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Kinetic Study of 4-Chlorophenol Biodegradation by Acclimated Sludge in a Packed Bed Reactor

Yen-Hui Lin

Department of Safety, Health and Environmental Engineering, Central Taiwan University of Science and Technology, 666, Bu-zih Road, Bei-tun District, Taichung 406053, Taiwan; yhlin1@ctust.edu.tw; Tel.: +886-4-22391647 (ext. 6861)

Abstract: In this study, batch experiments were conducted to evaluate the degradation of 4-CP using acclimated sludge. The Monod and Haldane models were employed to fit the specific growth rate with various initial 4-CP concentrations of 67–412 mg/L in the batch experiments. Haldane kinetics showed a better fit to experimental results than Monod kinetics. The kinetic parameters were obtained from a comparison of Monod and Haldane kinetics with batch experimental data. The values of μ_m and K_S were found to be 0.691 d^{-1} and 5.62 mg/L, respectively, for Monod kinetics. In contrast, the values of μ_m, K_S, and K_I were 1.30 d^{-1}, 8.38 mg/L, and 279.4 mg/L, respectively, for Haldane kinetics. The kinetic parameters in Haldane kinetics were used as input parameters for the kinetic model system of the packed bed reactor (PBR). The continuous flow PBR was conducted to validate the kinetic model system. The model-simulated results agreed well with experimental data in the PBR performance operation. At the steady-state stage, the removal efficiency of 4-CP was 70.8–96.1%, while the hydraulic retention time (HRT) was 2.5 to 12.4 h. The corresponding removal of 4-CP was assessed to be 94.6 and 96.1% when the inlet 4-CP loading rate was increased from 0.11 to 0.51 kg/m^3-d. The approaches of kinetic models and experiments presented in this study can be applied to design a PBR for 4-CP treatment in wastewater from the effluents of various industries.

Keywords: kinetic study; 4-chlorophenol; biodegradation; acclimated sludge; batch experiments; packed bed reactor

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1. Introduction

Chlorophenols that exist in industrial wastewater are discharged from industries such as pulp and paper, steel, oil refinery, pesticides, resin and dye, and pharmaceuticals [1–3]. Chlorophenolic compounds can cause carcinogen, teratogen, and mutagen [4,5]. Among chlorophenols, the effluent concentration of 4-chlorophenol (4-CP) from various industries ranges from 100 to 1000 mg/L [6]. Without proper treatment, the effluent may contaminate the soil and water bodies. 4-CP has been listed by the United States Environmental Protection Agency (USEPA) as one of the most precarious contaminants owing to its malignant property [7]. The toxicity of 4-CP can cause bioaccumulation, abnormal metabolism, and DNA damage to aquatic living organisms [8,9]. Even at low levels of approximately 3–4 mg/L, 4-CP can cause the death of aquatic animals [8]. Moreover, 4-CP provokes health risks to human beings, such as exhaustion, abnormalities, convulsion, and cancer [10,11]. Therefore, the USEPA set the 4-CP concentration level in natural water to less than 1 ppb. The toxicity of 4-CP and its persistence lead to adverse effects on the environment. Hence, technologies that can remove 4-CP from industrial wastewater are urgently needed.

Physical and chemical processes such as adsorption, solvent extraction, chemical oxidation, advanced oxidation, and photo-degradation have been successfully employed for 4-CP removal [12–14]. However, the drawbacks of these methods include high cost, complexity, and formation of undesirable by-products [15]. Biological treatment methods

are widely used to remove 4-CP because complete mineralization can be achieved. Additionally, these methods are less energy intensive, more environmentally friendly, and cost-effective, and are unlikely to form secondary toxic products [16–18].

