Kinetics Modelling of the Biodegradation of Benzene, Toluene and Phenol as Single Substrate and Mixed Substrate by Using *Pseudomonas putida*

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In the present work, kinetics of the biodegradation of benzene, toluene and phenol by using a pure culture of *Pseudomonas putida* (MTCC 1194) was determined by measuring the specific growth rate and degradation rate with substrate concentration as a function of time in a batch reactor. In general, the degradation rate of benzene, toluene and phenol increased with the increase in the initial substrate concentration and then decreased after reaching a maximum, showing substrate inhibition kinetics. The degradation rates of benzene, toluene and phenol were highest, 0.108, 0.133, and 0.0705 mg L^{-1} h^{-1} at γ = 148.2, 202.6, and 97.8 mg L^{-1} initial substrate concentration, respectively. Toluene degradation rate was highest, followed by benzene and phenol at the optimum temperature and pH. In the mixtures of benzene, toluene and phenol, toluene was the preferred substrate, but degradation of each compound was competitively inhibited by other compounds. This paper also deals with the study of Haldane, Andrews and Noack, and Han and Levenspiel model equations for substrate inhibition. An attempt has been made to study the applicability of three model equations for substrate inhibition systems by fitting their experimental data. It was found that the Han and Levenspiel model is best suited to the system.

Key words:
Benzene, toluene, phenol, *Pseudomonas putida*, growth kinetics

Introduction

Enormous quantities of aromatic compounds as pollutants are being released into the environment by various industries. Due to their natural abundance and by virtue of their broad range of applications of aromatic compounds in all spheres of life in various chemical forms such as ingredients of several compounds in the form of solvent, aromatic compounds are regarded as an integral part of the modern society. Aromatics compounds such as benzene, toluene and phenol are important industrial raw materials for paints, pesticides, resins, fiber glass unit, varnish, phenolic resin manufacture, textile unit, making of organic dyes and are also used as solvents for rubber and plastics.[1–2]

Benzene, toluene and phenol are highly toxic, and carcinogenic compounds commonly found as contamination linked to human activities.[3–9] Among these, benzene is more carcinogenic. Owing to its high volatility (distribution coefficient of 0.229 at ϑ = 25 °C), higher water solubility with respect to other aromatics (0.174 − 0.187 %), and high mobility, benzene is a widespread environmental contaminant, commonly found in soils, aquifers, and in the atmosphere.[10] Because of its confirmed carcinogenic properties,[11–12] the standard set by USEPA 2002, for benzene in drinking water is γ = 5 μg L^{-1}.[13] The U.S. Public Health Service 1989 has also recommended that drinking water contain no more than γ = 2 mg L^{-1} of toluene for lifetime exposure.[14] Phenol is also very toxic in nature and is highly soluble in water. Its solubility in water is γ = 98 g L^{-1} and its melting point is ϑ = 181 °C. The Ministry of Environment and Forests (MOEF), Government of India and EPA, USA, have listed phenol and phenolic compounds as priority-pollutants and have set a maximum concentration level of γ = 1.0 mg L^{-1} of phenol in the industrial effluents for safe discharge into surface water, the WHO recommends the permissible phenolic concentration of γ = 1.0 μg L^{-1} in potable waters.[15]

Various physico-chemical methods were reported in the literature for the treatment of benzene, toluene and phenol. However, biological treatment is an attractive approach for removing benzene, toluene and phenol from contaminated water. Biological treatment appears to be an economical, energy efficient and environmentally sound approach for

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treating benzene, toluene and phenol contaminated water. Microorganisms are able to degrade benzene, toluene and phenol under aerobic, as well as anaerobic conditions. Benzene and toluene is extremely toxic, and there are very limited reports on bacteria able to degrade benzene, toluene in the presence of concentrations of phenol. Further, literature survey reveals that few researchers[16–23] have attempted to study the biodegradation of single or combination of benzene, toluene and phenol using microbial free cells and immobilized cells. The literature on the kinetics of the removal of aromatic compounds from wastewaters for free cell system is vast and varied. Hence, evaluation of biokinetic parameters has its significance in respect of understanding the capacities of the microorganism for the degradation as well as for the operation of biological reactors.

The purpose of the present study is to investigate the degradation kinetics of benzene, toluene and phenol in single and mixed substrate biodegradation system by *Pseudomonas putida*. In addition, various kinetic constants, used in Haldane model (1965),[24] Andrews and Noack model (1968),[25] and Han and Levenspiel (1988)[26] model have been evaluated by the kinetic data, generated in the batch experiments in the growth of suspended microbial culture on benzene, toluene and phenol and are compared.

