



## KINETICS OF PHENOL BIODEGRADATION IN AF2B REACTORS

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**ABSTRACT** Several models of biodegradation kinetics of phenol in wastewater have been developed, but for this study the biodegradation kinetics of phenol were solved using the Gear's and Orthogonal Kollocation methods. The experiment was carried out using an Aerated Fixed Film Biofilter (AF2B) reactor containing a bacterial consortium. The reactor operates in batches to determine the parameters of the phenol biodegradation kinetic. Furthermore, the kinetics model is simulated in an AF2B reactor that operates continuously. The experimental results show that the phenol biodegradation kinetics model follows the first order reaction kinetics with kinetic parameters as follows:  $b = 0.12 \text{ day}^{-1}$ ;  $\mu_{\max} = 2.78 \text{ day}^{-1}$ ;  $K_s = 1.85 \text{ mg phenol L}^{-1}$ ;  $K_i = 94.3 \text{ mg phenol L}^{-1}$ . The simulation results show that the phenol removal efficiency is 98%, where in the steady conditions phenol concentration of the simulation results is lower than the experimental.

**KEYWORDS :** Aerated Fixed Film Biofilter, Bacterial Consortium, Biodegradation, Kinetic Parameters, Simulation.

## INTRODUCTION

Phenol and its derivatives are toxic compounds and one of the important parameters in hospital wastewater, where phenol in hospital wastewater must not exceed 0.01 ppm, and if it exceeds the quality standards it can cause environmental pollution and health problems in humans [1,2,3,4,5]. Phenol in hospital wastewater is produced from the use of floor cleaning materials such as creasols, kreolin and other antiseptic ingredients[6]. Several technologies for hospital wastewater treatment have been developed to reduce phenol concentrations, both physical - chemical and biological processing [7,9,10,11,12,13,15]. The biological processing combined with ozonation in an aerated fixed film biofilter (AF2B/O3) reactor could reduce phenol concentration by 88% [4]. The ability of a single bacterium or consortium to degrade phenol by using a batch reactor has been widely researched [16,17,18,19,20]. Likewise, the mechanism and kinetics of phenol biodegradation reactions are also widely studied [21,22,24,25]. The aim of the study was to determine the parameters and models of phenol biodegradation kinetics in AF2B reactors using an indigenous bacterial consortium and compare the results of the experiment with the simulation model.

The kinetic model of phenol biodegradation in the biofilm can be found using a microenviroments approach from a substrate diffusion process in the biofilm that use boundary systems and initial conditions. Some assumptions used in determining the phenol biodegradation kinetics model, among others: (1) a stagnant layer covers the biofilm; (2) concentration of substrate within the biofilm are assumed to vary only in the direction normal to biofilm surface; (3) the substrate is transported from bulk liquid to biofilm phase

through the stagnant liquid layer by molecular diffusion; and (4) biofilm growth does not effected the flow pattern of liquid in a reactor [26,27]. Since phenol is an only growth-limiting substrate and all other nutrients are present in excess amounts and oxygen is sufficiently supplied so the phenol utilization rate in the biofilm based on diffusion (Fick's law) and biological inhibition reaction (Haldane kinetics) can be expressed as [25,28,29].

$$\frac{\partial S_f}{\partial t} = D_f \frac{\partial^2 S_f}{\partial z_f^2} - \frac{k S_f}{K_s + S_f + (S_f^2/K_i)} X_f$$

Where  $S_f$  is the concentration of phenol in the biofilm ( $M/L^3$ );  $D_f$  the diffusion coefficient of phenol in the biofilm ( $L^2/T$ );  $k$  the maximum utilization rate of phenol by biofilm ( $1/T$ );  $K_s$  the half-velocity coefficient of phenol by biofilm ( $M/L^3$ );  $K_i$  the inhibition constant for phenol ( $M/L^3$ );  $X_f$  the density of biofilm ( $M/L^3$ ); and  $z_f$  the radical distance in biofilm ( $L$ ).

