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Biodegradation of phenol and cyanide by *Pseudomonas putida* MTCC 1194: an experimental and modeling study

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ABSTRACT

In this study, the biodegradation of phenol and cyanide from aqueous solution by *Pseudomonas putida* MTCC 1194 was carried out. The effect of initial concentration of phenol and cyanide on the microbial growth was estimated in the range of initial concentration 100–1,500 mg/L of phenol and 10–150 mg/L of cyanide. A maximum experimental specific growth rate was observed 0.0958 h^{-1} at 250 mg/L of phenol and 0.1590 h^{-1} at 25 mg/L of cyanide concentration in the growth medium. Specific growth rate was observed to follow substrate inhibition kinetics. Non-linear regression analysis technique with error function Marquardt's percent standard deviation was used to predict the model parameters. Seven growth kinetic models for the mono substrate system were calculated and corresponding interaction parameters were predicted. The experimental data was found well fitted with Haldane, Edward, Yano and Koga, Luong and Han and Levenspiel models as compared to Monod and Powell model. For binary substrate system, sum kinetics with interaction parameters model was used. Three-half-order degradation kinetic model was found to be fit, well with experimental data. This study presented that *P. putida* MTCC 1194 was very effective for the removal of phenol and cyanide from aqueous solutions.

Keywords: Biodegradation; Cyanide; Degradation kinetics; Growth kinetics; Phenol

1. Introduction

Phenol and cyanide, from the release of a wide variety of industrial and anthropogenic activities, frequently co-exist in polluted environments [1,2]. Cyanide present in the environment in different forms, containing HCN, salts (such as KCN or NaCN) and cyanide complexes such as $Zn(CN)_2$ and potassium ferricyanide, whereas cresol, xylenol, alkyl phenols and resorcinol are the derivatives of phenol [3,4]. Strict environmental regulations are applied to waters containing phenol and cyanide. In general, these wastewaters can be discharged into the environment only after decontamination [2,5,6]. Phenol and cyanide are found in the effluents generated from many industries which comprises steel plants, coal conversion process plants, phenolic resin, oil refineries, paper and pulp, fertilizers, plastics, petrochemical, pesticides, explosive production and rubber industries. The effluent concentration ranges of phenol and cyanide from a few industries have been described by Babich and Davis [7], Dash et al. [2], Gonzalez-Munoz et al. [8], Kira et al. [9]. Once the phenol and cyanide compounds existing in industrial effluents, are released

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into the environment, they harmfully affect human and aquatic life. According to US Environmental Protection Agency (USEPA) phenol and cyanide has been categorized as possible human carcinogens and registered it as a pollutant [10]. USEPA the disposal standards in Mexico and the Minimum National Standards (MINAS) of the Central Pollution Control Board (CPCB) in India has set a limit level of 0.2 mg/L of cyanide and 0.5 mg/L of phenol to control the concentration of these compounds in drinking water to protect the possible harmful effects on human health [1,11]. Many treatment technologies have been considered for the removal of phenol and cyanide from contaminated wastewater to decrease the toxicity of wastewater. In the biotreatment technique reports on the biotreatment of phenol and cyanide by microbes are available in the literature [2,12–19]. When biotreatment of a mono substrate of a toxic compound take place, the growth inhibition of the biomass occurs owing to the high toxicity of substrate up to a certain initial concentration. This substrate inhibition affects the biomass growth rate and biomass yield.

Generally, the effluents generated from industries contains a mixture of pollutants in effluent generates difficulties for their biotreatment due to different operating conditions such as temperature and pH. Further, the biotreatment of mono substrate may be inhibited by the occurrence of additional substrate in the wastewater. The interaction of multiple substrates is difficult owing to their competition for bacterial enzymes and toxicity [20]. So, it is essential to study the mode of interaction of substrates in multi substrate system and its influence on their bioremoval. In multi substrate system the biodegradation rate remains slow, due to toxicity of substrate, competitive inhibition and the development of toxic intermediates [17]. Different types of substrate interaction methods comprising non-competitive and competitive inhibition have been detected in various multi substrate biotreatment system [21–23]. Phenol and cyanide are harmful to growing cells, therefore they stop the growth of cells at higher concentrations and inhibiting their metabolism. The information on biotreatment dynamics is important to design the biotreatment unit and to calculate the concentration effect of a component throughout its elimination by using biotreatment technology.

The current study presents the growth kinetics and phenol and cyanide degradation in batch process using a microbial culture namely *Pseudomonas putida* MTCC 1194. Moreover, appropriate substrate inhibition models were applied to the experimental data to evaluate the bio-kinetic constants. The results presented in this study provide knowledge of the degradation and growth kinetics of phenol and cyanide.