Materials and methods

Microorganism, media and chemicals

The bacterium *P. putida* (MTCC 1194) was procured from Institute of Microbiology Technology (IMTECH), Chandigarh, India as lyophilized form. For the biodegradation, a basal salts mineral (BSM) medium was used as carbon free medium. The strain was grown on BSM prepared with deionized water (Milli-Q Millipore 18.2 MΩ cm resistivity). The BSM was sterilized in three parts to avoid precipitation of solution during autoclaving. The compositions of BSM were described in author's previous study.[27]

Acclimatization of culture and inoculum development

Initially *P. putida* was revived on nutrient Agar (γ/g L^{-1}: peptone 5; beef extract 3; agar 20; NaCl 5; pH 7–7.2) Petri dish and then in liquid medium. *P. putida* was cultivated in 500 mL flask containing $V = 100$ mL of the BSM with toluene as the sole carbon. The cultures were acclimatized to toluene by exposing the culture in a series of shake flasks. The startup of acclimatization was obtained by inoculating $V = 100$ mL of BSM (toluene concentration, $\gamma = 10$ mg L^{-1}) with *P. putida* from nutrient agar slants under sterile conditions. After 48 h of incubation at 30 °C, 5 mL of this culture was added to fresh BSM ($\gamma = 10$ mg L^{-1} toluene) as inoculum. 48 h later, a third fresh BSM was also inoculated with 5 mL of the last culture to insure that the bacteria were already adapted to toluene.

Analytical methods

Benzene and toluene were analyzed by using a Netel India Limited (model- MICHRO 9100) gas chromatograph equipped with a capillary column type HP 5 (30 m × 0.249 mm × 0.25 μm film thickness) and a flame ionization detector. The injector, oven and detector temperature were maintained at $\vartheta = 210$, 60 and 230 °C, respectively. The fuel was hydrogen gas and carrier gas was nitrogen. The calibration curve was prepared by injecting known amounts of the benzene and toluene into a sealed bottle equipped with Teflon septum according to the standard procedure.[28] The injected amount of benzene and toluene was allowed to evaporate in the air space within the bottle at $\vartheta = 30$ °C temperature. Benzene and toluene concentrations were determined by headspace analysis. Samples of the headspace gas ($V = 250$ μL) were withdrawn from the bottles using 1 mL gas tight syringe (Hamilton-Bonaduz-Schweiz) and analyzed by gas chromatograph. Aqueous samples of phenol were measured by same GC as described by Abuhameda *et al.,* 2003.[21] The concentrations of benzene and toluene in the liquid phase were calculated as described in author's previous study.[27] Growth of the microorganisms was measured by monitoring the optical density (OD) at $\lambda = 600$ nm by using a spectrophotometer (Model Lamda 35, Parkin-Elmer, USA).

Biodegradation studies

Benzene and toluene biodegradation in this study is a two-phase reaction. Most benzene and toluene are present in the gaseous phase and should be transported to the liquid phase to be degraded. Therefore, an experimental program was designed to avoid the possibility of volatilization of benzene and toluene while conducting of the batch experiments. In this study, similar type of designed batch reactor was used as reported in author's previous study.[27] During the biodegradation studies, experiments were conducted with only $V = 100$ mL working volume in a $V = 500$ mL bottles to avoid the deficit of oxygen. Control experiments were also performed without microorganisms. For biodegradation studies, each bottle containing $V = 100$ mL of the BSM was aseptically injected with one substrate (benzene or toluene) directly from the pure

solution using a $V = 10$ μL syringe to give a desired final concentration. Phenol was added from an autoclaved phenol stock solution. Liquid sample aliquots were periodically withdrawn to measure OD. Using a sterilized syringe, 3 mL of aqueous samples were drawn from the shake bottles at regular intervals. A sample of 1 mL was then transferred to a gas-tight centrifuge tube (Eppendorf, Germany) and centrifuged (Biofuse Stratos, Germany) at 8 000 rpm for 10 min at 4 °C and the supernatant was transferred to a separate eppendorf tubes which were stored at $\vartheta = 4$ °C before residual phenol analysis. The remaining $V = 2$ mL sample was used for monitoring the OD in the measurement of the growth of the organisms.

Model equations used for single substrate

Numerous studies were directed to evaluate the behavior of the organisms in various concentrations and under varying environmental conditions, so that the result may be useful in the design and optimization of biological reactors treating aromatic compounds. Knowledge of both the rate and extent of degradation is essential for the understanding the behavior.

For initial substrate concentration, degradation rate was determined from initial or maximum slope of the concentration curve, while specific growth rate was estimated from the slope of the semi-logarithmic plot of OD vs. time. The specific growth rate of benzene, toluene and phenol were calculated using the cell concentration in the exponential phase.