Growth substrates such as phenol sodium salicylate (SA), and sodium glutamate (SG) were added to growth medium and induced enzymes to co-metabolize carbazole or 4-CP [19–22]. Loh and Yu [19] found that carbazole can only be degraded by *Pseudomonas putida* cells in the presence of SA. Most studies reported that 4-CP biodegradation by special strains can be achieved through the supplementation of phenol and SG as a growth substrate [20–22]. The supplementation of phenol, SA, or SG for special strains to degrade recalcitrant aromatic hydrocarbons, such as carbazole or 4-CP, increased the treatment cost and, furthermore, resulted in pollution [23]. Moreover, pure culture as a single organism may not be able to accomplish full mineralization of xenobiotic compounds due to the accumulation of toxic intermediates during biodegradation [24]. Therefore, the biodegradation of 4-CP using a mixed culture would become more significant and appropriate [25]. With an undefined mixed culture, such as the activated sludge, many kinds of species are cultivated simultaneously to form flocks. The biodegradation of chlorophenols using activated sludge without the addition of a special growth substrate would be more practical and cost-effective [23]. To enhance the capability of the activated sludge in degrading the chlorophenols, the acclimation of activated sludge is needed [26]. The acclimation of activated sludge has been proven to increase the capability in the biodegradation of phenolic and chlorophenolic compounds [26,27]. The acclimated activated sludge had a 1- to 2-fold faster degraded rate than pure strains for chlorophenol mixtures [27].

Bacterial immobilization has been extensively used as a typical approach for the biodegradation of organic contaminants present in industrial wastewater [28,29]. Various bioreactors with immobilized bacteria, including sequencing batch and airlift reactors, rotating biological contactors, moving bed biofilm reactors, and packed bed reactors, (PBRs) have been employed for 4-CP biodegradation [30,31]. Among various reactors, PBRs were considered the most convenient bioreactors for the removal of 4-CP present in water and wastewater [6]. The merits of PBRs include better reactor performance stability, less sludge production, no requirement for sludge recycling, high resistance to shock loading, avoidable washout, and higher biodegradation efficiency [32,33].

The goals of this work were to conduct the performance of PBRs packed with ceramic particles as the supporting media for biofilm attachment to treat 4-CP synthetic wastewater. The objectives were to (1) carry out batch experiments to determine the kinetic parameters of acclimated sludge in Monod and Haldane kinetics, (2) fit experimental data using Monod and Haldane models in the batch experiments, (3) select the kinetic parameters as the input parameters for PBRs from better-fit kinetics, (4) develop the kinetic model of PBRs under continuous flow conditions, (5) carry out the continuous flow experiments to verify the kinetic model system of PBRs, and (6) investigate the effect of hydraulic retention times (HRTs) and inlet 4-CP loading rates on 4-CP removal at the steady-state stage.

2. Kinetic Model

2.1. Kinetic Model for Batch Experiments

The Monod and Haldane models were applied to describe the growth of acclimated sludge for 4-CP biodegradation. The Monod model was stated as the following equation [34]:

$$\mu = \frac{1}{X}\frac{dX}{dt} = \frac{\mu_m S}{K_S + S} \quad (1)$$

where μ = specific growth rate (h^{-1}), X = biomass concentration (mg/L), μ_m = maximum specific growth rate (h^{-1}), S = 4-CP concentration (mg/L), and K_S = half-saturation constant

(mg/L). Under the inhibition circumstance, the specific growth rate of acclimated sludge can be represented by the following equation:

$$\mu = \frac{\mu_m S}{K_S + S + S^2/K_I} \tag{2}$$

where K_I = inhibition constant (mg/L).

2.2. Kinetic Model for PBR

The non-steady-state mass balance for 4-CP biodegradation using Haldane kinetics in the biofilm can be expressed by [35]:

$$\frac{\partial S_f}{\partial t} = \frac{D_f}{r_f^2}\frac{\partial}{\partial r_f}\left(r_f^2 \frac{\partial S_f}{\partial r_f}\right) - \frac{\mu_m X_f S_f}{Y\left(K_S + S_f + S_f^2/K_I\right)} \tag{3}$$

where S_f = 4-CP concentration in the biofilm, D_f = diffusion coefficient (cm^2/d), and r_f = coordinate distance in biofilm (μm).

The growth thickness of a biofilm at non-steady-state using Haldane kinetics can be expressed by:

$$\frac{dL_f}{dt} = \int_0^{L_f}\left(\frac{\mu_m S_f}{K_S + S_f + S_f^2/K_I} - b - b_s\right) X_f dr_f \tag{4}$$

where L_f = biofilm thickness (μm), b_s = biofilm shear-loss constant (d^{-1}), and X_f = biofilm concentration (mg SS/L).