2. Materials and methods

2.1. Chemicals and reagents

Aqueous phenol and cyanide solutions were prepared by diluting phenol and sodium cyanide (NaCN) in distilled water. All chemicals used in this study were of an analytical grade (AR) and purchased from Himedia Laboratories Pvt. Ltd. Mumbai, India.

2.2. Microorganism and culture medium

The microorganism used for biodegradation was *P. putida* MTCC 1194 obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The culture used for phenol and cyanide degradation is essential to be acclimatized to the phenol and cyanide environment. The growth medium was arranged in two parts to escape precipitation of salts during autoclaving. The first part of growth medium contained K_2HPO_4 (1 g/L), KH_2PO_4 (1 g/L), $FeSO_4{\cdot}7H_2O$ (0.01 g/L), and the second part of growth medium contained NH_4NO_3 (3 g/L), KCl (0.5 g/L), $MgSO_4{\cdot}7H_2O$ (0.5 g/L), glucose (20 g/L). These parts of medium were mixed at room temperature and pH of 7.1 ± 0.1. For acclimatization, 2% glucose was added as a carbon source for the growth of *P. putida* MTCC 1194 into the growth medium. The concentration of glucose was decreased and content of phenol and cyanide was increased slowly in the growth medium. The *P. putida* MTCC 1194 was acclimatized in phenol and cyanide environment up to a concentration 1,500 mg/L of phenol and 150 mg/L of cyanide at pH 8 and temperature 30°C. The complete acclimatization phase of the *P. putida* MTCC 1194 to degrade phenol and cyanide is revealed in Fig. 1. Therefore, the inoculum was established for current biodegradation studies. The growth curve of *P. putida* MTCC 1194 was revealed in Fig. 2. When microorganisms are developed using two

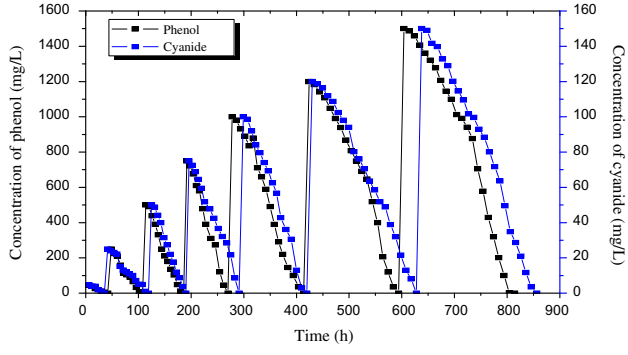

Fig. 1. Phenol and cyanide degradation profile followed throughout the acclimatization period.

compatible substrates, e.g. two carbon sources, they have a tendency to exhaust specially the one that tolerates a maximum growth rate which is reliable with this study where microorganism first utilize glucose and then phenol and cyanide. In addition, glucose was completely utilized by microorganism within 20 h, as a carbon source after that microorganism start to utilize the phenol and cyanide. This phenomenon was known as diauxic behavior shown in Fig. 2.

2.3. Batch biodegradation experimental design

Batch biodegradation experiments by using the acclimatized culture were achieved in 250 mL round flask comprising 100 mL of working solution. The batch study was carried out with initial concentrations of substrates up to phenol and cyanide concentration 1,500 mg/L and 150 mg/L, respectively. Flask at varying initial concentration were incubated at 30˚C with agitation speed 120 rpm in an incubator-cum-orbital shaker. The mixture ratio of phenol and cyanide were as 50:5, 100:10, 250:25, 500:50, 750:75, 1,000:100, 1,200:120, and 1,500:150. The samples were taken out at a fixed time interval, centrifuged at 10,000 rpm for estimation of remaining pollutant concentration. To obtain the optimum pH for the phenol and cyanide biodegradation, the pH of the growth medium comprising 1,500 mg/L of phenol with 150 of mg/L cyanide was adjusted to 5, 6, 7, 8, 9, and 10 with 0.1 N NaOH and 0.1 N HCI. To achieve the optimum temperature for the phenol and cyanide biodegradation, the temperature of the growth medium comprising 1,500 mg/L of phenol with 150 mg/L of cyanide was adjusted to 15, 20, 25, 30, 35, 40, and 45˚C. All batch experiments were conducted in triplicate form under the same conditions and average results are reported.