In the literature, two approaches are encountered for representing the kinetics of bacterial growth for single substrate. In the first approach, substrate has been considered non-inhibitory compound and is being represented by Monod's non-inhibitory kinetics equation as given below.

$$\mu = \frac{\mu_{\max}\gamma_S}{K_S + \gamma_S} \qquad (1)$$

Second approach considers the single substrate to be growth inhibitory compound. To represent the growth kinetics of inhibitory compounds, several kinetic models were fitted to the experimental data for selecting the best models. The various inhibition kinetic models for a single substrate are given in Table 1. In this study, Haldane model (1965), Andrews and Noack model (1968), and Han and Levenspiel (1988), inhibition growth models are used due to their mathematical simplicity and wide acceptance for representing the growth kinetics of inhibitory substrates. Values of K_S indicate the ability of microbes at low substrate concentration[20] and

Table 1 – *Models used for substrate inhibition*

Model	Form of normalized kinetics
Haldane (1965)	$\frac{\gamma_S}{K_S + \gamma_S + \gamma_S^2/K_I}$
Andrews and Noack (1968)	$\frac{\gamma_S}{(\gamma_S + K_S)(1+\gamma_S/K_I)}$
Han and Levenspiel (1988)	$(1-\gamma_S/K_I)^n \frac{\gamma_S}{\gamma_S + K_S(1-\gamma_S/K_I)^m}$

K_I values indicate the sensitiveness of the culture to substrate inhibition.[29] The higher K_I value physically means, the culture is less sensitive to substrate inhibition and vise versa. In mathematical models γ_S, the substrate concentration; μ, the specific growth rate; μ_{max}, the maximum specific growth rate; K_S, the half-saturation constant and K_I is the substrate inhibition constant.

Results and discussions

Effects of substrate concentration

The batch experiments at various initial benzene, toluene and phenol concentration ranging from $\gamma = 10$ to 400 mg L^{-1} were carried out under aerobic conditions using a *P. putida* microbial culture. The effects of single and mixed substrate concentration on the specific growth rate and degradation rate were studied. Fig. 1 and Fig. 2 show the specific growth rate and degradation rate of benzene, toluene and phenol for various initial concentrations of benzene, toluene and phenol using free cells of *P. putida.* During the batch experiments the pH was maintained at 7.0 ± 0.1 and the temperature was kept at 30 ± 0.1 °C. As shown in Fig. 1 and Fig. 2, both specific growth rate and degradation rate increase, with the increase in substrate concentration until it reaches to maximum value. However, after reaching a particular concentration,

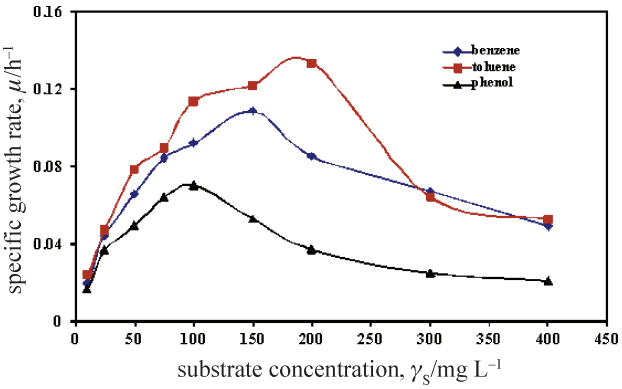
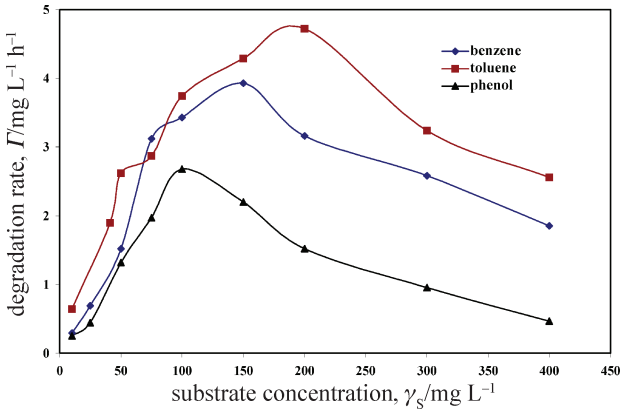


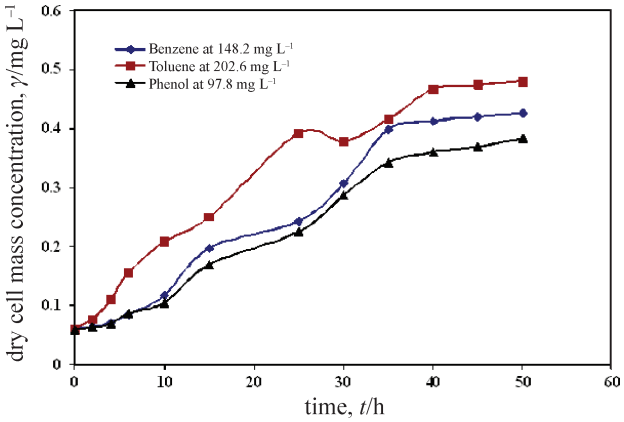
Fig. 1 – *Effects of substrate concentration on specific growth rate of benzene, toluene and phenol*



