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Phenol Biodegradation Kinetics in an Anaerobic Batch Reactor

[1]Tahere Taghizade Firozjaee, [2]Ghasem D.Najafpour, [3]Maryam Khavarpour,
[1]Zeinab Bakhshi, [1]Roya Pishgar and [1]Nafise Mousavi

[1]Faculty of Civil Engineering, Noushirvani Institute of Technology,
University of Mazandaran, Babol, Iran
[2]Faculty of Chemical Engineering, Noushirvani Institute of Technology,
University of Mazandaran, Babol, Iran
[3]Faculty of Chemical Engineering, Islamic Azad University,
Ayatollah Amoli Branch, Amol, Iran

Abstract: Biodegradation of phenol with mixed consortia of bacterial was studied in an anaerobic batch reactor. The culture was acclimatized for a period of two months to grow in mineral salt medium contained phenol as the sole carbon source. Minimum and maximum concentrations of 50 and 1000 mg/l of phenol in the medium were used for the purpose of culture adaptation. The goals of these experiments were to determine the kinetics of biodegradation by determination of biomass growth rates and phenol concentrations with respect to incubation time. Substrate inhibition kinetics and specific growth rate were defined while data were fitted in Haldane model. The specific growth rate, half-saturation and inhibition coefficients for Haldane model were 0.067 h^{-1}, 25.32 and 200 mg/l, respectively. The biokinetic parameters were used to predict the biodegradation profile, which was quite consistent with the obtained experimental data. The results were useful for the estimation of relations between growth rate and substrate utilization, which may be used to evaluate mass balance for a wastewater treatment plant contained phenol compound.

Key words: Anaerobe % Biodegradation % Haldane model % Inhibition % Kinetic model % Phenol

INTRODUCTION

Phenol is present in effluents of many industries such as petroleum refining, resin and plastic, leather and textile manufacturing, chemical and petrochemical plants, coke ovens, foundry operations, pulp and paper plants, rubber reclamation plants, pharmaceutics and agro-industrial operations. The level of phenol in these effluents may be as high as 6,000 mg/l [1]. Phenol is a toxic compound which may cause serious health hazards such as blood changes, liver and kidney damage and cardiac toxicity including weak pulse, cardiac depression and reduced blood pressure. These damages have been reported in humans acutely exposed to phenol by oral route. Ingestion of 1 g phenol is reported to be lethal for humans [2]. Because of their environmental significance, phenol has been the targets of a number of investigations focused on its physical, chemical and biological treatment of wastewater [3-6]. Bioprocesses are preferred method to physical and chemical technologies for pollution control due to their ability to destroy effectively the pollutants. Biological processes are able to mineralize organic pollutants in an environmental and economical way, rather than chemically oxidize or physically transfer pollution from one phase to another [7].

Efforts are now being made to remove organic contaminants as far as possible without external resources of energy, taking advantage of the biogas produced, where the desired level of purification is ultimately achieved with or without the aid of a subsequent aerobic biological clarification step. Above instances are advantages of anaerobic wastewater treatment.

Corresponding Author: Dr. Ghasem D. Najafpour, Faculty of Chemical Engineering, Noushirvani Institute of Technology, University of Mazandaran, Babol, Iran, Tel/Fax: +98111-3210975, E-mail: najafpour8@yahoo.com.

The fundamental advantages with the anaerobic wastewater treatment are considered as energy intensive, very less sludge is formed, while at the same most of the organic contaminants are converted into combustible fuel gas [8, 9]. Aerobic degradation of phenol with pure cultures has been extensively investigated; for instance *Pseudomonas putida* has been widely used for the biodegradation of phenols [10-14]. Also several investigations have studied anaerobic degradation of phenol [15-17]. However, a mixed consortium of microbes is required for complete mineralization [13].

In this article, high initial phenol concentrations were utilized in presence of mixed consortia of microorganisms. The kinetic parameters of anaerobic degradation of phenol with mixed strains of microorganisms in a batch reactor were investigated.