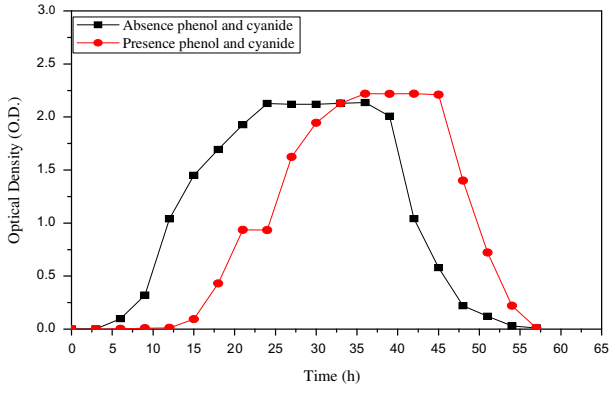

Fig. 2. Growth curve *P. putida* MTCC 1194 in the absence and presence of phenol and cyanide.

To escape the volatilization losses throughout the experiments, flasks were covered with rubber stoppers.

The degradation efficiency (%) was calculated from Eq. (1):

$$\text{Degradation efficiency}\,(\%) = \frac{(C_0 - C_f)}{C_0} \times 100 \quad (1)$$

where C_0 and C_f are the initial and final concentration of substrate (mg/L).

2.4. Estimation of substrate and biomass concentration

To measure the substrate and biomass concentration, the sample of 2 mL volume was centrifuged at 10,000 rpm for 10 min at 30˚C. Supernatant was collected for the analysis of phenol and cyanide concentrations by 4-aminoantipyrine and colorimetric picric acid [24], respectively, using an UV/visible spectrophotometer (Lambada 35; PerkinElmer, MA 02451, USA). Oven dry methods were used for analysis of biomass growth. Cells were pelleted via centrifugation at 30˚C temperature and 120 rpm and taken out in a petri plate and then oven dried at 80˚C for 24 h. After oven dry, the calibration curve for biomass concentration (1–1,000 mg/L) was obtained by plotting the optical density (OD) vs. biomass concentration. The batch experiments were repeated three times and average results were used.

2.5. Modeling of the growth and biodegradation kinetics

In this study, the non linear form of growth and biodegradation kinetic models was fitted by non linear least squares methods using Ms office 2012 and summarized in Table 1.

The specific growth rate of *P. putida* MTCC 1194 at different initial concentration of phenol and cyanide were designed by the subsequent equation:

$$\mu_s = \ln(x_2 - x_1)/(t_2 - t_1) \quad (2)$$

where x is the biomass concentration (mg/L), at time t (h), and μ_s is the specific growth rate (h^{-1}) [25].

Further, the specific substrate degradation rate q, (h^{-1}) was determined by the subsequent equation:

$$q = -\ln(x_2 - x_1)/(S_2 - S_1) \quad (3)$$

where x and S are the biomass and substrate concentrations, respectively in mg/L at time t(h) [26].

Table 1
List of growth and degradation kinetic models

Growth kinetic model	Mathematical expression	Refs.
Monod	$\mu_s = \mu_{max} S/K_s + S$	Monod [27]
Haldane	$\mu_s = \mu_{max} S/K_s + S + (S^2/K_i)$	Haldane [28]
Edward	$\mu_s = \mu_{max}(S/S + K_s + (S^2/K_i)(1 + S/K))$	Edward [29]
Yano and Koga	$\mu_s = \mu_{max} S/\left[S + K_S + \frac{S^2}{K_i}\left(1 + \frac{S}{K_i}\right)\right]$	Yano and Koga model [30]
Luong	$\mu_s = \frac{\mu_{max} S}{K_s + S}\left[1 - \frac{S}{S_m}\right]^n$	Luong [31]
Han and Levenspiel	$q_s = q_{max} S[1 - (S/S_m)]^n/K_s + S - [1 - (S/S_m)]^m$	Han and Levenspiel [32]
Powell	$\mu_s = \frac{(\mu_{max} + m) \times S}{K_S + S} - m$	Powell [33]
Degradation kinetic models		
Zero order model	$S = S_0 - k_0 t$	Schmidt et al. [34] Brunner and Focht [35]
First Order Model	$S = S_0 \exp(-k_1 t)$	Schmidt et al. [34] Brunner and Focht [35]
Second order Model	$K_s \ln \frac{S}{S_0} + S - S_0 = -k_2 t$	Schmidt et al. [34]
	$k_2 = \mu_{max} X_0$	Brunner and Focht [35]
Three-half-order kinetic Model	$Y = -k_{31} - \frac{k_{32} t}{2} = \frac{1}{t} \ln [S_0 - P + k_0 t S_0]$	Schmidt et al. [34]
	$P = S_0 - S + K_0 t$	Brunner and Focht [35]

The growth kinetic models for the mono substrate system were used as: Monod, Haldane, Edward, Yano and Koga, Han and Levenspiel, Powell and Loung [27–33] and biodegradation kinetic models as: zero order, pseudo-first-order, pseudo-second-order and three-half-order equations well defined in Schmidt et al. [34], Brunner and Focht [35] and Costa et al. [36].