F i g . 2 – *Effects of substrate concentration on degradation rate of benzene, toluene and phenol*

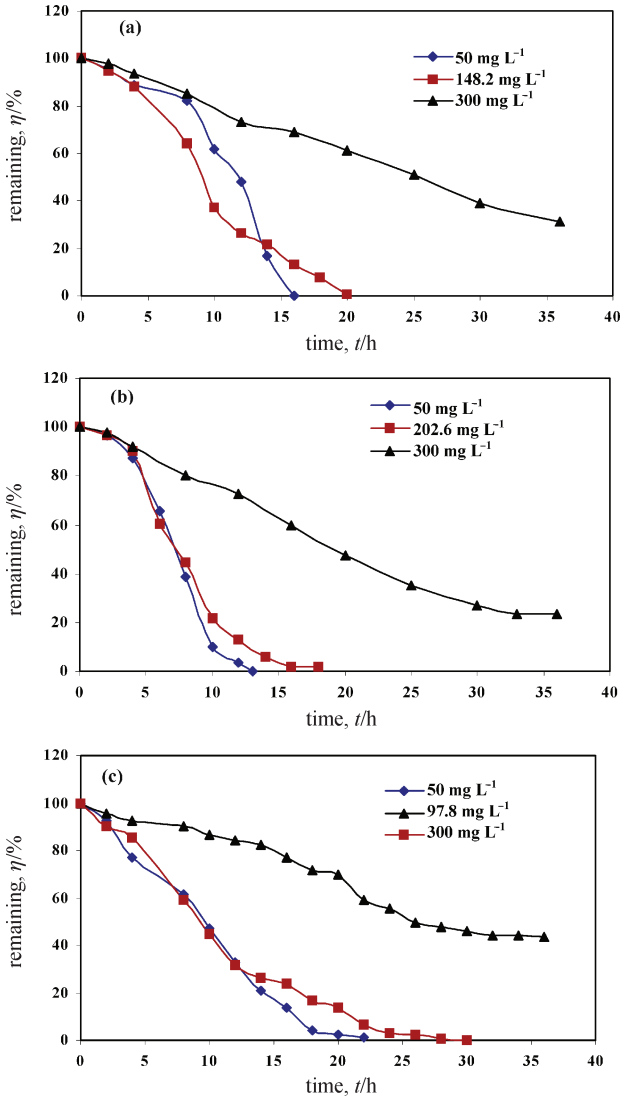
both the specific growth rate and degradation rate started to decline with the increase in substrate concentration, indicating substrate inhibition. The results showed that the effects were similar for both specific growth rate and degradation rate, but inhibition was more severe to specific growth rate than degradation rate. The degradation time of toluene was the lowest and the degradation rates of benzene and toluene were similar but the biodegradation of phenol was the lowest. It appeared that benzene and phenol were more toxic than toluene. The maximum specific growth rates of benzene, toluene and phenol were $\mu_{max} = 0.108$, 0.133, and 0.0705 h^{-1} at $\gamma = 148.2$, 202.6, and 97.8 mg L^{-1} initial substrate concentration, respectively. The substrate inhibition concentrations were observed. A further increase in substrate concentration decreased the degradation rate due to substrate inhibition. For phenol, both specific growth rate and degradation rate were lower than those of other substrates studied. Many researchers have also reported inhibition by benzene, toluene and phenol compounds at relatively low concentration and high mass concentration.[20,29–30] At high concentrations, it was observed that towards the end of the substrate consumption curve, there is a region of relatively less rate of substrate removal. Two possible explanations may be offered at this stage. One is the deficit in availability of oxygen as these experiments were done in serum bottles of $V = 500$ mL with 100 mL as working volume. These bottle experiments clearly showed that the culture was not able to degrade high concentration benzene, toluene and phenol compounds efficiently under hypoxic conditions in bottles. Morgan *et al.,* 1993[31] also reported that oxygen supply was the factor limiting benzene, toluene, ethyl benzene and xylenes (BTEX) biodegradation in ground water environments. After exponential phase, a drop in oxygen concentration was also advanced as possible reason for low growth rate by Yang and Humphrey, 1975.[32] The fall in pH of the solution may be another reason. The fall in the pH of the solution had previously been reported when the phenol was metabolized by mixed culture composed of *Pseudomonadaceae*, *Vibrionaceae*, etc. Low values of both oxygen and pH may affect the kinetics of substrate consumption adversely.[1]

Fig. 3 shows the growth profile of benzene, toluene and phenol compounds at their respective inhibition concentration by using *P. putida.* It is clear from the figure that the culture utilized benzene, toluene and phenol compounds as sole carbon source and was able to degrade them quite effectively. But the microorganism used toluene and benzene as sole carbon and energy source better than phenol.