MATERIALS AND METHODS

Microorganism and Culture Conditions: The mixed microbial culture for the phenol degradation was isolated and enriched from the pulp and paper wastewater treatment plant, Sari, Iran. Phenol-degrading bacteria are required to be adapted to the phenol environment. In order to remove phenol inhibition behavior in the growing media, the seed culture was acclimated. The acclimation was carried out for two months under anaerobic condition in a 250 ml Erlenmeyer flask contained phenol and mineral salt medium. During acclimatization process, certain enzymes in the bacteria were induced so that they are available for taking part in the metabolic reaction [11]. The synthetic media consist of glucose, yeast, KH_2PO_4 and K_2HPO_4, with concentration of 1.5, 0.8, 0.35 and 0.55g/l, respectively. The composition of the mineral salts presence in the synthetic medium is given in Table 1. The initial concentration of glucose was 1.5g/l which was gradually replaced by stepwise increasing the phenol concentration.

Experimental System and Analytical Techniques: Phenol was determined by a direct photometric method using 4-amino antipyrine [18]. Before quantifying phenol, samples were centrifuged (Hermele Z233 M-2, Germany) at 6,000rpm for 10 min. The calibration curves were prepared and the incorporated data were found linearly fitted with R^2 value of 0.999. Biomass concentration in the samples was determined by measuring its absorbance at 600 nm wavelength using a UV–Visible Spectrophotometer (Unico, 2100 series, USA). According to standard calibration curve, the cell dry weight concentration was

Table 1: Composition of the mineral salts in the synthetic medium

Constituents	Concentrations(mg/l)
$MgSO_4$	5
$FeSO_4$	6
$CuSO_4$	0.01
$CoCl_2$	0.88
$NiCl_2$	1
$MnCl_2$	5
$ZnSO_4$	0.1
H_3BO_3	

also determined based on turbidity of the media by light absorbance as a function of cell dry weight. The temperature in all batch experiments was maintained at 26±1°C.

Mathematical Model: It is necessary to evaluate the relationship between the specific growth rate, μ and the phenol concentration, S. Monod equation is a simple biokinetic model which was chosen for modeling of two parameters. According to the model, phenol is considered as non-inhibitory compound. Monod's non-inhibitory kinetics equation is presented in the following equation:

$$m = \frac{m_{\max} s}{K_2 + S} \tag{1}$$

Where μ is specific growth rate (hG^1), S is limiting substrate concentration, $\mu_{\max}$ is maximum specific growth rate (hG^1), K_2 is half-saturation coefficient (mg/l). Phenol biodegradation by microbes has generally been known to be inhibited by phenol itself. Hence, Monod equation is unable for describing inhibitory growth of microorganism at higher substrate concentrations. To present the growth kinetics of inhibitory compounds, several kinetics models were fitted to the experimental data for selecting the best models. These models have been discussed in detail in the literature by Edwards [19]. Haldane's growth model also is selected due to its mathematical simplicity and wide acceptance for representing the growth kinetics of inhibitory substrates. Haldane's inhibitory growth kinetics equation is as follows:

$$m = \frac{m_{\max} s}{K_2 + S + \left(\dfrac{S^2}{K_i} \right)} \tag{2}$$

where, K_i is Haldane's growth kinetics inhibition coefficient (mg/l). The specific growth rate (μ) in the exponential phase is calculated using Eq. (3).

$$m\frac{dX}{X.dt} \qquad (3)$$

where X is microorganism concentration (mg/l).

RESULTS AND DISCUSSION

Effect of Initial Concentration on Phenol Biodegradation and Cell Growth: Cell growth and phenol degradation at initial concentrations ranging from 50 to 1000 mg/l were measured as a function of time (Figure 1a &b). It was observed that phenol concentrations with the values of 50 to 400 mg/1 did not show any obvious inhibition effect on growth of the microorganisms.

In other to have the acclimatization stage for the culture, the cell growth curve did not show any significant lag phase. Moreover, phenol concentration had influenced on cell growth to reach to stationary phase. The media with phenol concentration of 400 mg/l required more time to grow than the media with low phenol concentrations. This behavior was due to substrate inhibition. Hill and Robinson [20] reported that not only inhibitory effect of the substrate but also the size of inoculums might be affected on duration of the lag phase. Therefore, to avoid lag-phase a large size of inoculum may be used.

Although high phenol concentrations provide more carbon sources which may lead to a high final biomass; but the cell growth was out of proportion to phenol degradation. It was expected that final biomass concentration became doubled when phenol concentrations changed from 200 to 400 mg/l.