As the phenol diffuses into and through the biofilm during biodegradation, the biofilm utilizes phenol as carbon source for biosynthesis and respiration. The biomass in the biofilm can increase or decrease with time until the growth rate is balanced by the decay rate and shear loss rate [26,27,30,31]. Since the density and volume of biofilm are assumed constant so the thickness of biofilm will be increase with time as the biofilm grows. Therefore, the phenol diffuses through a boundary which can be moving with time. The boundary is liquid/biofilm interface. Since the growth rate of biofilm can be expressed by the following equation [23]:

$$\frac{dL_f}{dt} = \int_0^{L_f} \left( \frac{YkS_f}{K_s + S_f + (S_f^2/K_i)} - b - b_s \right) dz_f$$

Where  $L_f$  is the biofilm thickness ( $L$ );  $Y$  the yield coefficient of biomass ( $M_x/M_s$ );  $b$  the decay coefficient of biomass ( $1/T$ ); and  $b_s$  the shear-loss coefficient of biofilm ( $1/T$ ). The initial biofilm thickness must be set to a small value in order that the biofilm can start grow.

A fixed biofilm reactor in which the kinetic model can be applied is a completely mixed biofilm reactor. All suspended biomass at the liquid/biofilm interface are exposed to the same phenol concentration [32]. The mass balance of phenol and suspended biomass in a fixed biofilm reactor can be described by the following equations [23].

$$\frac{dS_b}{dt} = \frac{Q}{V\varepsilon} (S_{b0} - S_b) - k_f (S_b - S_s) \frac{A}{V\varepsilon} - \frac{kS_b}{K_s + S_b + (S_b^2/K_i)} X_b$$

$$\frac{dX_b}{dt} = \left( \frac{YkS_b}{K_s + S_b + (S_b^2/K_i)} - b - \frac{Q}{V\varepsilon} \right) X_b + \frac{A}{V\varepsilon} b_s L_f X_f$$

Where  $S_s$  is the concentration of phenol in the bulk liquid ( $M/L^3$ );  $S_{b0}$  the concentration of phenol in the feed ( $M/L^3$ );  $S_s$  the concentration of phenol at liquid/biofilm interface ( $M/L^3$ );  $X_b$  the concentration of suspended biomass in the bulk liquid ( $M/L^3$ );  $Q$  the flow rate of the feed substrate ( $L^3/T$ );  $V$  the effective reactor volume ( $L^3$ );  $A$  the total surface area of media ( $L^2$ ); and  $\varepsilon$  the porosity of the reactor.

The Legendre polynomials (an even function) in planar geometry were used to approximate the exact phenol concentration profile in the

biofilm. The root of Legendre polynomials was used as the collocation points. The number of internal collocation points in biofilm was fixed at 6. The partial differential equation in dimensionless form can be converted to six ordinary differential equations by orthogonal collocation method. The entire model system including nine ordinary differential equations was solved by using Gear's method to determine the phenol concentration profile in biofilm, the growth of biofilm, phenol concentration in bulk liquid and concentration of suspended biomass in bulk liquid.

The parameters of kinetic for phenol biodegradation was performed in a batch reactor to evaluate yield coefficient (Y) and decay coefficient (b), as described by Haldane kinetic model. The concentration data of phenol and suspended biomass facilitate a priori estimation of kinetic parameters for evaluating the growth rate of suspended biomass and the utilization rate of phenol. The yield coefficient (Y) for suspended biomass is assumed approximately constant over the range of phenol concentration encountered in the growth phase. The yield coefficient (Y) for phenol utilizing bacteria can be determined by the following expression:

$$Y = - \frac{\Delta X}{\Delta S}$$

Where X is the increase of biomass concentration; and S the change in substrate concentration. The slope of a linearized plot for the increase in the concentration of suspended biomass (X - X<sub>0</sub>) versus decrease in phenol concentration (S<sub>0</sub> - S) is the yield coefficient. The data in the endogenous phase can be used for estimating the decay coefficient of suspended biomass. The decay coefficient (b) can thus be determined from the following equation

$$b = - \frac{\ln \left( \frac{X_2}{X_1} \right)}{t_2 - t_1}$$

Where t<sub>1</sub> and t<sub>2</sub> are the initial and final time; X<sub>1</sub> and X<sub>2</sub> are the initial and final biomass concentration, respectively. The slope of a linearized plot of ln X versus time in the endogeneous phase.

**EXPERIMENT**

**Supporting Media**

The media used for the growth of a bacterial consortium known as biofilter are wasp nest biofilter made of plastic material. Wasp nest biofilter has a large surface area for biomass accumulation and prevents plugging and maintains even air distribution conditions. The specifications of the wasp nest biofilter are as follows: density of 0.125 g/cm<sup>3</sup>, specific surface area of 150-240 m<sup>2</sup>/m<sup>3</sup>; The total volume of AF2B reactor continuously is 20 L[27].