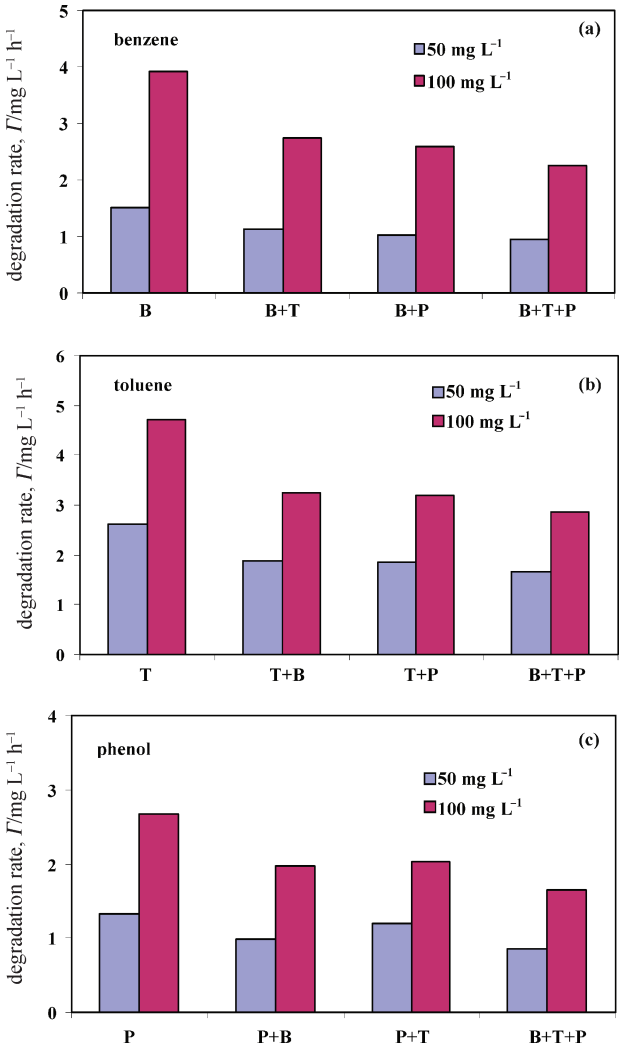


F i g . 3 – *Biodegradation and cell growth at inhibition concentrations of benzene, toluene and phenol*

Fig. 4 shows the degradation profile of the benzene, toluene and phenol compounds in terms of its percentage remaining with time at three significant concentration ranges. The profiles of degradation of three compounds were similar. According to the results shown in Fig. 4 (a–c), the degradation time at $\gamma = 50$ mg L^{-1} of toluene is less, followed by benzene and phenol. According to Fig. 4, the degradation time at $\gamma = 50$ mg L^{-1} of benzene, toluene and phenol compounds were 16, 13, and 22 h, respectively. Results show that the degradation times of these compounds were low at low substrate concentration, because of the degradation rate increased at low substrate concentration. It is clear that at the higher concentration of benzene, toluene and phenol compounds, the lower the degradation rate and increased the degradation time. This was also observed using mixed cultures for phenol degradation.[33–35] Other researchers using a pure culture of *P. putida*, Dapaals and Hill, 1992[36] observed that the length of lag phase increased exponentially with phenol concentration.



F i g . 4 – *(a) Degradation rate profile of benzene in terms of percentage remaining with time (b) degradation rate profile of toluene in terms of percentage remaining with time (c) degradation rate profile of phenol in terms of percentage remaining with time*



F i g . 5 – *Substrate interaction in binary and three-component mixture (a) effects of single substrate and mixture of toluene and phenol on benzene biodegradation; (b) effects of single substrate and mixture of benzene and phenol on toluene biodegradation; (c) effects of single substrate and mixture of benzene and toluene on phenol biodegradation. Substrate interaction shows at 50 mg L^{-1} and 100 mg L^{-1} of each component.*

