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# Biodegradation kinetics of phenol by predominantly *Pseudomonas* sp. in a batch shake flask

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ABSTRACT

Biodegradation of phenol by predominantly *Pseudomonas* species isolated from a sewage wastewater treatment plant was investigated in batch shake flasks. Phenol with a lower concentration of 100 mg/L was degraded in 10 h and a highest of 800 mg/L in 69 h. The phenol degradation rate was observed to vary largely with the concentrations of phenol used and was found to be less than 10 mg/L/h at both the extremes of the initial concentrations. The degradation kinetics was found follow the three half-order kinetic model with the regression greater than 0.97. The specific substrate utilization rates of the culture at various initial phenol concentrations were fitted to modified substrate inhibition kinetic models of Edward, Haldane, Luong, Han–Levenspiel and Yano–Koga. Among these models the Edward was found to fit the data well with a minimum Root Mean Square error value of 0.0039.

***Keywords:*** Biodegradation; Kinetics; Phenol; *Pseudomonas* species; Substrate inhibition models; Three half-order model

## 1. Introduction

Generation of gaseous, liquid and solid wastes is an unavoidable consequence of industrial, agricultural and domestic activities. Nevertheless, the environmental impact of these activities must be minimized to ensure sustainable quality of life. While conservation and better utilization of resources would ultimately have the greatest influence on sustainability of the planet; reduced generation, improved treatment and utilization of waste will remain the essential components of an overall strategy for maintenance of environmental quality.

One of the most alarming phenomena is the growth of industrial sectors, which discharge a huge amount of wastewater contaminated with the toxic organic substances like phenolics. The major industries that discharge phenolic wastewater include petroleum refineries, petrochemicals, textile, dye manufacturing, phenolics resin manufacturing, glass fiber units, varnish industries and smelting related to metallurgical operations [1–4]. Phenol and cresol are the two most important phenolic compounds that are present in wastewater discharged from these industries [5,6]. These are toxic to human beings and aquatic biota [7,8]. Humans, who are acutely exposed to phenol by the oral route, suffer damage in blood, liver, kidney and cardiac toxicity including weak pulse, cardiac

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depression and reduced blood pressure. Moreover, these are carcinogenic compounds lead to cancer. Therefore, removal of these phenolics to sufficiently low levels in wastewater is mandatory.

For the last two decades, rigorous pollution control and legislation in many countries have resulted in an intensive search for new and more efficient water treatment technologies. Important wastewater treatment technologies that have come up in recent times, include but are not limited to flocculation, precipitation, adsorption on granular activated carbon, reverse osmosis and Advanced Oxidation Process (AOP) *viz*., Photo Fenton, photocatalysis, sonication, etc. Among the various techniques available for removing phenol, bio-oxidation has been shown to be economical, and the most promising with versatile approach. Moreover a complete mineralisation of such organic waste could be achieved in shorter duration. Biological treatment with pure and mixed microbial strains is an attractive alternative for the treatment of contaminated ground, surface, and wastewaters containing recalcitrant substances such as phenolics [9–14]. Phenolics biodegradation using pure strain has been studied widely; for example, *Pseudomonas putida* has been widely studied for its potential for phenol biodegradation [1,9,10,12,13,15,16]. Though, pure cultures can achieve 100% biodegradation but they leave many hazardous residuals as intermediates [17]. On the other hand, mixed microbial culture may be well competent for the complete degradation of phenolics with generating only $CO_2$ and $H_2O$ as a result of complete degradation of organic pollutants [4,18]. Therefore, a mixed microbial culture would always be preferred for better applicability in wastewater treatment. Modeling the kinetics of wastewater system forms the crucial step. Hence in this study degradation kinetics of phenol by predominantly *Pseudomonas* sp isolated from a sewage treatment plant has been investigated and appropriate modeling techniques have been reported along with different substrate inhibition models.

## 2. Materials and methods

### 2.1. Chemicals, reagent and microorganism and culture conditions

Phenol used in the study was of analytical grade and purchased from Merck, India. All the other chemicals and reagents were also of analytical grade purchased from Merck or Sisco Research Laboratories, India.

A phenol degrading mixed microbial culture was isolated and enriched from a sewage treatment plant located in Guwahati, India. Detailed and routine biochemical characterization tests carried out with the indigenous mixed culture further confirmed the predominant presence of *Pseudomonas* group of species in the mixed consortium [19]. The culture was initially grown in 250 mL Erlenmeyer flask containing 100 mL of Mineral Salt Medium (MSM) having the composition [19]. The completed enrichment of the indigenous mixed culture was carried out for a period of more than one month.