However, according to Figure.1a this trend wasn't truly observed in practical case. The phenol substrate was mainly consumed for assimilation into biomass and energy for cell growth and maintenance [21]. However, at high substrate concentrations, the inhibition effects on cell growth were stronger than low substrate concentration and also more energy was required to maintain the cell viability, but may not be enough to synthesize new cells. Another possible reason for the decreased cell mass was the production and accumulation of various intermediates [22].

Determination of Kinetic Parameters: Using the microorganism concentration values during the exponential growth phase, the values for µ were obtained from Eq. (2). Haldane equation has shown by several researchers to give an adequate fit to plot of µ versus S. The model equations were solved with the aid of MATLAB 7.4 software for the nonlinear regression method. The model fitted with the data and also estimated the values of three biokinetic constants of Haldane equation. The dependence of specific growth rate on phenol concentration is shown in Figure 2. From this plot, the specific growth rate was increased with an increase in the initial phenol concentration to a certain level and then a decrease was started with a more increase in the concentration. This trend suggested that the phenol is inhibitory substrate.

In general, Haldane's growth kinetics model is used to represent growth kinetics data of an inhibitory compound such as phenol. The obtained specific growth rates for the media with low-concentration were also fitted
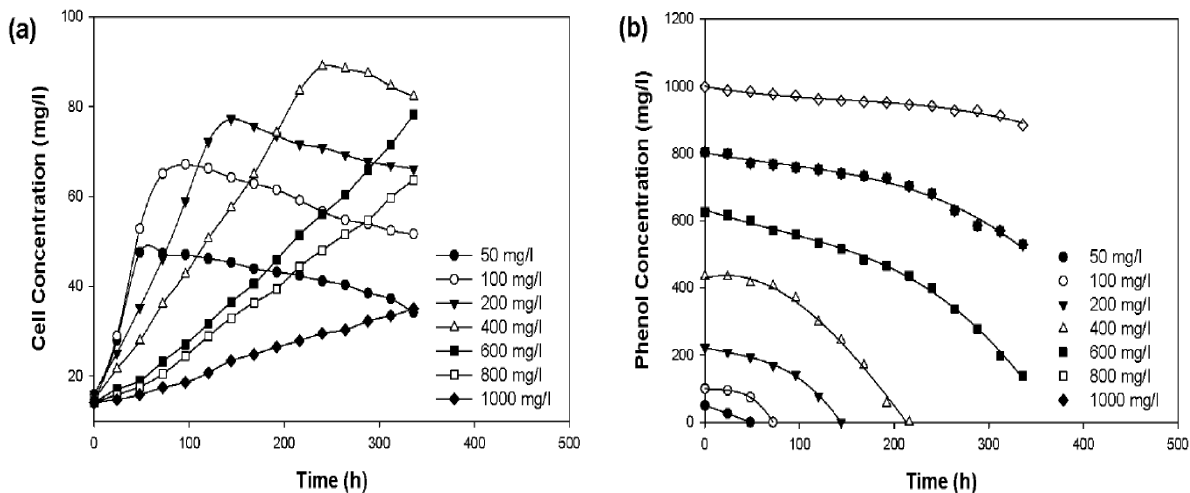

Fig. 1: Concentration profiles of different parameters: (a) Cell concentration as a function of time at various initial phenol concentrations, (b) Phenol degradation at different initial concentrations
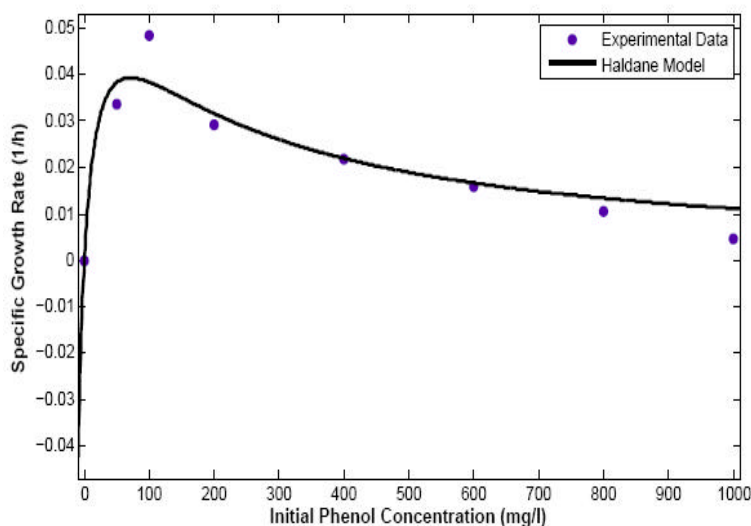
Fig. 2: Biokinetics parameter of culture media due to Haldane's growth model