In binary substrate system, the interaction parameters were observed by the sum kinetics with interaction parameters (SKIP) model showed by the following equation:

$$\mu_s = \frac{\mu_{max,1S} S_1}{K_{s1} + S_1 + \frac{S_1^2}{K_1} + I_{2,1} S_2} + \frac{\mu_{max,2S} S_2}{K_{s2} + S_2 + \frac{S_2^2}{K_2} + I_{1,2} S_1} \quad (4)$$

where interaction parameter $I_{2,1}$ indicates how substrate 2 affects the biodegradation of substrate 1 and vice versa [37] and the subscript 1 signified phenol and 2 signified cyanide. The values of kinetic parameters (μ_{max}, K_{si}, K_i) were evaluated from the mono substrate system kinetics.

2.6. Model validation

In this study Marquardt's percent standard deviation (MPSD) was used for model validation. The MPSD can be calculated by the following equation:

$$\text{MPSD} = 100 \sqrt{\frac{1}{N - p} \sum_{i=1}^{p} \left(\frac{Q_{e,i}^{exp} - Q_{e,i}^{cal}}{Q_{e,i}^{exp}} \right)^2} \quad (5)$$

3. Results and discussions

To investigate the growth kinetics and degradation kinetics of *P. putida* MTCC 1194 in the occurrence of the phenol and cyanide, experiments were completed in batch mode. This biodegradation study led to the removal of the phenol and cyanide. Kinetics of growth and degradation process were also modeled. The non-linear form of growth and degradation kinetics models were revealed in Table 1.

3.1. Effect of pH and temperature

To examine the effect of pH, the biodegradation experiments were conducted at varying initial pH values, from 5 to 10 at temperature 30°C and the initial concentration at 1,500 mg/L of phenol and 150 mg/L of cyanide (Fig. 3a). Mostly, microorganisms cannot survive in pH range above 9.5 or below 4.0 [37]. The samples were collected at the end of 60 h of incubation. The high basic and high acidic environment, enhance the toxicity [38,39]. From Fig. 3a, it was

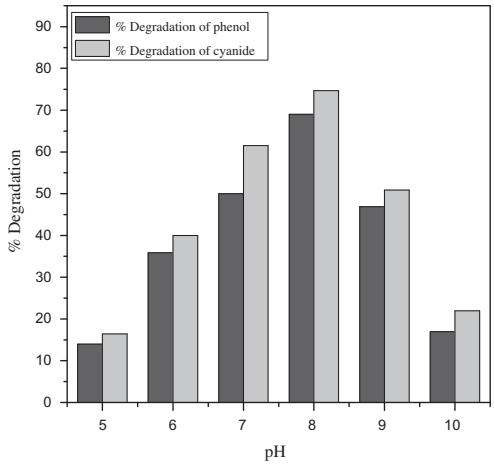

Fig. 3a. Effect of initial pH on phenol and cyanide degradation by *P. putida* MTCC 1194 at 1,500 mg/L of phenol and 150 mg/L of cyanide (Temp: 30˚C; incubation period: 60 h).

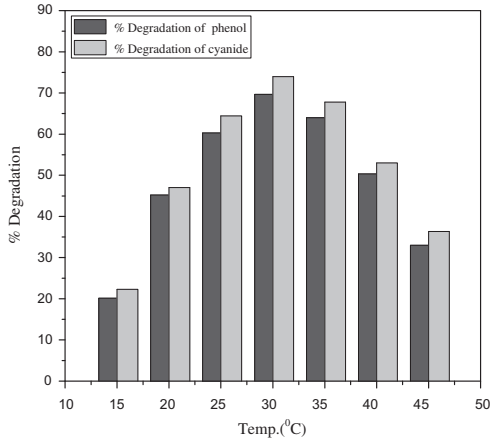

Fig. 3b. Effect of initial temperature on phenol and cyanide degradation by *P. putida* MTCC 1194 at 1,500 mg/L of phenol and 150 mg/L of cyanide (pH 8; incubation period: 60 h).

observed that the phenol and cyanide degradation rate becomes inhibited at high and low pH. Kulkarni and Chaudhari [39] and Kotresha and Vidyasagar [40] obtained an alkaline pH range for biodegradation of phenol. The maximum phenol and cyanide biodegradation by *P. putida* MTCC 1194 occurred at pH 8. Therefore, the pH of growth medium was set to be 8 for further biodegradation studies. To observe the effect of temperature, the biodegradation experiments were conducted at varying initial temperature values, from 15 to 45˚C at pH 8 and the initial concentration at 1,500 mg/L of phenol and 150 mg/L of cyanide (Fig. 3b). The percentage removal of phenol and cyanide by biodegradation mostly depends on the optimum growth condition of the microbes. The optimum temperature for removal of phenol and cyanide was the result of a balance between the stability of the complex and the optimum temperature for microbial growth [41]. Since at industrial level, narrow temperature ranges are preferred for pollution abatement, temperature of 30˚C was taken as the optimal temperature for all biodegradation studies.

