (Saravanan et al., 2008). Profile of the different initial concentrations of phenol on growth of microorganism is shown in Figure 4 where OD_{600nm} is plotted against time (h) at phenol concentrations ranging from 140 mg L^{-1} to 750 mg L^{-1}. It was observed from the profile that there was no inhibitory effect on microbial growth upto 300 mg L^{-1} of initial phenol concentration as there was a very short lag phase up to this concentration (Kumar et al., 2005; Gabriela et al., 2006). This behaviour of culture can be caused by toxicity of phenol above a certain limit (300 mg/L) where cell death starts because of cell lyses (Banerjee and Ghoshal, 2010). In this study toxicity limit was found to be 300 mg L^{-1}.

Specific growth rate (μ) of mixed microbial culture at different phenol concentration was calculated as per the equation given below:

$$\mu = \frac{1}{X}\frac{dX}{dt} \tag{8}$$

where μ is the specific growth rate (h^{-1}), t is time(h) and X is concentration of biomass (mg/L). Plot of experimental data between specific growth rate and initial

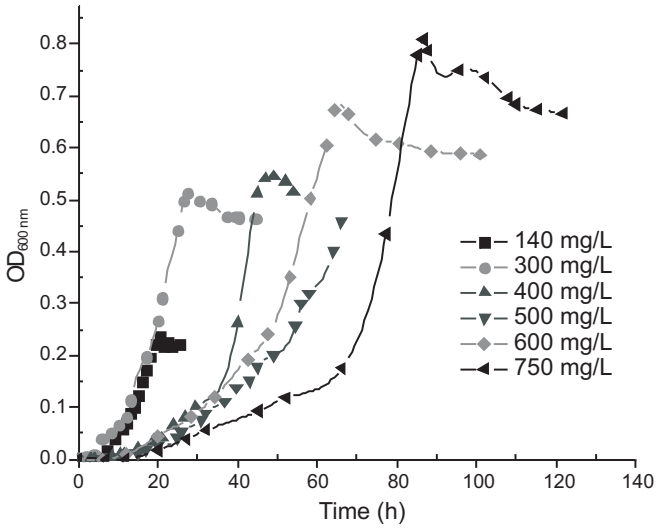

Figure 4: Microbial growth profile with time during phenol consumption at 37 °C and pH 7.0.

phenol concentration (Figures 5 and 6) showed that the value of μ increased from phenol concentration 140 mg L^{-1} (μ = 0.0681) to 300 mg L^{-1} (μ = 0.0962) and thereafter it started decreasing from 300 mg L^{-1} upto 750 mg L^{-1} (μ = 0.037) of phenol. The decrease in the specific growth rate is a clear indication of the substrate inhibition as reported by other authors (Saravanan et al., 2008).

Growth Kinetic Studies for Mixed Microbial Culture

To study the kinetics of mixed culture, there are some theoretical equations which represent the relationship between specific growth rate of microbes and substrate concentrations. In this study seven models were considered to predict the experimental data on specific growth and are represented in Figures 5 and 6.

To obtain the simulated growth profile for the seven tested models at different initial phenol concentrations, kinetic parameters related to respective models were calculated. Initial guess for biokinetic constants like μ_{max}, K_S, K_I, K and S_m were carefully assumed to predict the specific growth rate curves for different models. To get the best fit model among the seven models, Root Mean Square Error (RMSE) and R^2 values were generated for respective models. Values of different kinetic parameters for these models are summarized in Table 1. Among all these models used for fitting the experimental data between specific growth rate and initial phenol concentration, Edward and Aiba models were fitted best as clearly shown by the RMSE and R^2 values for both these models. Amongst all, the Monod model was the poorest fit as it does not consider inhibition effect.