**Bacterial consortium**

Bacterial consortium is a mixture of several types of bacteria obtained from the selection and isolation of bacteria found in hospital wastewater, namely from the initial reservoir of a Hospital Wastewater Treatment Plant (HWWTP). The bacterial consortium consists of Pseudomonas capica, Pseudomonas diminuta, Bacillus sp. While the nutrient composition used for biomass growth is Glucose (100 mg/L), Amonium phosphat (50 mg/L), Kalium phosphat (1 mg/L).

**Method of Analysis**

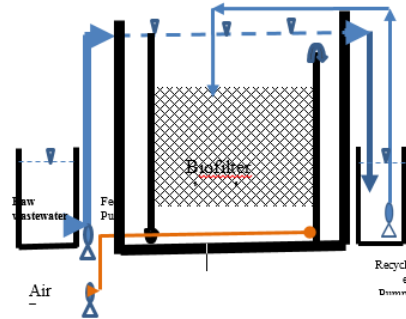
UV Vis (Brand: Hitachi U 2900) method is used to measure phenol. The membrane fiber method was used to measure the Volatile Suspended Solids (VSS) of the samples. Scanning Electron Microscope (SEM) was used to measure thickness of biofilms. This method is described in the section APHA of Standard Methods[34].

**Experiment System**

a. The experiments on AF2B reactor batches  
This experiments employed 2 L glass reactor batch contain biofilter shapes wasp nest. The consortium bacterial isolates are fed into reactor then added as much as 2 liters of distilled water, nutrient 5% (v) and aerated. Starter bacteria consortium that has been growing optimally in the reactor then added of phenol in variation concentration. Samples were withdrawn at the onset of the reactor for measurements of phenol and the concentration of suspended biomass at regular intervals.

b. Wastewater Treatment in the AF2B Reactor  
This experiment uses AF2B reactor which had a volume of 20 L as Fig

2. Reactor AF2B operates continuously and contains an biofilter shaped wasp nest and a bacterial consortium that has been acclimatized with the hospital wastewater. Set influent flowrate at a certain flowrate so as to have HRT for 5 hours and air flowrate of the blower at a certain flowrate and setting the recycle flowrate of 10% of the influent flowrate. Set the pH at normal conditions i.e: 6.5 - 7.5. The hospital wastewater containing phenol is fed into the reactor continuously then measuring phenol concentration in the effluent at certain time intervals.



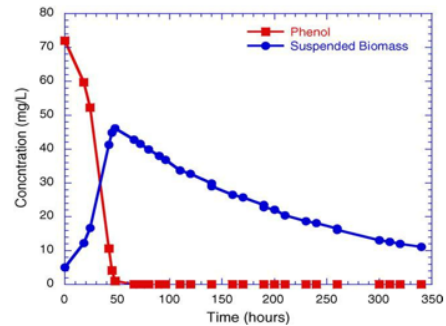
**Fig 1. Sketch an AF2B reactor**

**RESULT AND DISCUSSION**

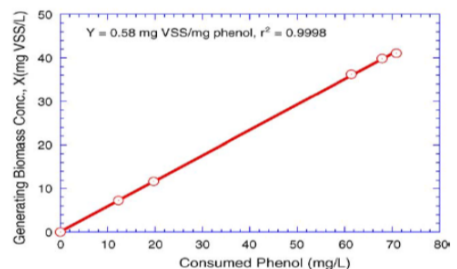
**Parameters Kinetic**

The batch kinetic test provided data on the variation of phenol and the concentration of suspended biomass versus time, as depicted in It can be seen that the data of suspended biomass represent a typical growth and decay curve with a well defined growth phase followed by a constant growth and endogeneous phase. On the other hand, the concentration of phenol stagnated in hours to 50 while the substrate concentration is at a maximum growth. This shows that phenol is biodegradable by a bacterial consortium to form a biomass where phenol is biodegradable along with the amount of biomass formed.

