

Kinetics of Biodegradation of Phenol and *p*-nitrophenol by Acclimated Activated Sludge

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Published online: 25 February 2018

To cite this article: Sam, S. P., Ng, S. L. & Rohana, A. (2018). Kinetics of biodegradation of phenol and *p*-nitrophenol by acclimated activated sludge. *J. Phys. Sci.*, 29(Supp. 1), 107–113, <https://doi.org/10.21315/jps2018.29.s1.14>

To link to this article: <https://doi.org/10.21315/jps2018.29.s1.14>

ABSTRACT: *The removal of highly toxic phenolic compounds, such as phenol and p-nitrophenol (PNP) from wastewater through biological approach provides a good cleanup strategy in wastewater treatment. It is recognised as an efficient green treatment with relatively low cost in comparison to conventional physicochemical processes. In this present study, acclimated sludge was acclimated to phenol and PNP, respectively, using sequencing batch reactor (SBR) system. The growth kinetics of both phenol- and PNP-acclimated activated sludge were well described by Haldane model. The values of the Haldane kinetic parameters, namely, maximum specific growth rate, μ_{max} , saturation constant, K_s , and inhibition constant, K_i , were found to be 0.4039 and 0.1346 h^{-1} , 5.393 and 110.3 $mg\ l^{-1}$, and 550.8 and 63.58 $mg\ l^{-1}$, respectively. The smaller ratio of K_s/K_i for phenol degradation compared to that of PNP biodegradation indicates the higher resistance of phenol-acclimated activated sludge to inhibition by its substrate. The pseudo zeroth-order equation was able to describe the phenol degradation kinetics at different initial concentrations. However, the equation was inadequate for the PNP degradation which was attributed to its higher toxicity compared to phenol.*

Keywords: Sequencing batch reactor, acclimated activated sludge, phenol and *p*-nitrophenol biodegradations, kinetics, Haldane model

1. INTRODUCTION

Wastewater is a worldwide concern as it presents a major health and environmental management challenge due to the presence of toxic and hazardous pollutants such as phenol and *p*-nitrophenol (PNP).^{1,2} Therefore, it needs to be treated properly

prior to being discharged to the environment. In contrast to physicochemical methods, biological approaches provide highly reliable and cost-effective treatment for the complete removal of toxic pollutants.³ Activated sludge process is one of the biological approaches in treating wastewater using mixed culture. Besides its simple operation, it is very efficient in mineralising toxic organic compound.⁴

It was reported that acclimation process could enhance the ability of activated sludge to achieve complete mineralisation.^{5,6} Complete removals can be achieved without experiencing inhibition at high concentrations of phenol and PNP at 1050 and 500 mg l⁻¹, respectively, using acclimated biomass were reported.^{5,6} In the study by Sam and Ng, phenol biodegradation time was found to decrease from 330–1260 min to 35–330 min for initial phenol concentration of 25–250 mg l⁻¹ after the acclimation of activated sludge to the target compound.⁷

Knowledge on biodegradation kinetics of toxic compounds is essential to improve the removal efficiency and process control. The degradation of phenolic compounds has been known to be inhibited by phenolic compounds themselves, especially at high concentrations.^{1,7} Thus, Haldane model is usually used to describe the growth kinetics of microorganism. Biomass growth can be defined as the increase of quantity of cellular constituents and structure which is accompanied by the increment of size and number of cells. It undergoes several phases, namely lag, exponential, stationary and death phases.³ In view of the wide range of concentrations of phenolic compounds in industrial wastewaters, the aim of this study is to investigate the growth and biodegradation kinetics of phenol and PNP at increasing concentrations in order to comprehend the effect of inhibition on the growth trends of acclimated activated sludges and thus their biodegradation performance.

2. EXPERIMENTAL

2.1 Culturing and Acclimation of Activated Sludge

The seed of activated sludge was collected from a local municipal sewage treatment plant at Batu Ferringhi, Penang, Malaysia. It was then cultured using two identical laboratory-scale sequencing batch reactors (SBRs), namely, R-P and R-PNP. Each reactor with the total working volume of 5 l was operated with five operational periods of FILL, REACT, SETTLE, DRAW and IDLE with the time ratio of 2: 8: 1: 0.75: 0.25 for a 24 h cycle. The reactors were fed with the base mix of the following compositions (in mg l⁻¹): bacto-peptone (188), sucrose (563), KH₂PO₄ (32), K₂HPO₄ (180), (NH₄)₂SO₄ (212), NaHCO₃ (500), MgSO₄ (49), FeCl₃•H₂O (18.8) and CaCl₂ (40). A volume of 3.5 l of feed solution was added into