The mass balance of 4-CP in the bulk liquid using Haldane kinetics can be written as:

$$\frac{dS_b}{dt} = \frac{Q}{V\varepsilon}(S_{b0} - S_b) - k_f(S_b - S_I)\frac{A}{V\varepsilon} - \frac{\mu_m S_b X_b}{Y\left(K_S + S_b + S_b^2/K_I\right)} \tag{5}$$

where Q = flow rate (cm^3/d), V = working volume (cm^3), ε = reactor porosity (dimensionless), A = area of media (cm^2), S_{b0} = 4-CP influent concentration (mg/L), S_b = 4-CP concentration in the bulk liquid phase (mg/L), k_f = mass transfer coefficient (cm^2/d), S_I = 4-CP concentration at the interface of the biofilm and bulk liquid phase (mg/L), and X_b = biomass concentration in the bulk liquid phase (mg SS/L).

The mass balance of the biomass concentration in the bulk liquid phase using Haldane kinetics can be written as:

$$\frac{dX_b}{dt} = \left(\frac{\mu_m S_b}{K_S + S_b + S_b^2/K_I} - k_d - \frac{Q}{V\varepsilon}\right) X_b + \frac{Ab_S L_f}{V\varepsilon} X_f \tag{6}$$

2.3. Kinetic Model Solution

The partial differential equation in Equation (3) was transformed into six ordinary differential equations using orthogonal collocation methods. Nine ordinary differential equations in the entire kinetic model system were simultaneously solved using Gear's method. The main and subroutine programs were coded in Fortran language and executed with a Fortran compiler in a Quadra Macintosh computer.

3. Materials and Methods

3.1. Nutrient and Minerals Salt Media

Synthetic wastewater containing 4-CP as well as nutrient and mineral salt media were prepared for batch and packed bed experiments. The ingredients of nutrient and minerals salt media were as follows (g/L) [36]: peptone (0.12), yeast extract (0.12), NaCl (0.007), $MgSO_4 \cdot 7H_2O$ (0.002), $CaCl_2 \cdot 2H_2O$ (0.004), $FeSO_4 \cdot 7H_2O$ (1.36), $Na_2MoO_4 \cdot 2H_2O$ (0.24), $CuSO_4 \cdot 5H_2O$ (0.25), $ZnSO_4 \cdot 7H_2O$ (0.58), $MnSO_4 \cdot H_2O$ (1.01), and $NiSO_4 \cdot 6H_2O$ (0.11). The

0.1 N NaOH and 0.1 N HCl were used to adjust the pH level to 7.0 ± 0.2 prior to each run of the tests [37]. Peptone and yeast extract were used as a carbon and nitrogen source during the batch and PBRs experiments where the biodegradation of 4-CP was sought in the presence of a primary substrate [23].

3.2. Inoculum Cultivation of Acclimated Sludge

The inoculum of 4-CP degrading bacteria was obtained from the activated sludge tank at the Shigangba Water Recycling Center (Taichung, Taiwan). The inoculum cultivation of 4-CP degrading bacteria was carried out in 250 mL Erlenmeyer flasks. The flasks contained 80 mL seed inoculum and 120 mL nutrient and mineral salt media with a nominal 4-CP concentration. The flask with 200 mL was then placed in a 30 °C rotary shaker moving at a rotary speed of 120 rpm. The feeding stepwise initial concentration of 4-CP at 3, 5, and 7 mg/L was used to acclimate the activated sludge for further use.

3.3. Batch Experiments

Batch experiments were carried out to determine the kinetic parameters of maximum specific growth rate (μ_m), half-saturation constant (K_S), inhibition constant (K_I), yield coefficient (Y), and decay coefficient (b). Batch tests were performed in a 5 L glass batch reactor containing 4 L nutrient medium. The various initial 4-CP concentrations of 67, 94, 158, 206, 245, 315, 357, and 412 mg/L and the initial biomass concentration of 116 mg SS/L were prepared in the batch reactor, respectively. Each batch experiment was conducted at 30 °C using a water jacket [37]. The pH was controlled at 7.0 ± 0.1 using 0.1 M NaOH.

3.4. Supporting Media

A ceramic particle with an average diameter of 1.14 cm was chosen as a supporting medium because its high surface area is easy for biomass accumulation and it is an inert medium. The adsorptive experiments were carried out to observe the adsorptive capability of ceramic particles with an initial 4-CP concentration of 200 mg/L. The experimental results indicated that no adsorptive phenomena occurred.