Biodegradation kinetics of mixture

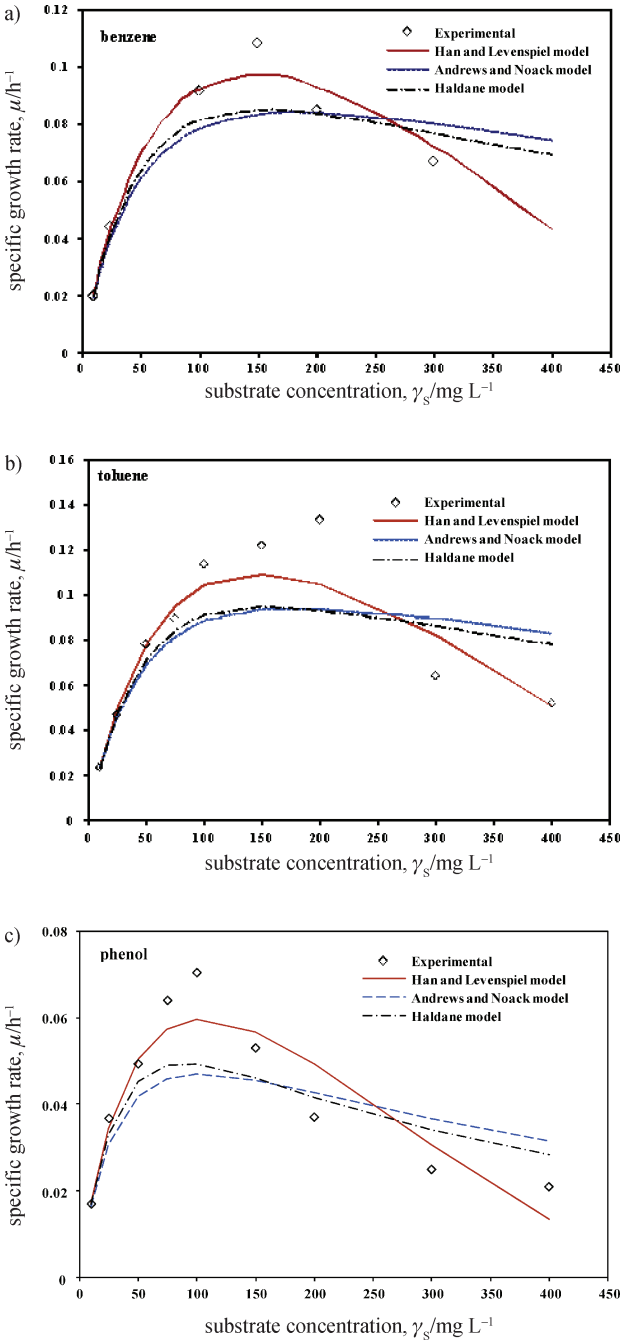
Substrate interaction was analyzed using binary or three component mixtures of benzene, toluene and phenol for two initial substrate concentrations, containing $\gamma = 50$ mg L^{-1} and 100 mg L^{-1} of each component. To understand the effect of the other pollutant on the microbial system, the growth kinetic parameters thus established were compared with those obtained from the experimental study involving single substrate degradation using microbial cultures enriched in the same pollutants. Fig. 5 shows the degradation rate with single, binary and three components of benzene, toluene and phenol compounds. For single substrate, the degradation rate of toluene was found 2.62 and 4.72 mg L^{-1} h^{-1} at concentration of $\gamma = 50$ mg L^{-1} and 100 mg L^{-1}, respectively. Fig. 5 shows that the degradation rate of toluene for single substrate is greater than benzene and phenol for same concentration. But after the addition of the benzene and phenol, the degradation rate decreased to 1.88 and 1.855 mg L^{-1} h^{-1} at concentration of $\gamma = 50$ mg L^{-1}. Similar results were obtained for 100 mg L^{-1} toluene concentration. In the three-component benzene, toluene and phenol mixtures, the toluene degradation rate decreased to 1.67 mg L^{-1} h^{-1} for 50 mg L^{-1} concentration, and also the degradation rate decreased from 4.72 to 2.87 mg L^{-1} h^{-1} for $\gamma = 100$ mg L^{-1} substrate concentration. This result was quite similar to the inhibitory effects of benzene on toluene by *Pseudomonas* sp. CFS251.[37] For benzene, the presence of toluene decreased its degradation rate by 26 % from 1.52 to 1.12 mg L^{-1} h^{-1} and for pres-

ence of phenol decreased by 32 % from 1.52 to 1.03 mg L^{-1} h^{-1} for 50 mg L^{-1} substrate concentration. The biodegradation times of benzene, toluene and phenol as a mixture were longer than that when benzene, toluene and phenol were present alone especially at high concentrations. However, the effects of inhibition on benzene to toluene and toluene to benzene are quite different.[37–39] The degradation of phenol was also inhibited by the supplemented toluene and benzene, and the inhibition effect of benzene was stronger (Fig. 5c). The results show that the phenol inhibition is more for benzene and toluene. For mixtures of binary and three components, a few researchers reported that the presence of phenol increased the degradation times of benzene or toluene and the presence of benzene or toluene decreased the biodegradation times of phenol. Degradation of phenol starts when benzene or toluene consumed completely.[20–21]