the reactor and equal volume of treated effluent was withdrawn from the reactor during the FILL and DRAW periods, respectively. The sludge age was maintained at 20 days by wasting 250 ml of mixed liquor at the end of the REACT period. The activated sludges were then acclimated to the final acclimation concentration of 200 mg l⁻¹ phenol and 250 mg l⁻¹ PNP, respectively, in reactors R-P and R-PNP, without supplementing sucrose and bacto-peptone. Both acclimated sludges were used in the biodegradation studies once the quasi-steady state was attained, indicated by the constant values of mixed liquor suspended solid (MLSS) of 1110 ± 50 and 600 ± 50 mg l⁻¹ for R-P and R-PNP, respectively.

2.2 Biodegradation Kinetic Study

Biodegradations of phenol and PNP were carried out by using phenol- and PNP-acclimated activated sludges, respectively, in a batch mode. The degradation studies were investigated by varying the initial concentrations of phenol (0 to 1000 mg l⁻¹) or PNP (0 to 500 mg l⁻¹) in the presence of 400 mg l⁻¹ of acclimated activated sludges and nutrients with the same composition as base mix. The residual concentration of phenol was determined using 4-aminoantipyrine method and measured spectrometrically at 506 nm while the PNP concentration was determined at 401 nm.^{8,9} The biomass growth in terms of mixed liquor vapour suspended solid (MLVSS) was determined using optical density measurement at 600 nm. The complete mineralisation of phenolic compounds was ascertained by chemical oxygen demand (COD) concentrations using closed reflux and titrimetric method.⁸

The specific biomass growth data was fitted into Haldane model using MATLAB software R2017a. The Haldane equation is given as below:

$$\mu = \frac{\mu_{\max} S_o}{K_s + S_o + \left(\frac{S_o^2}{K_i} \right)} \quad (1)$$

where S_o is the initial substrate concentration (mg l⁻¹), μ_{\max} is the maximum specific growth rate (h⁻¹), K_s is the half-saturation constant (mg l⁻¹) and K_i is the substrate inhibition constant (mg l⁻¹). The value of specific growth rate, μ , was determined using a graph of biomass versus time. The kinetic data of phenolic compound degradation was fitted into pseudo zeroth-order equation shown below:

$$[S] = [S_o] - kt \quad (2)$$

where S_o is the initial substrate concentration (mg l⁻¹), S is substrate concentration at time, t and k is the pseudo zeroth-order rate constant.

3. RESULTS AND DISCUSSION

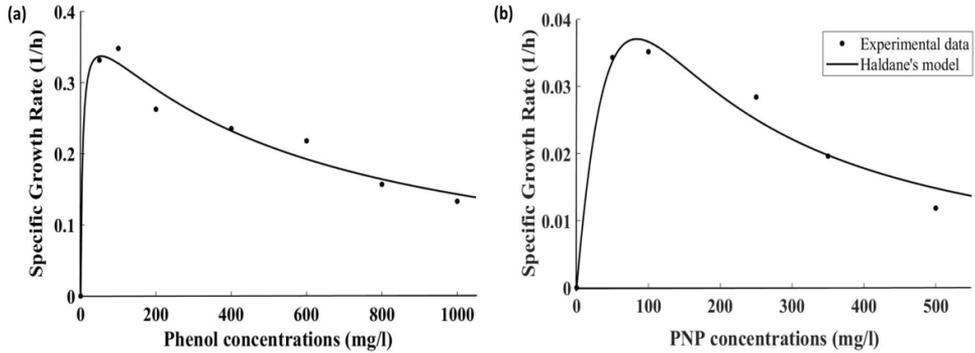


Figure 1: Experimental and Haldane predicted specific growth rates at different initial (a) phenol and (b) PNP concentrations.