The best fit Aiba and Edward models predicted the values of inhibition constant (K_I) as 400 and 209.9 which indicates the initial phenol concentrations without inhibiting the growth of mixed culture. Values of

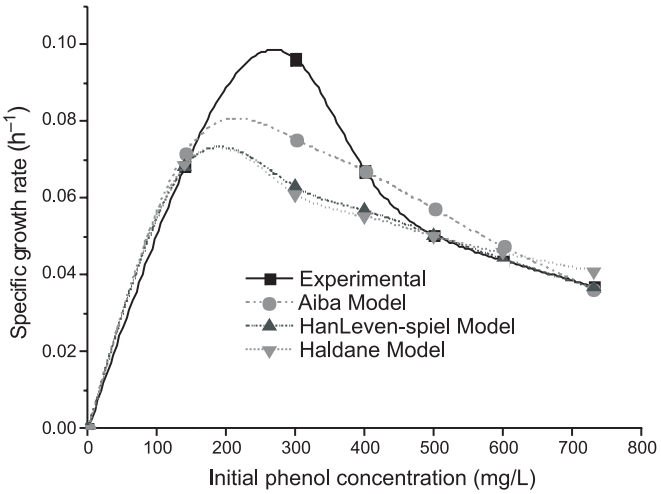

Figure 5: Experimental and predicted specific growth rate of the mixed culture for Aiba, Han-Levenspiel and Haldane models.

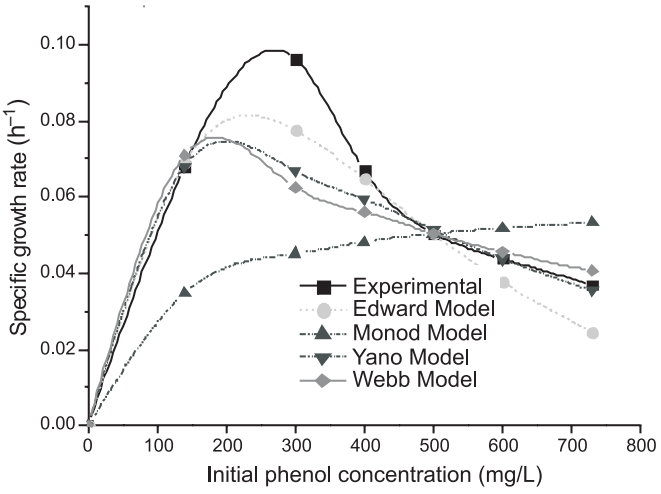

Figure 6: Experimental and predicted specific growth rate of the mixed culture for Edward, Monod, Yano and Webb models.

Table 1: Summary of growth kinetics parameter values obtained from different models during biodegradation of phenol by mixed microbial culture used in present work

Model	μ_{max} (h^{-1})	K_S *(mg/L)*	K_I *(mg/L)*	K	S_m *(mg/L)*	n	m	*RMSE*	R^2
Experimental	0.1042	107.98	-	-	-	-	-	-	-
Monod	0.0609	105	-	-	-	-	-	0.0264	0.355
Haldane	0.1097	40	450	-	-	-	-	0.0143	0.81
Yano	0.09675	50.152	1×10^5	3.2	-	-	-	0.0108	0.89
Aiba	0.3187	300	400	-	-	-	-	0.0095	0.915
Webb	0.1233	55.59	350	1×10^5	-	-	-	0.0139	0.82
Edward	0.0011	50.28	209.9	-	-	-	-	0.0079	0.942
Han and Levenspiel	0.0999	36.3	-	-	2395.23	2.63	3	0.0125	0.854

inhibition constant for Yano model was 100,000 which was very high and the same range was previously reported by Banerjee and Ghoshal (2010) for isolated culture giving the reason of developed capacity of microbes to withstand such a high concentration after sufficient acclimatization. Haldane model predicted growth rate (0.1097 h^{-1}) very near to the experimental value. Webb and Han and Levenspiel could fit partially as these models showed low R^2 values.

Conclusion

Kinetic modelling of phenol degradation was studied under static batch condition for the mixed microbes isolated from sludge of the wastewater treatment plant of a petroleum refinery. Kinetic study shows that mixed microbes could grow up to 750 mg L^{-1} after acclimatization. Seven different kinetic models were tested to predict kinetic constants out of which substrate inhibition models Aiba and Edward proved to be the best fit with experimental data. The acclimatized mixed culture used in this study under static batch condition showed the good potential to biologically degrade phenol. The biokinetic constants generated in this study can be very helpful to understand the capacities of mixed culture towards treatment of phenol-rich wastewater under tested conditions. Moreover, these kinetic parameters can be useful in designing a batch reactor under the static conditions where diffusion mass transfer predominates over convection mass transfer.

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