3.5. PBR Experiments

Figure 1 shows the PBR experimental setup. The PBR is packed with ceramic particles with an average diameter of 1.14 cm. The packed volume was 840 mL in the 1.568 L PBR. The acclimated activated sludge as inoculum was added into the nutrient medium and then injected into the bioreactor until it reached the effluent port where the influent was stopped. The peristaltic pump with a high recycle flow rate was used to mix the inoculum completely for 72 h and allow the inoculum to attach onto the ceramic particles. The feed tank containing 4-CP and nutrient medium was pumped into the PBR with a flow rate of 2.11–6.33 mL/min. A recycle flow with a recycle ratio of 15:1 was employed to maintain a completely mixed condition in the PBR. The effluent samples were collected at intervals of 1–4 days to measure the biomass and 4-CP concentrations. To maintain the PBR temperature at 30 ± 0.2 °C, the PBR was encased in a water jacket with a water bath. The hydraulic retention time was maintained at 4.1–12.4 h and the inlet 4-CP loading rate increased from 0.11 to 0.51 kg/m^3-d.

3.6. Analytical Methods

The 4-CP was measured using HPLC-UV instruments that contain an Alliance 2695 liquid chromatograph, Waters 2027 auto-sampler, and 2487 UV/Vis detector. The HPLC was equipped with a Symmetry® C18 column (250 nm × 4.6 nm, 5 µm) for 4-CP elution. The UV/vis detector was set at 254 nm. A mixture of potassium phosphate (50 mM) and acetonitrile (70/30, v/v) was employed to elute 4-CP at a flow rate of 1 mL/min [38]. The injection volume was 6 µL. The calibration equation for 4-CP was as follows:

4-CP (mg/L) = $8 \times 10^{-4} \times$ (Area) $- 3.3099$, $R^2 = 0.9999$. The concentrations of suspended solids (SS) were measured according to the standard procedure [39].