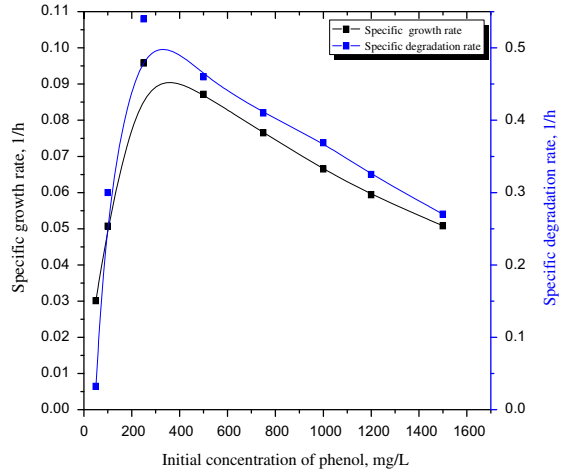

Fig. 4a. Comparison of specific growth and specific degradation rates at different initial phenol concentrations.

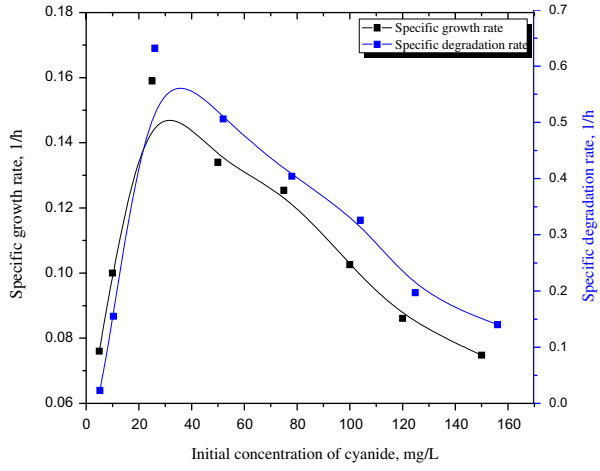

Fig. 4b. Comparison of specific growth and specific degradation rates at different initial cyanide concentrations.

3.2. *Effect of phenol and cyanide on the bacterial growth*

The specific growth rate and specific substrate degradation rate is calculated by using Eqs. (2)–(3), respectively. The experimental data of specific growth

rate and specific substrate degradation rate are plotted against the initial concentration of phenol and cyanide in Figs. 4a and 4b, respectively. The figure indicates a tendency that the value of both specific growth rate and specific substrate degradation rate increase with increased initial concentration of phenol and cyanide up to a specific value and finally decrease [14]. The maximum specific growth rate for phenol is found to be equal to 0.0958 h^{-1} at 250 mg/L. However, for cyanide the maximum specific growth rate is equal to 0.1590 h^{-1} at 25 mg/L. The maximum specific degradation rate is found to be 0.5412 h^{-1} for phenol at 250 mg/L and 0.6321 h^{-1} for cyanide at 25 mg/L. The declining trend beyond 250 mg/L of phenol and 25 mg/L of cyanide indicated that both substrates are inhibitory type substrates and the inhibition effect becomes predominant above this initial concentration [14,42].

3.3. Modeling of the growth kinetics

Numerous mono substrate growth kinetic models were obtained to characterize the experimental growth kinetics. In this study, seven models were selected and listed in Table 1 [27–33]. The parameters of growth kinetic models were achieved by observing the growth rate of biomass with time at initial concentrations of phenol (100–1,500 mg/L) and cyanide (10–150 mg/L) from batch experiments. The predicted value of model parameters and error function MPSD is also revealed in Tables 2a and 2b. The error function was used to measure the goodness of the fit of experimental data. Figs. 5a and 5b shows the comparative results of all seven growth kinetic models with experimental data. It is clear from Figs. 5a and 5b and Tables 2a and 2b that experimental values show Haldane, Edward, Yano and Koga, Luong and Han and Levenspiel are more appropriate models for both phenol and cyanide. The MPSD value indicates that Monod and Powell models do not fit properly with experimental data. From Table 2, it is evident that the μ_{max} obtained from the model is nearer to the μ_{max} obtained from experiments for Haldane, Edward, Yano and Koga, Luong and Han and Levenspiel models than for Monod and Powell models, which verifies that the inhibition models describe better kinetics in the presence of phenol and cyanide. The substrate inhibition constant (K_i) value predicted by Edward, Haldane and Yano-Koga models, above this value specific growth decline, and closely agreed with the experimentally found value of 250 mg/L of phenol and 50 mg/L of cyanide. Han and Levenspiel and Luong models predicted critical substrate concentration (S_m) value, at which critical growth rate fall to zero, at ~1,500 mg/L of phenol and 150 mg/L of cyanide, but this value was detected to be different from that achieved in the experiments [43]. In the binary substrate system, biodegradation of phenol and cyanide has been carried out at 10:1 ratio as follows; 50 mg/L phenol-5 mg/L cyanide, 100 mg/L phenol-10 mg/L cyanide, 250 mg/L phenol-25 mg/L cyanide, 500 mg/L phenol-50 mg/L cyanide, 750 mg/L phenol-75 mg/L cyanide, 1,000 mg/L phenol-100 mg/L cyanide, 1,200 mg/L phenol-120 mg/L cyanide, and 1,500 mg/L phenol-150 mg/L cyanide. The predicted values of interaction parameters $I_{2,1}$ and $I_{1,2}$ ($I_{2,1}$ = 0.13, $I_{1,2}$ = 23.98) indicate that the phenol inhibits cyanide degradation more than cyanide inhibiting the degradation of phenol [28]. The specific growth rate of *P. putida* MTCC 1194 in the binary component system can be stated as:

$$\mu_s = \frac{0.0416\,S_1}{50.09 + S_1 + \frac{S_1^2}{216.59} + 0.13\,S_2} + \frac{0.3569\,S_2}{0.97 + S_2 + \frac{S_2^2}{42.39} + 23.98\,S_1} \quad (6)$$

Table 2a
Model parameters for specific growth rate kinetic models during biodegradation of phenol by *P. putida* MTCC 1194

Model	Model parameters							
	μ_{max} (h^{-1})	K_s (mg/L)	K_i (mg/L)	K (mg/L)	S_m (mg/L)	n	m	MPSD
Monod	0.0687	50.0930	–	–	–	–	–	22.4850
Haldane	0.0416	640.0477	216.59	–	–	–	–	3.6966
Edward	0.4324	671.7012	215.11	27,021	–	–	–	3.9348
Yano and Koga	0.1875	252.7257	222.70	–	–	–	–	5.7371
Luong	0.2069	267.1838	–	–	20,000.00	1,335.10	–	6.4313
Han and Levenspiel	0.2158	283.0158	–	–	314,625.00	278.95	0.0102	6.4065
Powell	0.0679	0.0022	–	–	–	–	808.87	20.5820

Table 2b
Model parameters for specific growth rate kinetic models during biodegradation of cyanide by *P. putida* MTCC 1194

Model	Model parameters							
	μ_{max} (h^{-1})	K_s (mg/L)	K_i (mg/L)	K (mg/L)	S_m (mg/L)	n	m	MPSD
Monod	0.0999	0.9671	–	–	–	–	–	22.9970
Haldane	0.3569	19.8100	42.39	–	–	–	–	5.9499
Edward	0.3562	19.8040	42.41	870,377.00	–	–	–	5.9494
Yano and Koga	0.2408	11.4130	39.51	–	–	–	–	5.3791
Luong	0.2582	12.0040	–	–	317,667.00	25.39	–	5.5565
Han and Levenspiel	0.2576	12.9620	–	–	16,630.00	1.32	3.9987	5.5621
Powell	0.1003	0.0018	–	–	–	–	50.3060	22.8670

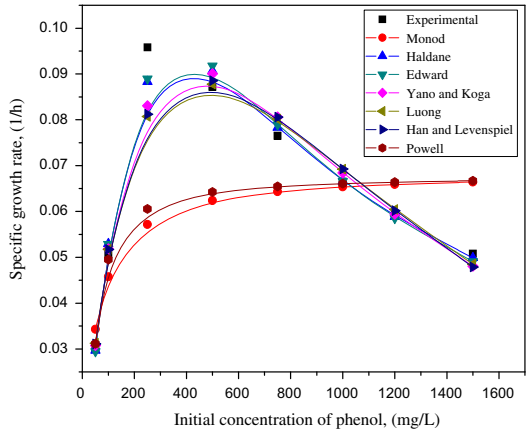

Fig. 5a. Comparison of experimental and predicted specific growth rate of the microbial at different initial concentration of phenol.

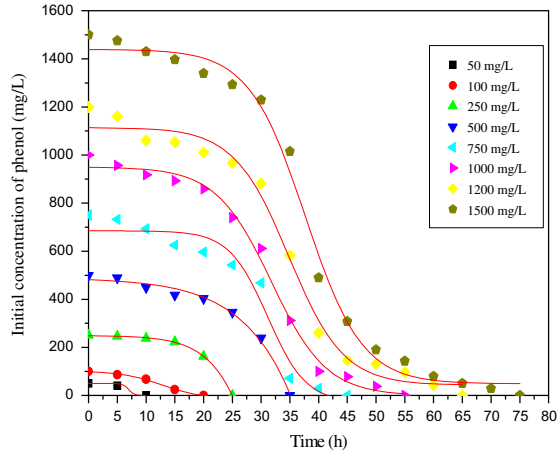

Fig. 6a. Time profile of phenol degradation by *P. putida* MTCC 1194.