Table 2: Summary of growth kinetics in various studies for the treatment phenolic wastes

S.N	Reference	Concentration rang (mg/l)	Monod's model		Haldane's model			Other conditions, temp.°C /pH
			$\mu_{max}(hG^1)$	K_2(mg/l)	$\mu_{max}(hG^1)$	K_2(mg/l)	K_i(mg/l)	
1	Aerobic- Mixed culture [23]	0-900	-	-	0.260	25.4	173.0	28±0.5/6.6
2	Aerobic- Mixed culture [23]	0-1000	-	-	0.223	5.586	934.5	28±0.5/6.6
3	Anaerobic- Mixed culture [15]	0-10000	-	-	0.027	0.03	363	35
4	Aerobic- Mixed culture [24]	60-500	0.465	30.96	0.542	36.2	145	Not mentioned
5	Aerobic- Pure culture [12]	0-100	-	-	0.436	6.19	54.1	26/608
6	Aerobic- Pure culture [11]	0-1000	0.216	20.59	0.305	36.33	129.79	29.9±0.5/7.1
7	Aerobic- Pure culture [25]	750-1750	-	-	0.026–0.078	2.2–29.31	868.0–2434.7	34-30/5-9
8	Aerobic- Mixed culture [13]	0-800	0.37	144.68	0.3085	44.92	525.00	27±1/7.0
9	Anaerobic- (this work)	50-1000	0.099	18.85	0.067	2524	200	26±1/7.0

to Monod model. Monod model was unable to describe the inhibitory growth of microorganism at higher substrate concentrations. The values of biokinetic constants (μ_{max} and K_s) predicted by Haldane model slightly differed from Monod model. Since, Haldane model represent the substrates inhibitory effect, therefore the fit of Haldane model was better than Monod's fit. The value of K_i, the inhibition coefficient, is a measure of sensitivity to inhibition by inhibitory substances. It should be noted that when K_i is very large, Haldane equation is simplifies to Monod equation. The value of K_s, half-saturation coefficient, is defined as the substrate concentration at

which μ is equal to half μ_{max}. The smaller it is the lower the substrate concentration at which μ approaches μ_{max}. Also, K_s values show the affinity of microorganisms to substrate [2].

A comparison of these kinetic constants with those reported in others studies are summarized in Table 2. The biokinetic constant values obtained for this study almost lie in the range of literature anaerobic values. However, the obtained inhibition coefficient, ($K_i = 200$ mg/1) is small which was compared to the value reported by Suidan [15]. This result indicates that the inhibition effect is observed even in a low concentration range.

The half-saturation coefficient was 25.32 mg/l that was a low value. This range of the value of K_s for phenol indicates the ability of a microorganism to grow at low substrate levels. Beside, a comparison of these kinetic parameters with the results achieved in aerobic studies show that the values of $\mu_{max} = 0.067$ hG^{1} and $K_s = 25.32$ mg/l are smaller than the value obtained in aerobic process. Since, the anaerobic process need to longer time for biodegradation, thus, the biomass grows slowly and this value is smaller than the value was reported in other aerobic studies. The evident variability seen in the literature for the biokinetic constant values may be due to various factors such as inoculums adoption, variation in predominating microbial species and different environmental factors [23].

CONCLUSION

In the present study, Monod equation was unable to describe the growth inhibition of the microorganism at higher substrate concentrations. The phenol exhibited inhibitory behavior on the growth media. In other to have the acclimatization stage for the culture, the organism showed a short lag phase at high substrate concentration, whereas in the low concentrations the lag phase was absent. Specific growth rates obtained for the media with low-concentration and high-concentration phenol were fitted to Monod's and Haldane's, respectively. For Monod model, the specific growth rate and half-saturation coefficient was obtained 0.099 hG^{1} and 18.85 mg/l, respectively. The specific growth rate, half-saturation coefficient and inhibition coefficient for Haldane model were 0.067 hG^{1}, 25.32 and 200 mg/l, respectively. Haldane model was successfully applied and perfectly fitted with the experimental data for anaerobic degradation of phenol. The results also showed that mixed cultures were able to degrade the phenol at high concentration.

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