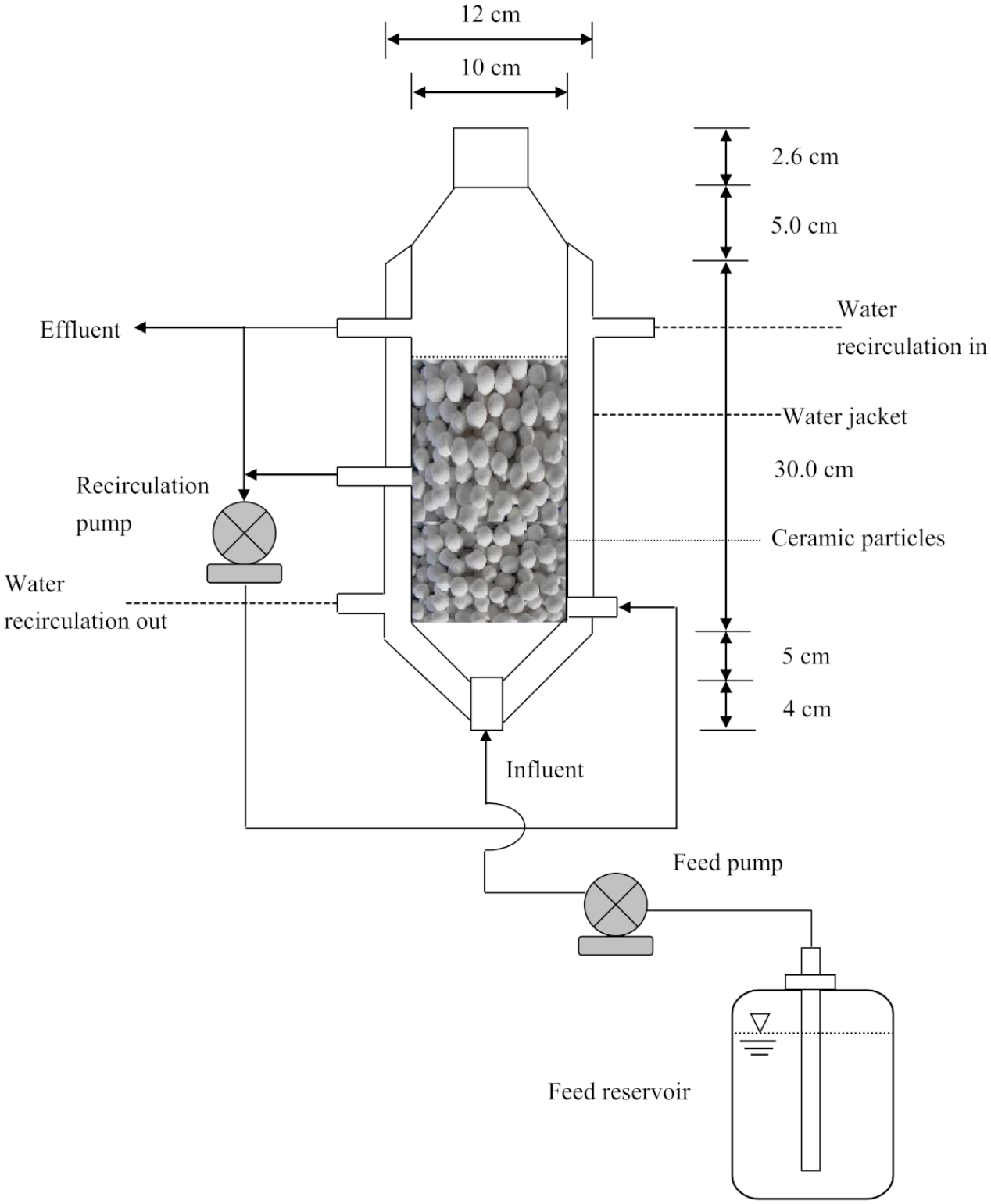
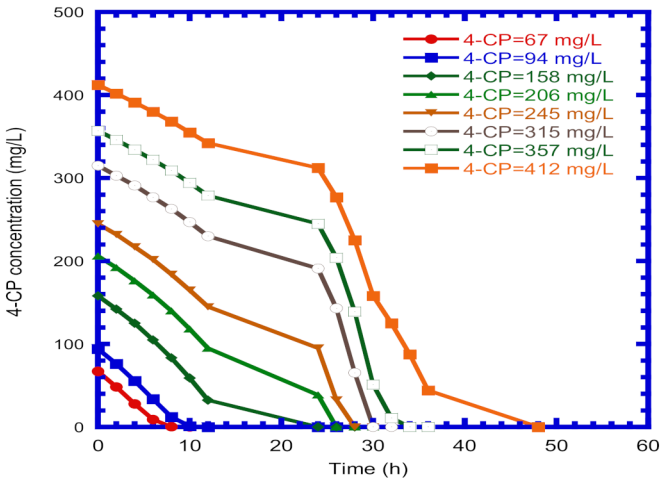


Figure 1. Laboratory-scale packed bed reactor.

4. Results and Discussion

4.1. Degradation of 4-CP and Biomass Growth

The degradation of 4-CP by acclimated sludge at an initial concentration of 67–412 mg/L is presented in Figure 2a. At the beginning of the batch experiments, the degradation rate for 4-CP was nearly the same under various initial concentrations, which indicated the activated sludge was successfully acclimated with feeding 3, 5, and 7 mg/L 4-CP. It was observed that the complete degradation for 4-CP took place during 8–48 h under different initial 4-CP concentrations of 67–142 mg/L. Sahinkaya and Dilek [23] used batch reactors to examine the degradation of 4-CP using unacclimated and acclimated activated sludge. The results of their study indicated that no adverse effect on COD removal by acclimated sludge was found although an increase in 4-CP concentration up to 300 mg/L. However, a considerable decrease in COD removal by unacclimated sludge was observed. They also found that complete removal of 4-CP was achieved with acclimated sludge at an initial concentration of 4-CP up to 300 mg/L. The time-course variation of biomass growth on 4-CP is plotted in Figure 2b. It was found that the biomass growth rate for various 4-CP initial concentrations was slightly different at the onset of the experiments. The biomass growth profiles were followed by log-growth, constant-growth, and decay phases. The time required for maximum biomass concentration was observed from 10 to 48 h at initial 4-CP concentrations of 67–412 mg/L. A constant decay rate was also found under different initial 4-CP concentrations.



