Journal of Hazardous Materials B129 (2006) 216–222

Journal of Hazardous Materials

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Kinetics of high strength phenol degradation using *Bacillus brevis*

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Received 26 May 2005; received in revised form 21 August 2005; accepted 25 August 2005
Available online 3 October 2005

Abstract

A strain of *Bacillus brevis* has been isolated and identified based on biochemical results. Phenol biodegradation in a batch reactor was studied using the pure culture of *B. brevis*. The isolated strain was optimized for various environmental conditions and the biodegradation of phenol was maximum at pH 8.0, 5% (v/v) of inoculum size and without any co-substrate. The kinetics of biodegradation according to Haldane's equation $\mu = \mu_{max} S/(K_s + S + (S^2/K_i))$ adequately describes cell growth with kinetic constants in the ranges $\mu_{max} = 0.026–0.078\ h^{-1}$, $K_s = 2.2–29.31$ mg/l, $K_i = 868.0–2434.7$ mg/l. These values are specific for this organism and we have compared with literature for pure or mixed cultures degrading phenol.

Keywords: Phenol; Biodegradation; *Bacillus brevis*; Batch reactor; Kinetics

1. Introduction

Phenolic compounds are hazardous pollutants and they enter the environment through the decomposition of attached algae, phytoplankton [1] and through wastewater discharges from a variety of industries like leather, phenol–formaldehyde resin, oil refinery, coking plant, pharmaceutical, coal conversion, etc. [2–7]. Phenolic compounds are toxic to aquatic life, plants and many other organisms. When phenol-containing water is chlorinated, toxic polychlorinated phenols can result thereby contributing to off flavors in drinking and food processing waters [8]. As a consequence, it is necessary to treat phenolic wastewater before disposing into stream, so as to reduce the phenol content to a standard value or less than 1 ppb, as described by the EPA.

Different treatment methods are available for reduction of phenol content in wastewater. The technologies for the treatment of wastewater containing phenol include chlorination, ozonation, adsorption, solvent extraction, membrane process, coagulation, flocculation and biological treatment [9]. But physicochemical methods have proven to be costly and have the inherent drawbacks due to the tendency of the formation of secondary toxic materials such as chlorinated phenols, hydrocarbons, etc. Thus the biological method of treatment has turned out to be a favorable alternative for phenol degradation. Different microorganisms were used by the different researchers for the degradation of phenol [10–15].

We have isolated a novel strain of *Bacillus brevis* from the phenol–formaldehyde resin manufacturing industrial wastewater and utilized for biological degradation of phenol. The main goal of this paper is to investigate the biodegradation kinetics with respect to (i) higher phenol concentration and (ii) the environmental conditions like pH, inoculum concentration, and co-substrate.

2. Kinetics

The biodegradation of phenol and its derivatives by microbial cultures has been the focus of research for more than three decades [8,16–20]. These studies have been conducted with different micro-flora, under varied environmental conditions of pH, temperature, addition of co-substrate, in the presence or absence of oxygen, in batch and continuous flow reactors. Determination of growth and degradation kinetics of organism has been one of the main issues considered in these studies. As the knowledge of the growth kinetics is essential for the understanding of the

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doi:10.1016/j.jhazmat.2005.08.040

Table 1
Comparison of growth kinetic parameters observed by various researchers with the present study