### 2.2. Batch biodegradation study

All biodegradation experiments using the mixed culture were performed in 250 mL Erlenmeyer flask containing 100 mL of MSM containing phenol at concentrations ranging from 100 to 800 mg/L. The flasks were incubated at 27 °C in a rotary orbital shaker; samples were withdrawn at regular time interval, centrifuged (10,000×*g* for 3 min) and the samples analyzed for residual phenol concentration. Each assay was carried out for a period up to residual concentration of phenol in flask was found to reach 0 mg/L. The representative samples collected during the middle and end of the experiment were analyzed for the intermediates concentrations. Each experiments were performed in triplicates and the standard deviation of the results obtained from each set was found to $\pm$0.25–4.5%.

### 2.3. Analytical methods

Cell density in the samples was estimated with Diode Array Spectrophotometer (Spekol 1200, Analytik Jena, Germany) by measuring its absorbance (OD) at 600 nm wavelength. $OD_{600}$ was then converted to dry cell weight by a calibration curve, which was obtained by plotting dry weight of biomass per milliliter vs. $OD_{600}$. For determination of biomass concentration as dry weight, samples were centrifuged for 10 min at 10,000*g* at room temperature (Biofuge Stratos, Heraeus instruments, Kendro, Hanau, Germany) in eppendrof tubes. The pellets were dried at 50 °C for 24 h, cooled in an exsiccator at room temperature and weighed. The tubes were dried weighed to determine its final weight with biomass. The difference between the first (empty tube weight) and second (with biomass) weight was used to determine the dry weight of biomass in mg/L.

The amount of phenol and intermediates in the biomass free samples was determined by High Performance Liquid Chromatograph (HPLC) (Perklin Elmer 200Series) with UV detection equipped with a C-18 column (Chromopack 150 mm × 4 mm × 5 mm). The mobile phase was a mixture of acetonitrile and water (60/40). The eluent was delivered at a rate of

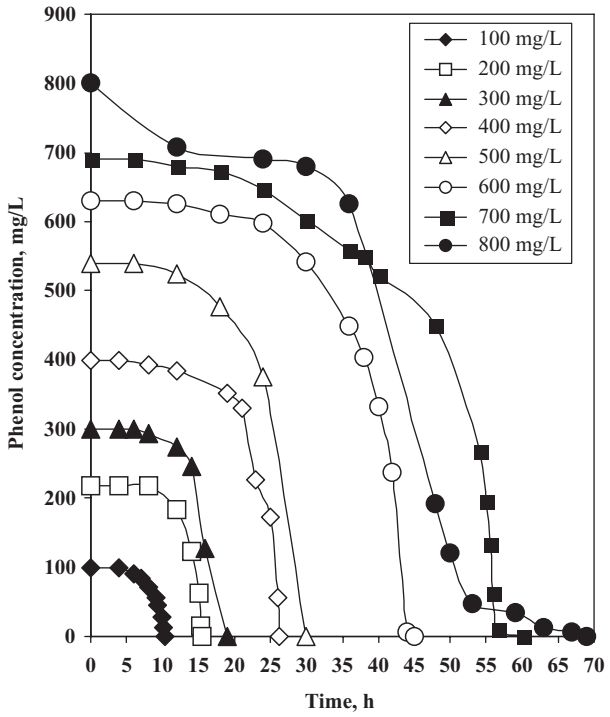


Fig. 1. Time profile of phenol degradation by the mixed culture [data as shown in Saravanan et al., 2008].

1.0 mL/min and the wavelength of detection was at 275 nm. For these operating conditions, the retention times for hydroquinone, benzoquinone, phenol and catechol were 2.3, 2.7, 3.0 and 3.25 min, respectively. The organic acids present in the samples were identified using an Acclaim OA column by Dionex (150 mm × 4.0 mm × 5 μm) and the mobile phase was 100 mM $Na_2SO_4$, pH 2.65 adjusted by using methanesulfonic acid at a flow rate of 0.6 mL/min and the detection wavelength was 210 nm.