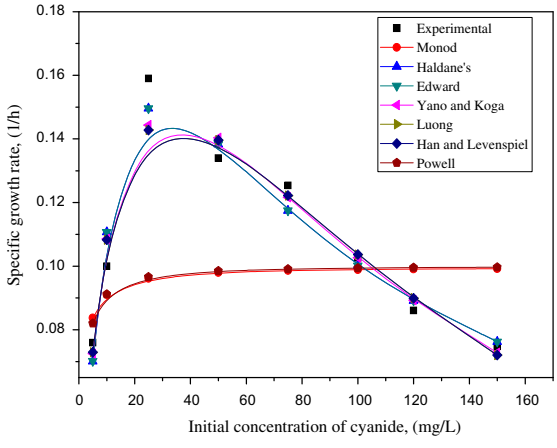

Fig. 5b. Comparison of experimental and predicted specific growth rate of the microbial at different initial concentration of cyanide.

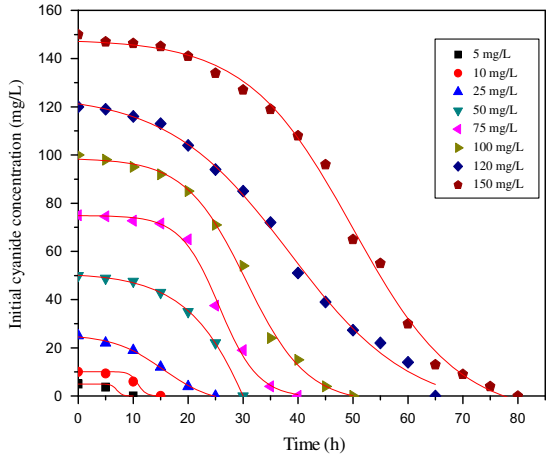

Fig. 6b. Time profile of cyanide degradation by *P. putida* MTCC 1194.

Table 3a
Parameter list of zero-order, pseudo-first, pseudo-second and three-half-order kinetic models for phenol

Initial concentration (mg/L)	Zero-order model			Pseudo-first-order model		pseudo-second-order model		Three-half-order model		
Phenol	S_0 (g/L)	K_0 (g/L h)	MPSD	K_1 (h)	MPSD	K_2 (g/(mg h))	MPSD	K_{31} (h^{-1})	K_{32} (h^{-2})	MPSD
50	50.01	1.9357	1.7×10^{-6}	0.0430	2.1×10^{-5}	0.0200	70.71	0.001	0.0172	2.1×10^{-6}
100	112.14	5.7260	11.57	0.0898	21.07	0.0003	74.49	0.000	0.0130	10.43
250	267.62	4.5805	7.50	0.0200	8.32	0.0015	51.02	0.000	0.0023	5.50
500	541.09	9.1705	7.88	0.0220	9.98	0.0002	54.92	0.010	0.0012	6.26
750	791.41	19.2019	35.26	0.0740	53.53	1.6×10^{-6}	88.41	0.000	0.0039	34.47
1,000	801.71	15.5236	40.29	0.0532	51.02	1.9×10^{-6}	85.76	0.000	0.0023	32.16
1,200	893.47	14.3586	36.91	0.0427	48.78	3.6×10^{-6}	82.40	0.000	0.0016	29.45
1,500	1,142.33	17.1632	37.37	0.0431	56.83	4.7×10^{-7}	96.68	0.000	0.0014	33.15

Table 3b
Parameter list of zero-order, pseudo-first, pseudo-second and three-half-order kinetic models for cyanide

Initial concentration (mg/L)	Zero-order model			Pseudo-first order model		Pseudo-second order model		Three-half-order model		
Cyanide	S_0 (g/L)	K_0 (g/L h)	MPSD	K_1 (h)	MPSD	K_2 (g/(mg h))	MPSD	K_{31} (h^{-1})	K_{32} (h^{-2})	MPSD
5	4.99	0.1800	1.9×10^{-4}	0.0397	1.5×10^{-4}	0.1822	70.71	0.034	0.0021	1.9×10^{-4}
10	10.54	0.4345	6.84	0.0513	8.31	0.0255	63.28	0.000	0.0112	3.24
25	28.61	1.2206	11.18	0.0870	27.29	0.0004	78.90	0.000	0.0095	13.15
50	56.38	1.2757	10.64	0.0308	13.81	0.0063	61.72	0.001	0.0027	8.45
75	92.32	2.3587	18.05	0.0787	58.65	1.8×10^{-5}	88.49	0.000	0.0047	31.31
100	118.12	2.5386	16.19	0.0650	50.09	9.3×10^{-6}	88.92	0.000	0.0030	27.08
120	133.98	2.0231	10.50	0.0315	26.66	2.7×10^{-5}	78.39	0.000	0.0012	7.18
150	152.61	1.9990	30.07	0.0411	55.02	3.8×10^{-6}	89.65	0.000	0.0012	31.27