S. no.	Authors	Bacterial strain	System	Concentration range (mg/l)	Haldane's model			Other conditions: temperature (°C)/pH
					μ_{max} (h^{-1})	K_s (mg/l)	K_i (mg/l)	
1	Pawlowsky and Howell [19]	Mixed culture 1	Batch	0–900	0.260	25.4	173.0	28 ± 0.5/6.6
		Mixed culture II (filamentous organism)	Batch	0–1000	0.223	5.86	934.5	28 ± 0.5/6.6
2	Hill and Robinson [26]	*Pseudomonas putida* (ATCC 1784, Stainer 110)	Batch/Continuous	0–700	0.534	0.015	470.0	30/6.2–6.7
3	Yang and Humphrey [8]	*P. putida* (ATCC 17514)	Continuous	0–500	0.567	2.39	106.0	30/60
		T. cutaneum	Continuous	0–900	0.464	1.66	380.0	28 ± 0.5/6.8 ± 0.1
4	Chi and Howell [18]	*P. putida*	Continuous	Up to 700	0.369	5.94	227.0	30/6.2–6.8
5	Livingston and Chase [27]	NCIB8250 (*Acinetobacter* sp.) + NCIBI0535 (*Pseudomonas* sp.) + NCIB1015 (*Pseudomonas* sp.)	Batch	0–500	0.418	2.9	370.0	30/6.2–6.7
6	Kumaran and Paruchuri [28]	*Acinetobacter calcoaceticus* (phenol only)–*Pseudomonas fluoroscens* 2218 (phenol only)–pooled culture (phenols) (*P. fluoroscens*, *P. putida*, *P. cepacia*, *A. calcoaceticus*, *Candida tropicalis*)	Batch	60–500	0.542	36.2	145.0	Not mentioned
				85–890	0.618	71.4	241.0	
				300–710	0.456	53.9	516	
7	Abuhamed et al. [29]	*P. putida* F1 ATCC 700007	Batch	50	0.051	18.0	430	30/7.0
8	This study	*B. brevis*	Batch	750–1750	0.026–0.078	2.2–29.3	868–2434.7	34 ± 1

capacities of the microorganisms for the degradation and the operation of the treatment units, therefore, several degradation studies related to phenol degradation are reviewed (Table 1).

Cell growth kinetics in a batch reactor may be modeled by the following equation:

$$dX/dt = \mu_g X - K_d X = \mu_{net} X \quad (1)$$

For substrate,

$$dS/dt = -1/Y(dX/dt) \quad (2)$$

μ_g is a function of S. Two viewpoints exist regarding the use of equations relating to specific growth rate, μ, to phenol concentration. One view is that phenol can be considered as non-inhibitory compound for an adapted population and was represented by Monod's non-inhibitory kinetics equation as given below:

$$\mu_g = \mu_{max} S/(K_s + S) \quad (3)$$

The other view considers the phenol as 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. Out of the models, Haldane's model was used due to its mathematical simplicity and wide acceptance for representing the growth kinetics of inhibitory substrates. The Haldane's inhibitory growth kinetics equation is as follows:

$$\mu_g = \mu_{max} S/K_s + S + (S^2/K_i) \quad (4)$$

At higher substrate concentrations, $S \gg K_s$, the above equation reduces to the following equation:

$$\mu_g = \mu_{max} S/S + (S^2/K_i) \quad (5)$$

or

$$1/\mu_g = 1/\mu_{max} + S/(K_i \mu_{max}) \quad (6)$$

This is the linearized Haldane's equation.

3. Materials and methods

3.1. Microorganism

The bacterial strain was isolated from phenol–formaldehyde resin manufacturing industrial wastewater and identified based on the morphological, physiological, biochemical and carbon source utilization characteristics [21].

3.2. Growth medium

The growth medium [22] contained phenol as the sole carbon source, and mineral salts at the concentrations (per liter): $(NH_4)_2SO_4$, 325 mg; $MnSO_4$, 8.45 mg; $MgSO_4$, 65 mg; $FeCl_3$, 2.0 mg; $CaCl_2$, 9.0 mg; K_2HPO_4, 2627 mg; KH_2PO_4, 1436 mg. Phenol was sterilized and mixed in appropriate proportions with the sterilized medium at the start of experiment. The pH of the medium was adjusted to 8.0.

3.3. Acclimatization of culture and inoculum development

The acclimatization of the isolate was performed in a minimal medium. Initially, the isolate was grown in minimal medium with 500 mg/l phenol. Later, cell mass was centrifuged and inoculated to minimal medium with 750 mg/l phenol and incubated. The 3rd and 4th incremental addition of phenol to 1500 and 1750 mg/l, respectively, were made in minimal medium and the isolate was grown. The samples were analyzed regularly for phenol and growth. All the inoculum transfers were done in exponential phase. The temperature in all the batch experiments was maintained at $34 \pm 0.1\,^{\circ}C$.