## 3. Results and discussion

### 3.1. Kinetics of phenol biodegradation

Fig.1 [20] shows the time profile of phenol degradation by the culture. The culture took about 10 h to degrade completely when phenol in the media was 100 mg/L; it took about 68 h for degrading an initial phenol concentration of 800 mg/L. Hence it is clear that the time taken by the culture to degrade phenol was dependent up on its initial concentration. The degradation time taken in all the adopted phenol concentration may be concentration divided into two phases: initial lag phase and active degradation phase. The extent of two phases, in turn, depended on initial phenol concentration. In order to model such kinetic behaviors of phenol degradation by the mixed culture, several kinetic models such as; growth-associated models (logarithmic, logistic and Monod with growth), non-growth associated models (zero order, first order and Monod based) and three-half order model were modeled [21]. The mathematical form of these models is shown in Table 1 [20]. Where $t$ = time (h), $S_0$ = initial phenol concentration at $t = 0$ (mg/L), $s$ = phenol concentration at time $t$ (mg/L) and $k_0$, $k_1$, $k_2$ are zero, first and second order rate constants. Out of these seven different models tested, only a non-deterministic three-half-order kinetic model could fit the data well with coefficient of determination ($R^2$) values greater than 0.97. Table 2 shows the regression and rate constant values obtained from three-half-order kinetic model. Based on this observation, it is highly exceptive 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 [22]. Hence only the three-half order kinetic model could fit all the

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 | $S = S_0 + X_0[1 - \exp(\mu_{max} t)]$ | $S_0 >> K_S$ |
| | Logistic | $S = \frac{S_0 + X_0}{1 + \frac{X_0}{S_0}[\exp(K(S_0 + X_0)t)]}$ $\left(K = \frac{\mu_{max}}{K_S}\right)$ | $S_0 << K_S$ |
| | Monod with growth | $K_s \ln \frac{S}{S_0} = (S_0 + X_0 + K_s) \ln \frac{X}{X_0} - (S_0 + X_0)\mu_{max} t$ | $S_0 \sim K_S$ |
| Three-half-order kinetic model | | $\frac{1}{t} \ln \left[\frac{S_0 - S}{S_0}\right] = -k_1 - \frac{k_2 t}{2}$ | |

Table 2
Regression obtained from the three half order kinetic model at different initial phenol concentrations

| Initial phenol concentration ($S$) | Regression ($R^2$) | $k_o$ | $k_1$ ($\times 10^{-5}$) | $k_2$ |
|---|---|---|---|---|
| 100 | 0.9721 | −9.1608 | 65 | −0.016 |
| 200 | 0.9741 | −13.141 | 15 | −.0054 |
| 300 | 0.9625 | −13.848 | 10 | −.0042 |
| 400 | 0.9529 | −12.436 | 4 | −.0024 |
| 500 | 0.9701 | −12.314 | 2 | −.0014 |
| 600 | 0.9703 | −11.986 | 0.5 | −0.0008 |
| 700 | 0.9721 | −11.033 | 0.4 | −0.0006 |
| 800 | 0.9803 | −9.3999 | 0.5 | −0.0006 |

experimental data obtained at different initial phenol concentrations.

The degradation rate of phenol was determined by calculating the slope rate of the biodegradation curve except lag period (Fig. 1). Thus its variation with respect to its initial concentration is shown in Fig. 2. From the figure a maximum degradation rate of 15.7 mg/L/h was observed at 400 mg/L of phenol concentration. Moreover concentration below and above 400 mg/L gave lesser degradation rates by the culture indicating the influence of phenol concentration on its degradation rate. Hao et al. (2002) in their study on biodegradation of 4-chlorophenol (4-CP) using *Acitenobacter*n reported similar trend at 30 mg/L of 4-CP initial concentration [9].

### 3.2. Analysis of probable intermediates during phenol degradation

In order to understand whether the phenol has been probably completely degraded by the culture

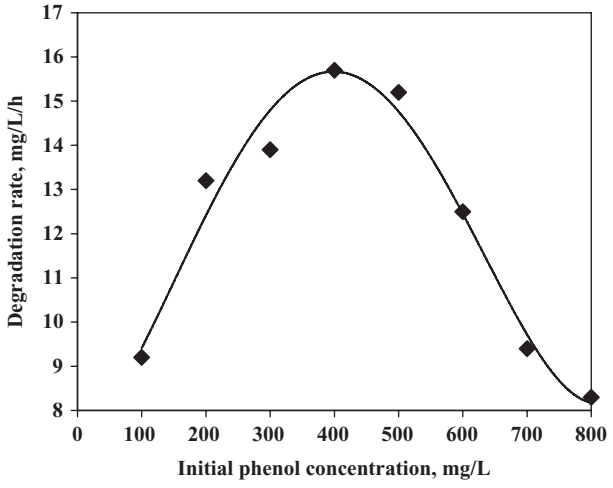


Fig. 2. Variation of phenol degradation rate with initial phenol concentration.