Growth kinetic model for single substrate

In order to predict the microbial kinetics at substrate versatility conditions, an attempt was made to fit the kinetic rate data to appropriate kinetic models. Having experimentally observed substrate inhibition, kinetic data were fitted with the most widely accepted Haldane (1965), Andrews and Noack (1968), and Han and Levenspiel (1988) inhibition models. Equations of the models are given in the Table 1. Values of K_s indicate the ability of microbes to grow at low substrate levels and K_I values indicate the sensitiveness of the culture to substrate inhibition. The higher K_I value physically means that the culture is less sensitive to substrate inhibition and vice versa.

The experimental specific growth rate data were plotted in Fig. 6 (a–c) against initial concentration of benzene, toluene and phenol to show the variation in the experimental specific growth rate against initial substrate concentration. Here a typical trend has been observed. Specific growth rate increases with the increase in initial benzene, toluene or phenol concentration up to a certain concentration level, and then it starts decreasing with the increase in concentration. This suggests that benzene, toluene and phenol are inhibitory type of substrates. In general, Haldane's growth kinetics model is used to represent growth kinetics data of an inhibitory compound. This model has been used on the premise that it has less parameters and tends to be used easily in model equations representing continuous biological reactors. However, the estimation of these three parameters requires the use of a non-linear regression technique. The estimation of parameters in Monod's and linearized Haldane's models being two parameter models is easy. Although these values may not be the correct values, they provide us with some estimates of parameters that are to be used as guess values of parameters for Haldane's growth kinetics model during estimation. Therefore, specific growth rate data from low-concentration region were fitted to Monod's model and those from high-concentration region were fitted to linearized-Haldane's model.[1] Table 2 presents the values of parameters of these models. It has been found that these models cannot represent the data over an entire concentration range.



F i g . 6 – *Experimental and model predicted profiles of specific growth rate for (a) benzene (b) toluene (c) phenol*

Table 2 – *Growth kinetics parameter values of Monod's and linearized-Haldane's model for biodegradation of benzene, toluene and phenol*

Substrate	Monod's model		Linearized-Haldane's model	
	μ_{max}/h^{-1}	K_s/mg L^{-1}	μ_{max}/h^{-1}	K_I/mg L^{-1}
benzene	0.1631	71.18	0.3626	64.12
toluene	0.1722	62.56	0.3003	57.40
phenol	0.1093	53.18	0.3562	24.14

It is seen that Han and Levenspiel gives the best fit for benzene, toluene and phenol. Haldane, and Andrews and Noack models gave almost similar values as the equation was similar (Table 1). Since $K_s\ \gamma_S/K_I$, the extra factor in the denominator in Andrews and Noack model is not large compared to the other factors, the two models yield similar results. The Han and Levenspiel model, gives a better fit especially at higher levels of substrate due to the following reasons. At low substrate concentrations, the second term in the denominator of Han and Levenspiel model, i.e. $[K_s\ (1-\gamma_S/K_I)]$, has a significant effect as the factor $(1-\gamma_S/K_I)$ is appreciable. However, as the substrate concentration increases, this factor keeps decreasing and at high substrate concentrations $[\gamma_S/[\gamma_S + K_s(1-\gamma_S/K_I)^m]$ tends toward 1. Hence, it declines as a function of $(1-\gamma_S/K_I)^m$. The Han and Levenspiel model gives a better fit. From the comparison of the various correlation coefficients, it was clear that the Han and Levenspiel model fitted the systems best. The results from Han and Levenspiel models were hence found to be more illustrative of the experimental behavior for benzene, toluene or phenol concentration degradation studies. Similar results also found in the literature for the degradation of phenol.[40] The corresponding μ_{max}, K_s, K_I and r^2 correlation coefficient values of the various models have also been listed out in Table 3.

Error analysis

In this study, four non-linear error functions were examined and in each case a set of parameters were determined by minimizing the respective error function across the concentration range studied. The error functions employed were presented in Table 4.