3.4. Analytical methods

Biomass concentrations were determined using UV–vis spectrophotometer [Model U-2001 Hitachi, Japan] at 550 nm by measuring the absorbance and correlated to standard biomass concentration. Phenol was estimated by direct photometric method based on rapid condensation with 4-aminoantipyrine, followed by oxidation with potassium ferricyanide under alkaline conditions to give a red colour product. The estimation was carried out according to standard methods [23].

3.5. Experimental design to study the free cell system

Experimental studies were carried out with shake flasks as batch reactors. A sample volume of 100 ml was taken in each 250 ml conical flask. Each sample contained the minimal medium with phenol of varying concentrations as the sole carbon source. The flasks were maintained at $34 \pm 0.1\,^{\circ}C$ and the shaker speed was maintained at 150 rpm.

4. Results and discussion

4.1. Effect of initial concentration

Shake flask experiments were conducted to examine the effect of various initial concentrations of phenol on the degradation behavior of *B. brevis* at $34 \pm 0.1\,^{\circ}C$. The various initial phenol concentrations were ranging from 750 to 1750 mg/l (Fig. 1). Results of these studies show that higher the initial concentration of phenol the more time it takes to be degraded completely. The results of the plot of cell biomass concentration versus time for

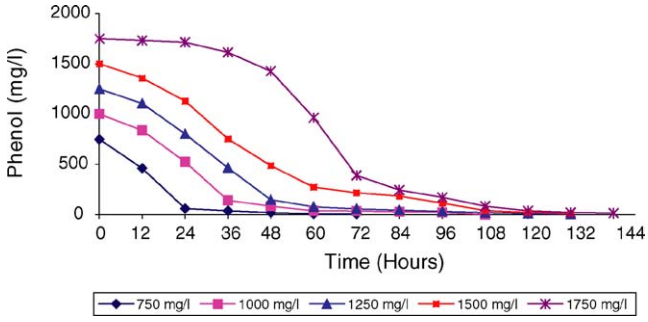

Fig. 1. Phenol degradation by *B. brevis* in batch reactor.

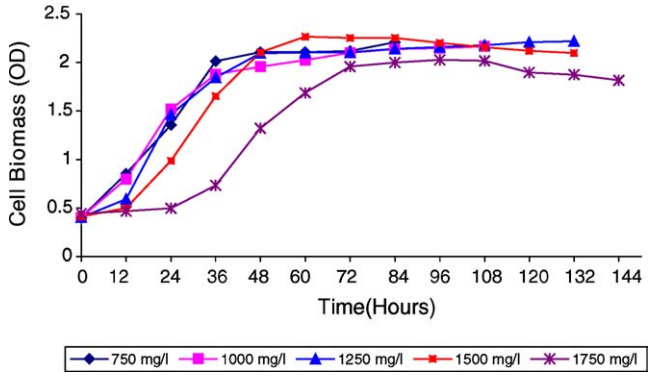

Fig. 2. Growth of *B. brevis* in phenol.

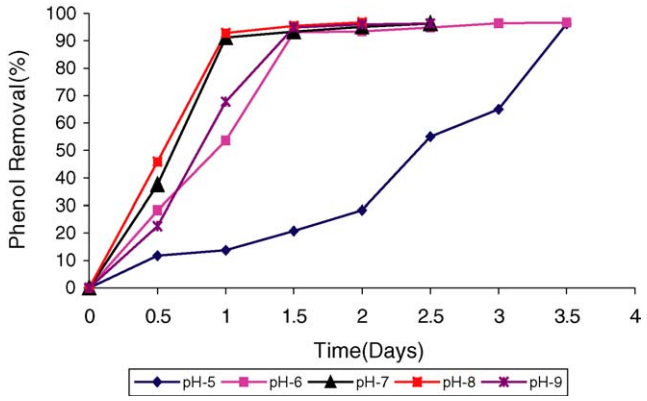

Fig. 3. The behavior of the strain for various pH in phenol degradation.