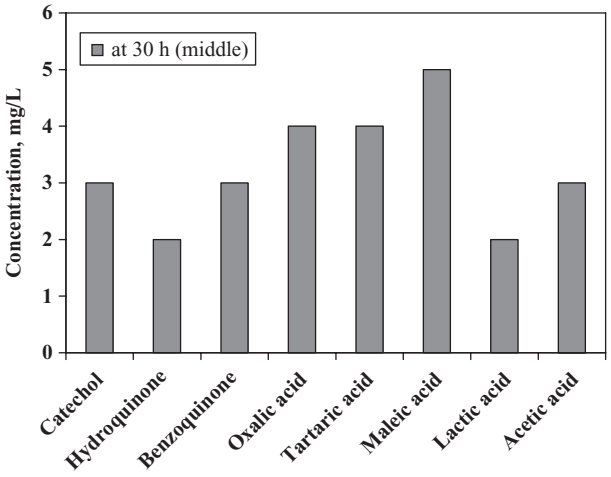


Fig. 3. Intermediates formed and their concentrations during (middle) and end of batch biodegradation of phenol.

with no intermediates at the end of each biodegradation experiments. The samples were collected during (middle) and end of the biodegradation experiments and analyzed for various intermediates using HPLC. At the middle of the biodegradation experiments some considerable amount of hydroquinone, benzoquinone, catechol and organic acids such as oxalic acid, maleic acid, acetic acid, formic acid and tartaric acid [23] in very low concentrations of less than 5 mg/L, in all the flasks. However, no such intermediates could be detected at the end of the each experiment ensuring complete degradation of phenol by the culture. Fig. 3 is a typical illustration of the concentrations levels of the various intermediates obtained during biodegradation of phenol at 800 mg/L initial concentration. The complete intermediates study was carried out on the based of literature report.

### 3.3. Effect of initial phenol concentration on its specific utilization rate

The kinetic profile at each phenol concentration was used to calculate the specific degradation rate, given by

$$q = -\frac{1}{x}\frac{\mathrm{d}s}{\mathrm{d}t} \tag{1}$$

where $x$ = amount of biomass at time $t$ (mg/L), $s$ = substrate concentration (mg/L) and $t$ = time (h). The variation of specific substrate utilization rate with initial phenol concentration is given in, Fig. 4. It is clear from the figure that the specific substrate utilization rate falls at higher concentration of the substrate, which indicates substrate inhibition on the specific

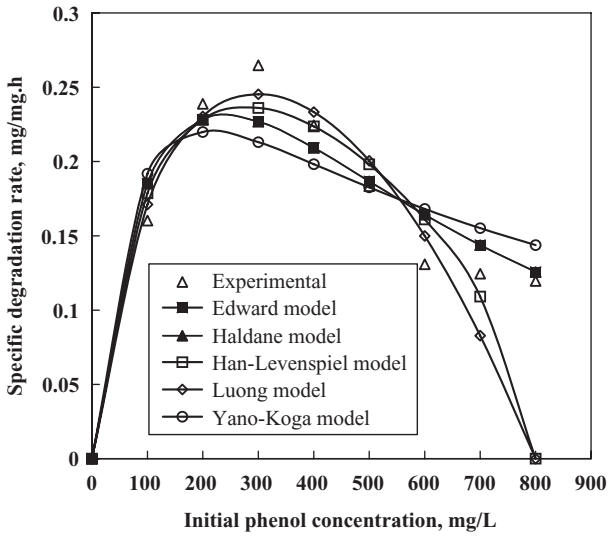


Fig. 4. Experimental and predicted substrate degradation rate of the culture due to different models.