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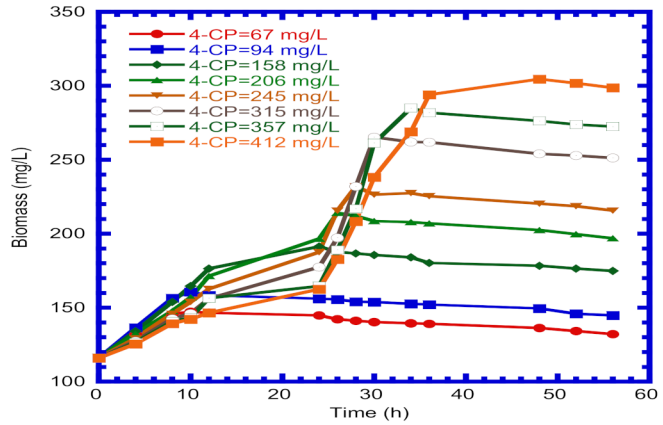


(**b**)

Figure 2. Batch experiments to observe the time-course variations of 4-CP and acclimated sludge: (**a**) 4-CP degradation; (**b**) biomass growth.

4.2. Kinetic Parameters

The specific growth rate against the initial concentration of 4-CP is illustrated in Figure 3. As displayed in Figure 3, the increase in the value of the specific growth rate made the initial 4-CP concentration increase up to a concentration level of 94 mg/L. The specific growth rate then began to decrease with the increase in initial 4-CP concentration. This indicated that the 4-CP is an inhibitory-type compound. A non-linear regression method was used to determine the kinetic parameters in Monod kinetics. The kinetic parameters, μ_m and K_S, for Monod kinetics were found to be 0.691 d^{-1} and 5.62 mg/L, respectively, with a correlation coefficient (R^2) of 0.711 (Figure 3a). The comparison of experimental results with Haldane's model showed a satisfactory agreement. The Haldane model (R^2 = 0.711) exhibited a better fit to experimental data than the Monod model (R^2 = 0.856) because Monod kinetics did not account for the endogenous phase during the whole growth phase due to 4-CP inhibition. However, Haldane kinetics effectively described the 4-CP inhibition on microbial growth. The values of kinetic parameters (μ_m, K_S, K_I) in Haldane's kinetics were evaluated using a non-linear regression technique. The respective values of μ_m, K_S, and K_I, were found to be 1.30 d^{-1}, 8.38 mg/L, and 279.4 mg/L with an R^2 of 0.856 (Figure 3b). Assadi et al. [25] carried out an aerobic sequencing batch reactor (SBR) to investigate the biodegradation of 4-CP by acclimated-mixed sludge. Their experimental results indicated that 4-CP biodegradation was inhibited at a critical concentration of 74.5 mg/L. In this study, microbial growth was inhibited at a critical concentration of 94 mg/L.

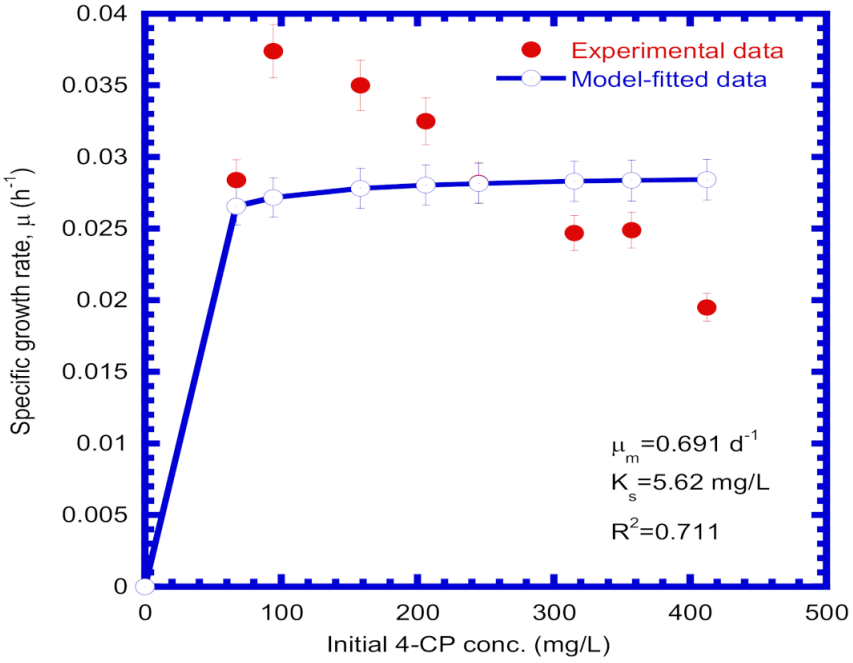
To determine the growth yield of biomass concentration (as SS) on 4-CP, the batch experiments were conducted till 4-CP initially present was fully consumed. The value of biomass concentration presented just at the end of the log-growth phase was used in computing the generated biomass concentration as a result of consumption of 4-CP [40] The value of biomass growth yield (Y) was calculated from the batch experimental results of all initial 4-CP concentrations. The value of biomass produced at the end of the log-growth phase versus 4-CP consumption was used to determine the value of Y. The calculated equation can be expressed as