B. brevis (Fig. 2) indicate that at lower initial phenol concentrations (750, 1000, 1250 and 1500 mg/l), *B. brevis* degrades phenol immediately have no lag phase, indeed the same *B. brevis* has a lag phase of 24 h (Fig. 2) at 1750 mg/l of initial phenol concentration. Lag phase has been observed up to 36 h and thereafter up to 72 h it follows log phase.

At each of the initial phenol concentrations there was a period of exponential growth period was observed which is further confirmed by substrate being consumed at faster rate. The same observation was made in our experimental studies during the exponential growth phase of the species. Further, the substrate removal rate was relatively reduced towards the end of the substrate consumption curve. This may be due to deficit in availability of oxygen as well as the substrate for the whole biomass, which in turn the fall in pH of the solution during the degradation period being noticed. The fall in the oxygen and pH of the solution during degradation has been reported when the phenol was metabolized by mixed culture composed of Pseudomonadaceae, Vibrionaceae, etc. [8,24,25].

4.2. Effect of physico-chemical parameters

4.2.1. Temperature and pH

The experimental temperature was maintained at $34 \pm 0.1\,^{\circ}C$. Previous studies reported in the literature were conducted at the temperature ranges from 28 to $30 \pm 0.1\,^{\circ}C$. The increase in temperature may also influence the rate of phenol degradation to a maximum of 1750 mg/l. The experiments were also carried out to optimize the pH (Fig. 3). The most favorable pH for the strain to achieve the maximum rate of phenol degradation was 8.0.

4.2.2. Inoculum size

The inoculum concentration in the shake flasks were varied from 2 to 6% (v/v). Five percent (v/v) of biomass concentration provided the best degradation in short duration, whereas, 6% (v/v) concentrations have an efficiency of degradation as equivalent as 5% concentration. The results show that the increased inoculum concentration reduced the lag phase and helping the system to reach the exponential growth phase quickly (Fig. 4). Hill and Robinson [26] concluded that not only the inhibitory effect of the substrate but also the size of the inoculum might affect the duration of the lag phase. But in this study the results show that after a optimum concentration of inoculum the effect only was marginal.

4.2.3. Addition of co-substrate

The behavior of the strain in the presence of co-substrate (dextrose) was also studied. The co-substrate was added at the ratios 0.2–0.8% (w/v). It was observed that the efficiency of phenol degradation was reduced comparatively in the presence of co-substrate. Thus the organism ignores phenol in presence of dextrose and phenol degradation period was delayed (Fig. 5).

4.3. Growth kinetics

The biokinetic parameters such as maximum specific growth rate (μ_{max}), Monod's constant (K_m) and inhibition constant (K_i), were determined by the plot of specific growth rate against a wide range of phenol concentrations for *B. brevis* strain. These have been depicted in Figs. 6 and 7. From these plots biokinetic constants were estimated using Monod's and Haldane equation for *B. brevis* are presented in Table 2.

Figs. 6 and 7 show the comparison of experimentally obtained specific growth rate (μ) with that predicted by the Monod's and Haldane's models. From both the graphs, it is evident that the growth kinetics of phenol represented by Haldane's growth kinetics model is very suitable than the Monod's model. The coefficient of correlation R^2 was found to be 0.953 in Haldane's model and 0.850 in Monod's equation. The values of the growth

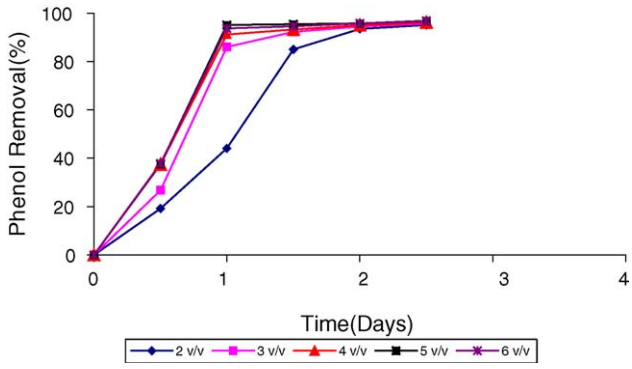

Fig. 4. Effect of the inoculum size on phenol degradation.