Table 3
Substrate inhibition and kinetic models

| Model | Mathematic equation | References |
|---|---|---|
| Monod | $\mu = \frac{\mu_{max} S}{K_s + S}$ | [25] |
| Haldane | $\mu = \frac{\mu_{max} S}{K_s + S + \frac{S^2}{K_i}}$ | [17] |
| Yano-Koga | $\mu = \frac{\mu_m}{(K_s/S) + 1 + \sum_{j=1}^{n} (S/K_j)^j}$ | [26] |
| Edward | $\mu_i = \mu_m \frac{S}{S + K_s + (S^2/K_{si})(1 + S/K)}$ | [27] |
| Luong | $\mu = \frac{\mu_m S}{K_s + S}\left[1 - \frac{S}{S_m}\right]^n$ | [28] |
| Han-Levenspiel | $q = \frac{q_{max} S\left[1 - \frac{S}{S_m}\right]^n}{K_s + S - \left[1 - \frac{s}{s_m}\right]^m}$ | [29] |

utilization rate similar to growth rate inhibition at higher substrate concentrations [24]. This variation of specific substrate utilization rate at all concentrations of the substrate was modeled in order to get the biokinetic constants. The data was fitted to the models that were originally developed for explaining substrate inhibition on the growth rate of microorganism, to predict the utilization rates at different concentrations. These models are shown in Table 3. The form μ and $\mu_{max}$ in the original model equation were replaced with $q$ and $q_{max}$ representing specific substrate utilization rate and maximum specific substrate utilization rate. These model equations were solved using nonlinear regression method using MATLAB® 7.0. Fig. 4 also shows the fit of these models to the experimental data points. From this figure it could be seen that except Yano-Koga model all other could fit the data very well.

The biokinetics constants obtained from the fit of these models, along with Root Mean Square (RMS) error value between the model predicted and experimental specific utilization rates are shown in Table 4. The table also shows the values of half-saturation constant ($K_s$) and maximum substrate utilization rate ($q_{max}$) estimated using the linearised form of Monods model. These values agree quite closely with those predicted by other models except that of Yano-Koga. Moreover Edward and Haldane models predicted the inhibition constants value above, which the specific substrate utilization rate declines, closely matched with the experimentally observed value of 300 mg/L.

Luong and Han-Levenspiel models also predicts the critical substrate concentration at which the rate falls to zero, but this value was not found to be vary from the experiments. This difference in the model predicted values of critical substrate concentrations could be reasoned based on the fact that the two models were originally developed for systems containing a different microorganism and substrate.

The kinetic parameter value predicted by different models clearly indicates the effectiveness of this predominantly *Pseudomonas* sp. in degrading the phenol.

Table 4
Estimated substrate degradation kinetic parameters obtained from different models during biodegradation of phenol

| Model | $q_{max}$ ($h^{-1}$) | $K_s$ (mg/L) | $K_i$ (mg/L) | $S_m$ (mg/L) | $n$ | $m$ | $K$ | RMS error |
|---|---|---|---|---|---|---|---|---|
| Monod | 0.4132 | 156.46 | – | – | – | – | – | – |
| Edward | 0.4321 | 121.49 | 210.00 | – | – | – | 20.00 | 0.0039 |
| Haldane | 0.4643 | 113.54 | 376.65 | – | – | – | – | 0.0074 |
| Luong | 0.4142 | 138.71 | – | 800 | 2.05 | – | – | 0.0173 |
| Han–levenspiel | 0.451 | 135 | – | 800 | 0.6 | 1.00 | – | 0.0333 |
| Yano–Koga | 0.4510 | 110.17 | – | – | 1.00 | – | 400.00 | 0.0076 |

## 4. Conclusions

A mixed culture of microorganism isolated from sewage treatment plant was shown to completely degrade phenol up to a maximum concentration of 800 mg/L in MSM. Phenol degradation kinetics followed the three–half-order kinetic models at all initial concentrations of phenol. The specific utilization kinetics was found to be inhibited at higher concentration similar to substrate inhibition on growth of microorganism. The substrate inhibition on specific phenol utilization rate was explained using modified Edward, Haldane, Han-Levenspiel, Luong and Yano-Koga substrate inhibition models. The bio kinetics constants were evaluated using the modified models. The obtained values showed the utilization potential of phenol by predominantly *Pseudomonas* sp. Over all the study showed the potential of the predominantly *Pseudomonas species* in utilizing phenol.

## Symbols

| | |
|---|---|
| $S$ | limiting substrate concentration (mg/L) |
| $q$ | specific substrate utilization rate ($h^{-1}$) |
| $K_s$ | half saturation coefficient (mg/L) |
| $q_{max}$ | maximum substrate utilization rate (mg/mg.h) |
| $K_i$ | inhibition coefficient (mg/L) |
| $S^*$ | substrate concentration at which specific growth rate is maximum (mg/L) |
| $S_m$ | critical inhibitor above which the reactions stops (mg/L) |
| $K$ | positive constants |
| $n$ | positive integer |
| $K_{si}$ | substrate inhibition constant (mg/L) |
| $K$ | constant (mg/L) |

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