Complete mineralisations of phenol and PNP were achieved as indicated by the COD concentrations below 20 mg l⁻¹. The experimental data of specific growth rate was plotted against various phenol (Figure 1(a)) and PNP concentrations (Figure 1(b)). The data was observed to be well-fitted into the Haldane model. The ability of Haldane model in describing the growth kinetics in the presence of inhibitory effects was also reported in other studies.^{1,10} In this study, it was observed that specific growth rates, μ , increased with the increase of phenol and PNP concentrations up to 100 and 50 mg l⁻¹, respectively. The μ values decreased subsequently with the increase in substrate concentrations. This revealed the inhibition behaviour of phenolic compounds on acclimated activated sludges at higher concentrations.^{7,11}

The values of kinetic parameters obtained from the fitting of Haldane model are: $\mu_{\max} = 0.4039$ and 0.1346 h⁻¹, $K_s = 5.393$ and 110.3 mg l⁻¹, $K_i = 550.8$ and 63.58 mg l⁻¹ with the $R^2 = 0.9677$ and 0.9764 for phenol and PNP degradations, respectively. Based on the K_i values obtained, the inhibition effect occurred at phenol and PNP concentrations higher than 550.8 and 63.58 mg l⁻¹, respectively. Furthermore, it was reported that the degree of inhibition can be illustrated by the K_s/K_i ratio, in which the resistance of acclimated activated sludge towards the inhibitory effects was indicated by a small K_s/K_i ratio.^{7,12} In this study, the K_s/K_i ratio for phenol degradation (0.0098) was found to be much smaller compared to that of PNP degradation (1.735). Thus, phenol-acclimated activated sludge was able to grow within the phenol concentration range studied without any observable inhibition.

The kinetics of biodegradation of phenol and PNP fitted well to the pseudo-zeroth-order equation with R^2 values > 0.9 . The value of rate constant, k , was determined from the slope of the plot of different phenol or PNP initial concentration against time and the values are presented in Table 1. For the cases of phenol biodegradation, the highest value of k was obtained at the initial phenol concentration of 50 mg l^{-1} . This result is in agreement with the study of growth kinetics in which the μ_{max} for phenol-acclimated activated sludge was obtained at this concentration (Figure 1(a)). In general, above 50 mg l^{-1} a gradual decrease of k value was observed with increasing phenol concentration. The decreasing trend is again in agreement with the Haldane fitting. For PNP biodegradation, the increasing trend of k values from 50 to 250 mg l^{-1} contradicts the growth kinetics results obtained using Haldane model (Figure 1(b)). Higher toxicity of PNP ($EC_{50}=64 \text{ mg l}^{-1}$) compared to phenol ($EC_{50}=270 \text{ mg l}^{-1}$) resulted in significant inhibitory effect at PNP concentration as low as 63.58 mg l^{-1} as predicted based on the Haldane model.¹³ Thus, the use of pseudo-zeroth order equation in describing the biodegradation kinetics of PNP is not satisfactory as it does not take into account the effect of inhibition at high PNP concentration.

Table 1: Mean values of the pseudo zeroth-order rate constants for phenol and PNP biodegradations.

Initial phenol concentration (mg l^{-1})	k ($\text{mg l}^{-1}\text{h}^{-1}$)	R^2	Initial PNP concentration (mg l^{-1})	k ($\text{mg l}^{-1}\text{h}^{-1}$)	R^2
50	104.5	0.9486	50	13.96	0.9522
100	89.1	0.9168	100	22.24	0.9307
200	73.6	0.9393	250	31.18	0.9325
400	63.7	0.9763	350	25.39	0.9393
600	49.5	0.9732	500	16.51	0.9342
800	55.8	0.9304	–	–	–
1000	39.9	0.9425	–	–	–

4. CONCLUSION

Specific biomass growth was well described by Haldane model with the kinetic parameters, $\mu_{\text{max}} = 0.4039$ and 0.1346 h^{-1} , $K_s = 5.393$ and 110.3 mg l^{-1} and $K_s = 550.8$ and 63.58 mg l^{-1} for both phenol and PNP degradations, respectively. Low degree of inhibition for phenol biodegradation in the concentration range studied was reflected by the smaller K_s/K_i ratio compared to that of PNP biodegradation. Therefore, phenol-acclimated activated sludge was able to grow

at phenol concentration up to 1000 mg l⁻¹ without experiencing inhibition. In addition, pseudo zeroth-order kinetics was used to describe the biodegradation kinetics of phenol biodegradation. However, this equation does not incorporate the inhibition effect and thus it is inadequate to explain the cases of PNP degradation at high concentrations which is attributed to its high toxicity.

5. ACKNOWLEDGEMENTS

The authors gratefully acknowledged the financial support from Universiti Sains Malaysia (grant number 304/PKIMIA/6313275).

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