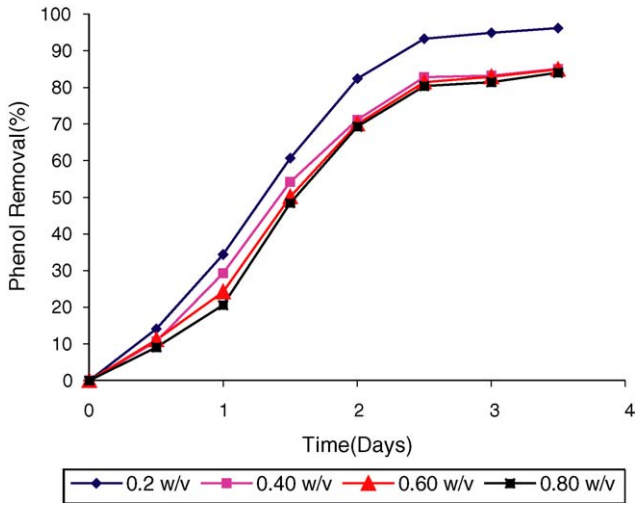

Fig. 5. Effect of addition of dextrose on phenol degradation.

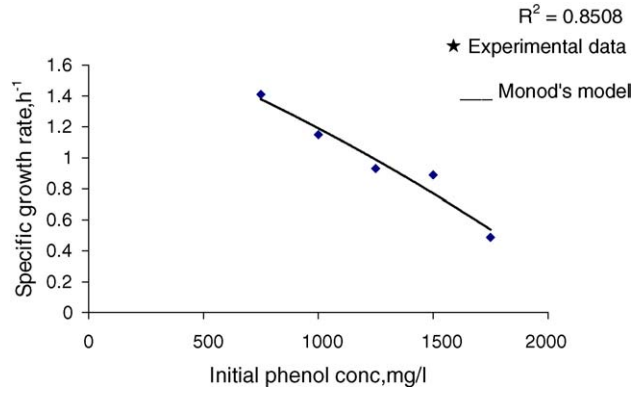

Fig. 6. Effect of initial phenol concentration on specific growth rate as determined by fitting the data to Monod kinetic model.

kinetic parameters for various species obtained from different researchers for phenol were compared with our results and given in Table 1. The maximum specific growth rate (μ_{max}, h^{-1}), the values of substrate half saturation coefficient (K_s, mg/l) and the substrate inhibitory coefficient (K_i, mg/l) were in the range of

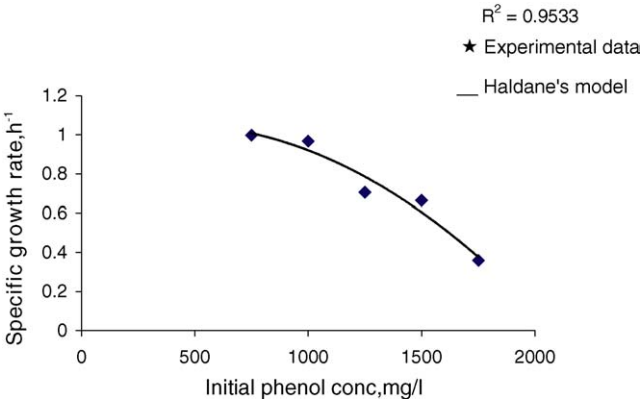

Fig. 7. Effect of initial phenol concentration on specific growth rate as determined by fitting the data to Haldane kinetic model.