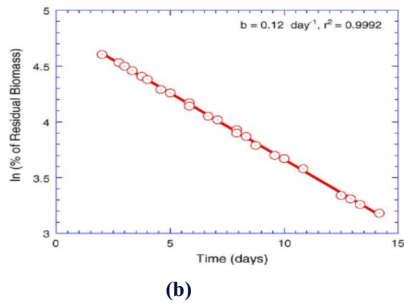
Furthermore, the experimental data in Fig. 2 was linearised and in the plot between the concentration of biomass produced with the concentration of phenol consumed. The slope of a linearized plot for the increase in the concentration of suspended biomass (X - X<sub>0</sub>) versus decrease in phenol concentration (S<sub>0</sub> - S) is the yield coefficient (Y) which was 0.58 mg VSS/mg phenol. The data in the endogenous phase can be used for estimating the decay coefficient of suspended biomass which decay coefficient (b) was 0.12 day<sup>-1</sup> shown in Fig. 3.



**Fig 2. Batch kinetic data on the variation of phenol and suspended biomass**



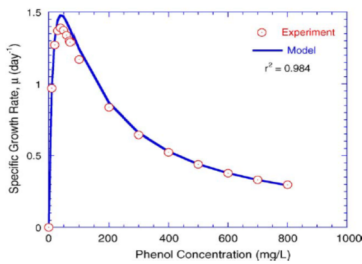
**(a)**



**Fig 3. Batch kinetic test to determine: (a) yield coefficient, and (b) Decay rate of suspended biomass**

Batch cultures of phenol-utilizing bacteria were conducted in media containing initial phenol concentrations ranging from 10 to 800 mg/L. The variation of specific growth rate ( $\mu$ ) with phenol concentration (S) obtained from batch tests are shown in The Haldane equation for substrate-inhibited growth was fitted to the data of specific growth rate of biomass ( $\mu$ ) as a function of phenol concentration (S) using a non-linear least-squares technique [25,31,33]. By using a non-linear least-squares regression analysis so a correlation coefficient ( $r^2$ ) of 0.984 was obtained. The data yielded the following parameters:  $\mu_{max} = 2.78 \text{ day}^{-1}$ ;  $K_s = 1.85 \text{ mg phenol/L}$ ;  $K_i = 94.3 \text{ mg phenol/L}$ .  $S_0$  that the biodegradation kinetics model of phenol in the AF2B reactor is as follows:

$$\mu = \frac{2.78 S}{1.85 + S + \frac{S^2}{94.3}}$$



**Fig 4. Specific growth rate of suspended biomass on phenol**

**Simulation model**

The model was verified by investigating phenol utilization by conducting an AF2B reactor with 72 mg/L phenol measured from hospital wastewater. The kinetic parameters of phenol biodegradation obtained from batch kinetic tests were used for model simulation.

The results of experiments using AF2B reactors that operate continuously using hospital wastewater containing phenol get data as shown in Fig 5.

(a) shows that the effluent of phenol varied with time. The curve of phenol concentration was described in three parts. First, the phenol concentration steadily increased to about 54 mg/L ( $0.75 S_{pp0}$ ) at 3 days. At this period of time, there was no significant biodegradation of phenol by biofilm. The phenol concentration curve was a typical dilute in curve, which was a characteristic of completely mixed bioreactor while the bioreactor was filled with clear water at time zero [29,30].

The second part of the phenol curve ran from 3 to 13 days, when the curves started to deviate from the top of dilute in curve. The effluent phenol concentration leveled off and then decreased. Apparently, biofilm was vigorously utilizing phenol during this period of time. Biofilm was also actively growing during this period.

The third part of phenol concentration curve ran from 13 to 40 days. At this period, the system reached a steady-state condition and the effluents of phenol was about 4 mg/L ( $0.06 S_{pp0}$ ). The removal efficiency for phenol was 98%. As can be seen, the experimental data are slightly higher than simulation results for the effluent of phenol at this period. One reason was the phenol degrading bacteria grew

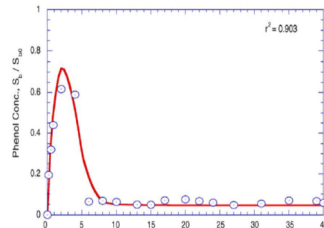
enough to form biofilm.

Thus, the effect of shear loss was more significant as biofilm became thicker. The higher shear loss of biofilm resulted in higher suspended biomass. The suspended biomass decomposed and released soluble microbial products (SMP) which slightly increased the effluent phenol concentration [1,10,31]. While biodegradation of phenol by a bacterial consortium in an AF2B reactor that operates in batches is 88% [27].