3.4. Effect of initial concentration on phenol and cyanide biodegradation

Time profile for effect of initial concentration on phenol (100–1,500 mg/L) and cyanide (10–150 mg/L) degradation by *P. putida* MTCC 1194 is revealed in Figs. 6a and 6b. It is clear from figure that the degradation time increased with increase in initial concentration of both phenol and cyanide. The higher concentration of compound takes more time to complete degradation. Such as, for complete degrading 50 mg/L of phenol the microbes took about 10 h but for 1,500 mg/L it took 75 h. Similarly, for degrading 5 mg/L of cyanide the microbes took 10 h but for 150 mg/L it took 80 h for complete degradation. The lag phases were observed at high initial concentrations, however the acclimatized culture was used for experiments. Hill and Robinson [44] determined that the inhibitory effect of the pollutants as well as the inoculum size might affect the length of the lag phase. Therefore, a large amount of inoculum should be used to avoid lag phase. It was also detected that at near the end of the substrate consumption curve, the substrate removal rate is less. The fall in pH and shortage in the availability of oxygen may affect the substrate removal rate [45]. Yang and Humphrey [46] concluded that after an exponential phase a reduction in oxygen was a reason for low growth rates.

3.5. Modeling of the biodegradation kinetics

The biodegradation kinetics define the rate of pollutant uptake onto microbes and it also controls the equilibrium time. The kinetics of phenol and cyanide biodegradation by *P. putida* MTCC 1194 was applied to zero order, pseudo-first-order, pseudo-second-order and three-half-order kinetics models. The non-linear form of these models is given in Table 1. The parameters evaluated from models, were listed in Tables 3a and 3b. Three-half-order model was found to fit the experimental data with low MPSD value (Tables 3a and 3b). Three-half-order model could be measured as a superior model in comparison with other models because it integrates both the kinetics of

culture growth and substrate [35,43,47]. The degradation process could be divided into two phases such as: lag phase and active degradation phase as shown in Figs. 5a and 5b. According to this observation, a three-half-order kinetic model comprising zero-order, first order and second order rate to fit this model to a higher degree as compared to the other kinetic models [43,48]. The fitness of three-half order model to experimental data also indicates that phenol and cyanide degradation is growth-associated phenomenon, and the substrate is utilized for the growth of culture [48].

4. Conclusions

The results of this study indicated the potential of bacterial cultures of *P. putida* MTCC 1194 for biodegradation of phenol and cyanide. Different growth kinetic models such as: Monod, Haldane, Edward, Yano and Koga, Luong, Han and Levenspiel and Powell were used to define the kinetic constants for growth of biomass. The experimental data for phenol and cyanide biodegradation fitted well with Haldane, Edward, Yano and Koga, Luong and Han and Levenspiel models, providing a lower value of the error function MPSD. To define the biodegradation kinetics of phenol and cyanide zero order, pseudo-first-order, pseudo-second-order and three-half order kinetics models were used. Among these kinetic models, three-half-order kinetic model was established to define the degradation profile. The culture growth for binary substrate study, the sum kinetics model was also used. Results obtained from this study could be important for understanding the *P. putida* MTCC 1194 capacities for phenol and cyanide degradation.

Nomenclature

μ_s	—	is the specific growth rate (h^{-1})
μ_{max}	—	is the maximum specific growth rate (h^{-1})
S	—	*is* the concentration of substrate (mg/L) at time t
K_s	—	is the half saturation coefficient (mg/L)
m	—	is maintenance rate
K_i	—	is the substrate inhibition constant (mg/L)
q_s	—	is the specific substrate degradation rate (h^{-1})
q_{max}	—	is the maximum specific substrate degradation rate (h^{-1})
S_m	—	is the critical inhibitor concentration (mg/L)
m, n	—	are the empirical constants
S_0, K_0	—	are zero order model constant
K_1, K_2	—	are first and second order rate constants, respectively
x_0	—	is the concentration of biomass (mg/L) at time $t = 0$
P	—	is the rate of product formation
K_{31}, K_{32}	—	are three-half-order rate constants

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