Table 2
Growth kinetic parameters of Monod's and Haldane's model for biodegradation of phenol using *B. brevis* in batch system (temperature =34 ± 1 °C, pH 8)

Phenol concentration (mg/l)	Monod's model		Haldane's model		
	μ_{max} (h^{-1})	K_s (mg/l)	μ_{max} (h^{-1})	K_s (mg/l)	K_i (mg/l)
750	0.061	25.53	0.078	14.6	868.0
1000	0.048	8.56	0.066	2.2	1582.8
1250	0.040	14.97	0.057	8.9	1358.7
1500	0.039	82.45	0.051	9.28	1773.2
1750	0.021	2.88	0.026	29.31	2434.7

0.026–0.618, 0.015–71.4 and 106.0–934.5. The maximum specific growth rate obtained in this study also fall into these ranges except the μ_{max} (0.026 h^{-1}) value for the highest concentration of phenol (1750 mg/l), but the values of K_s obtained for all initial phenol concentrations fall with in the reported ranges. This range of the values of K_s for phenol indicates the ability of a microorganism even to grow at low substrate levels. In the present investigation the K_i values were in the range of 868–2434.7 and this indicates that the inhibition effect is observed only in a high concentration range.

4.4. Endogenous or decay coefficient

A typical growth curve shows a decline in cell population after the complete consumption of substrate. During this declining phase some part of the cell population becomes food for the rest of the cell population. This part of the growth curve in a batch reactor has been modeled by following equation:

$$dx/dt = -k_d X \quad (7)$$

In order to determine the value of k_d, the growth of culture was continued and the cell mass concentration was observed for few days even after the complete consumption of phenol. The selection of the particular growth run was arbitrary, assuming that the k_d is not dependent on initial concentration. Figs. 8–12 show the negative intercept in the graph plotted for the entire growth region as $1/\theta$ versus $S_0 - S/\theta X$, gives decay rate coefficient. The values of the decay rate coefficients obtained were in the range of 0.003–0.012 h^{-1} for phenol. Kumaran and Paruchuri [28] have

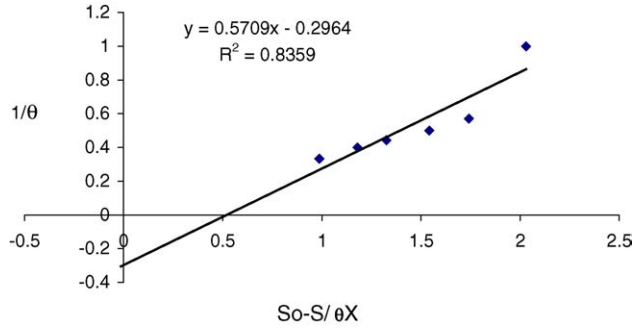

Fig. 8. Evaluation of decay coefficient K_d and yield coefficient Y for *B. brevis* growth on phenol initial concentration = 750 mg/l.

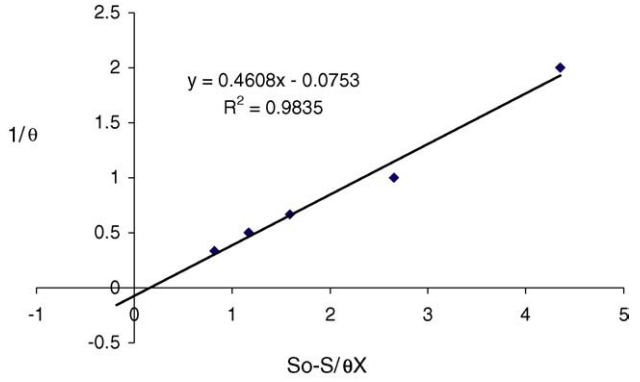

Fig. 9. Evaluation of decay coefficient K_d and yield coefficient Y for *B. brevis* growth on phenol initial concentration = 1000 mg/l.