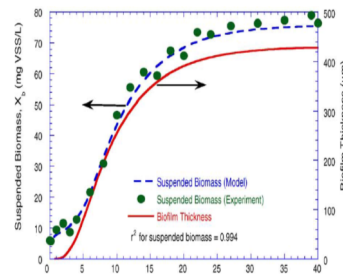
The growth curves of suspended and attached biomass were plotted in (b). One indicator of the generating biomass growth was the concentration of the suspended biomass in the effluent. A good agreement was observed between model simulation and actual experimental data for suspended biomass growth in the fixed biofilm reactor. The figure shows that a significant utilization of phenol by suspended biomass during the first 13 days. At this period, the suspended biomass actively utilized phenol for their growth.

The growth curve of suspended biomass steadily reached a steady-state condition from 13 to 40 days. The concentration of suspended biomass in the effluent was around 79 mg VSS/L at a maximum growth. The growth curve of biofilm by model prediction was also shown in Fig. 5 (b). As can be seen, the elapsed time required for biofilm to start to grow was about 2 days. The model predicted that the biofilm vigorously grew to utilize phenol at a transient-state period from 2 to 15 days. The growth of biofilm reached up to a maximum value of 430  $\mu\text{m}$  at a steady-state condition.

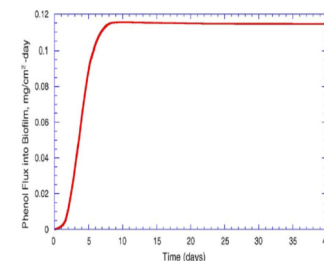
The model-predicted flux of phenol at different times was plotted in (c). In the beginning of the test, there was no significant phenol flux into the biofilm at the first 2 days because the growth of biofilm was negligible. The phenol flux was abruptly increased from 2 to 7 days. At this period, the biofilm grew thicker and vigorously consumed phenol. Thus, the difference of phenol concentration between bulk liquid phase and biofilm/liquid interface became higher, which significantly increased the phenol flux into biofilm due to biological activity. At the period of time from 7 to 40 days, the phenol flux maintained a maximum value because the phenol concentration in the effluent continued to decrease then reach a constant concentration at a steady-state condition [32].



(a)



(b)

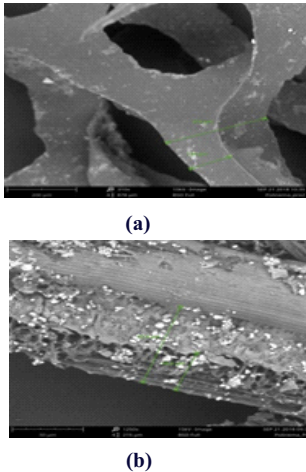


(c)

**Fig 5. Comparison of experimental data and model simulation: (a) phenol effluent concentration, (b) biomass concentration, (c) phenol flux into biofilm.**

#### **Bacterial consortium growth**

shows SEM photos of biomass trapped in the AF2B reactor contains bio-filter shaped wasp nest at different operating times. Rod and oval-shaped biomass could be distinctly identified from SEM photos at 0 and 5 days, respectively. There was no apparent difference in morphology at 0 and 5 days; however, the amounts of biomass at 5 days is greater than 0 days. The reactor system was in the exponential growth phase for biomass at 5 days, and then reached a constant growth phase for biomass at 40 days.



**Fig 6. SEM photo indicating the spatial distribution of biomass in the bio-filter wasp nest at different operating time: (a) 0 days, (b) 5 days.**

#### **CONCLUSIONS**

Phenol biodegradation kinetics model in a batch reactor has the equation:  $\mu = \frac{2.78 S}{1.85 + S + S^2/94.3}$

, which kinetic parameters:  $b = 0.12 \text{ day}^{-1}$ ;  $\mu_{\text{max}} = 2.78 \text{ day}^{-1}$ ;  $K_s = 1.85 \text{ mg phenol L}^{-1}$ ;  $K_i = 94.3 \text{ mg phenol L}^{-1}$ .

The model has been verified and developed in an AF2B reactor that operates continuously and shows good performance with phenol removal of 98%. Experimental and simulation results can be used to design phenol biodegradation in hospital wastewater at full scale.

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