Table 4 – *Equation used for error analysis*

Model	Equation
Sum of the squares of the errors (SSE)	$SSE = \sum_{i=1}^{N} (\mu_{cal} - \mu_{exp})_i^2$
Average relative error (ARE)	$ARE = \frac{100}{N} \sum_{i=1}^{N} \left\| \frac{(\mu_{cal} - \mu_{exp})}{\mu_{exp}} \right\|_i$
Hybrid fractional error function (HYBRID)	$HYBRID = \frac{100}{N-p} \sum_{i=1}^{N} \left[\frac{(\mu_{exp} - \mu_{cal})}{\mu_{exp}} \right]_i$
Marquardt's percent standard deviation (MPSD)	$MPSD = 100 \sqrt{\frac{100}{N-p} \sum_{i=1}^{N} \left[\frac{(\mu_{exp} - \mu_{cal})}{\mu_{exp}} \right]_i^2}$

Choosing best isotherm model

Since each of the error functions produces a different set of parameters, an overall optimum parameter set is difficult to identify directly. Thus, a normalization of each parameter is employed in order to have a better comparison between the parameter sets for the single model.[41] In the normalization

Table 3 – *Biodegradation kinetic parameters evaluated from substrate inhibition models*

Substrate	μ_{max}/h^{-1}	K_s/mg L^{-1}	Model	K_I/mg L^{-1}	n	m	r^2
			Haldane	340.15	–*	–	0.8978
benzene	0.1631	71.18	Andrews and Noack	468.9	–	–	0.841
			Han and Levenspiel	519.21	0.8963	2.0533	0.9775
			Haldane	380.62	–	–	0.8372
toluene	0.1722	62.56	Andrews and Noack	511.32	–	–	0.7894
			Han and Levenspiel	514.07	0.8023	2.1823	0.9455
			Haldane	148.65	–	–	0.9101
phenol	0.1093	53.18	Andrews and Noack	195.54	–	–	0.8965
			Han and Levenspiel	531.62	1.489	2.1758	0.9248

–*, not applicable

Table 5 – *Values of four different error analyses of models for biodegradation of benzene, toluene and phenol*

Substrate	SSE	ARE	HYBRID	MPSD	Model
	0.001241	11.239	$-8.4 \cdot 10^{-8}$	18.86	Haldane
benzene	0.00178	15.53	$-5.44 \cdot 10^{-8}$	18.864	Andrews and Noack
	0.00187	2.9771	$-1.26 \cdot 10^{-10}$	6.45	Han and Levenspiel
	0.00399	15.398	$-9.01 \cdot 10^{-10}$	28.38	Haldane
toluene	0.0047	19.381	$-8.9 \cdot 10^{-7}$	24.58	Andrews and Noack
	0.00122	10.2798	$-2.67 \cdot 10^{-10}$	15.33	Han and Levenspiel
	0.00089	15.669	$-2.97 \cdot 10^{-7}$	25.622	Haldane
phenol	0.00128	21.302	$-7.5 \cdot 10^{-7}$	20.711	Andrews and Noack
	0.000205	17.382	$2.49 \cdot 10^{-14}$	24.667	Han and Levenspiel

processes, each error function was selected in turn and the results for each parameter set were determined. Secondly, the errors determined for a given error function were divided by the maximum to obtain the normalized errors for each parameter set. Lastly, the normalized errors for each parameter set were summed up. The value of all four error functions is presented in Table 5. By comparing the results of the values for the error functions, contradictory 'best-fit' results for toluene are obtained. r^2 factor is based on the square of the difference between theoretical and experimental data points.

Conclusions

The following conclusions are drawn from the results presented in this study:

1. The kinetics studies of benzene, toluene and phenol biodegradation by using the pure culture *P. putida* appeared to be effective in the batch reactor with high substrate concentrations under aerobic condition.

2. The batch experiments were conducted to investigate the interaction between benzene, toluene and phenol for single and mixed components. The biodegradation times of benzene, toluene and phenol as a mixture were longer than that when benzene, toluene and phenol were present alone especially at high concentrations. The effects of inhibition on benzene to toluene and toluene to benzene are quite different. The degradation of phenol was also inhibited by the supplemented toluene and benzene, and the inhibition effect of benzene was stronger. Toluene and benzene were better substrate than phenol, resulting in faster growth.

3. In most of the cases, the Han and Levenspiel model gives a better fit with experimental data for benzene, toluene and phenol. It was found that the Han and Levenspiel model is best suited to the system.

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List of symbols

K_I – inhibition constant, mg L^{-1}
K_S – half-saturation constant, mg L^{-1}
m, n – exponent
N – number of data points
p – number of parameters
r^2 – correlation coefficient
t – time, h
V – volume, μL, mL, L
γ – mass concentration, mg L^{-1}
γ_S – substrate concentration, mg L^{-1}
Γ – degradation rate, mg L^{-1} h^{-1}
η – remaining degree, %
ϑ – temperature, °C
λ – wave length, nm
μ – specific growth rate, h^{-1}
μ_{cal} – specific growth rate, h^{-1} (calculated value from models)
μ_{exp} – specific growth rate, h^{-1} (experimental value)
μ_{max} – maximum specific growth rate, h^{-1}

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