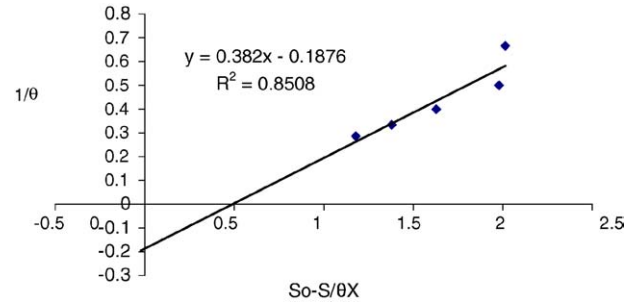

Fig. 10. Evaluation of decay coefficient K_d and yield coefficient Y for *B. brevis* growth on phenol initial concentration = 1250 mg/l.

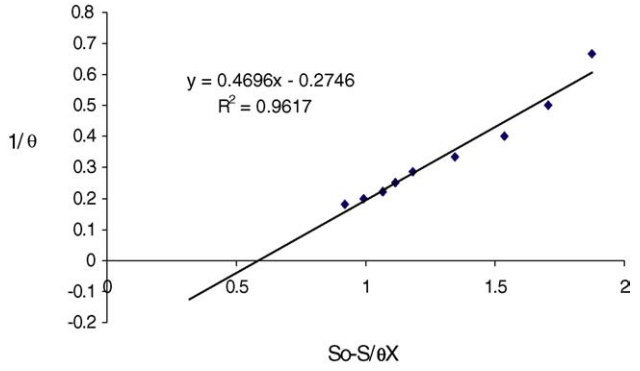

Fig. 11. Evaluation of decay coefficient K_d and yield coefficient Y for *B. brevis* growth on phenol initial concentration = 1500 mg/l.

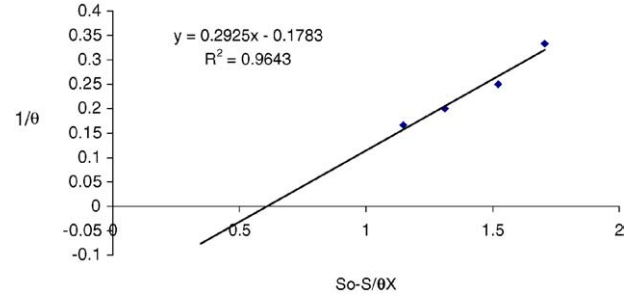

Fig. 12. Evaluation of decay coefficient K_d and yield coefficient Y for *B. brevis* growth on phenol initial concentration = 1750 mg/l.

reported the value of decay coefficient as 0.005 h^{-1} for phenol degradation by a mixed culture. The values obtained in this study are comparable with these values of decay coefficients. The decay coefficient affects the growth kinetics. The growth rate will be reduced by as much the value of decay coefficient so that the wash out condition would occur at lower dilution rates.

4.5. Yield coefficient

In Figs. 8–12, the slope of the graph plotted was used to determine yield coefficient for phenol of various concentrations. In all the cases, the values of the yield coefficient for phenol ranges from 0.293 to 0.571 (the coefficient of correlation R^2 ranges from 0.836 to 0.984). These values are comparably smaller than the values obtained by other researchers, this may due to high substrate concentrations.

5. Conclusion

The isolated strain *B. brevis* degraded phenol to a maximum concentration of 1750 mg/l. The duration taken up by the species was about 144 h. Results obtained for favorable degradation were pH 8.0, temperature 34 ± 0.1 °C and biomass concentration 5% (v/v). The addition of co-substrate has not shown any significant results either increase in phenol concentration or decrease in degrading duration. The organism shows a short lag phase at high substrate concentration, whereas in the low concentrations the lag phase was absent.

The phenol exhibited inhibitory behavior and its growth kinetics was correlated well by the simple Haldane's inhibitory growth kinetics model. The endogenous decay and yield coefficients for various phenol concentrations were also determined. The above-determined parameters are required for the design and simulation of batch and continuous bioreactors treating high strength phenolic wastewaters.

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