$$Y = \frac{X - X_0}{S_0 - S} \tag{7}$$

Figure 4 illustrates the value of Y determined from the slope of (X-X_0) versus (S_0-S) by conducting linear regression under eight different initial 4-CP concentrations in the batch experiments. The value of Y obtained in this study was 0.472 ± 0.006 mg SS/mg 4-CP. The declined curve of biomass concentration in the endogenous phase after the complete depletion of 4-CP was used to calculate the decay coefficient (b). The computed equation can be represented by

$$b = -\frac{\ln(X_2/X_1)}{t_2 - t_1} \tag{8}$$

In order to evaluate the value of b, the growth batch experiments were not stopped; rather, the measurement of biomass concentration (as SS) was continued further for another 46 h even after complete degradation of 4-CP [40]. The batch growth curves extended to the decay phase (Figure 2b) for determining b. The data on the decay phase were plotted as ln (biomass concentration) versus time. Figure 5 shows the negative slopes determined from various plots of ln (X_2/X_1) versus operation time by linear regression. The value of b obtained was 0.0540 ± 0.0047 d^{-1}. Konya et al. [41] developed a mathematical model for the activated sludge unit, treating 4-chlorophenol containing synthetic wastewater with a 4-CP content of 500 mg/L. Model-predicted and experimental data were compared to estimate the specific rate constant (k), saturation constant (K_S), inhibition constant (K_I), yield growth (Y), and decay coefficient (b). The values of k, K_S, K_I, Y, and b in their study were 1.44 d^{-1}, 25 mg/L, 17 mg/L, 0.206 g X/g 4-CP, and 4.7 × 10^{-5} d^{-1}, respectively. The kinetic constants determined in their study were different from those obtained in this study due to different supplements of nutrient media in the experiments.



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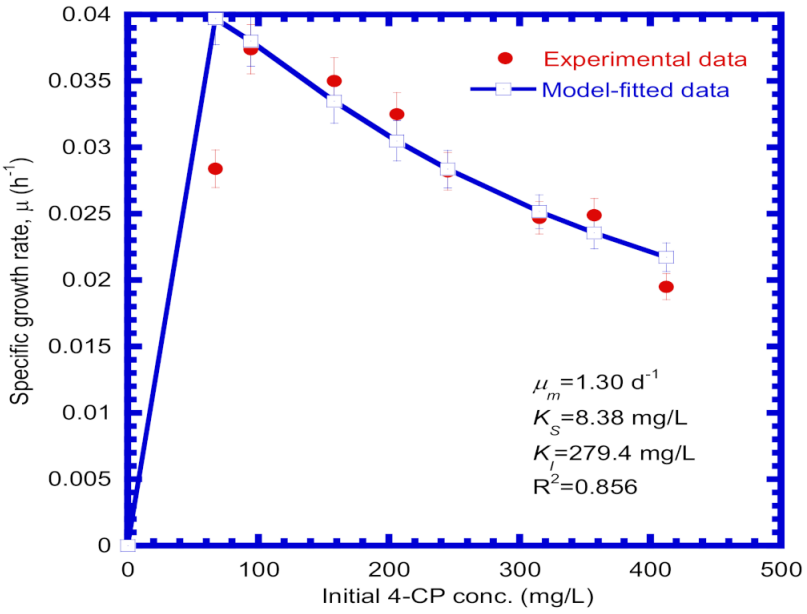


(**b**)

Figure 3. Comparison of experimental and model-fitted data to determine kinetic parameters: (**a**) Monod kinetics and (**b**) Haldane kinetics. The experimental data presented are means of three replicates.

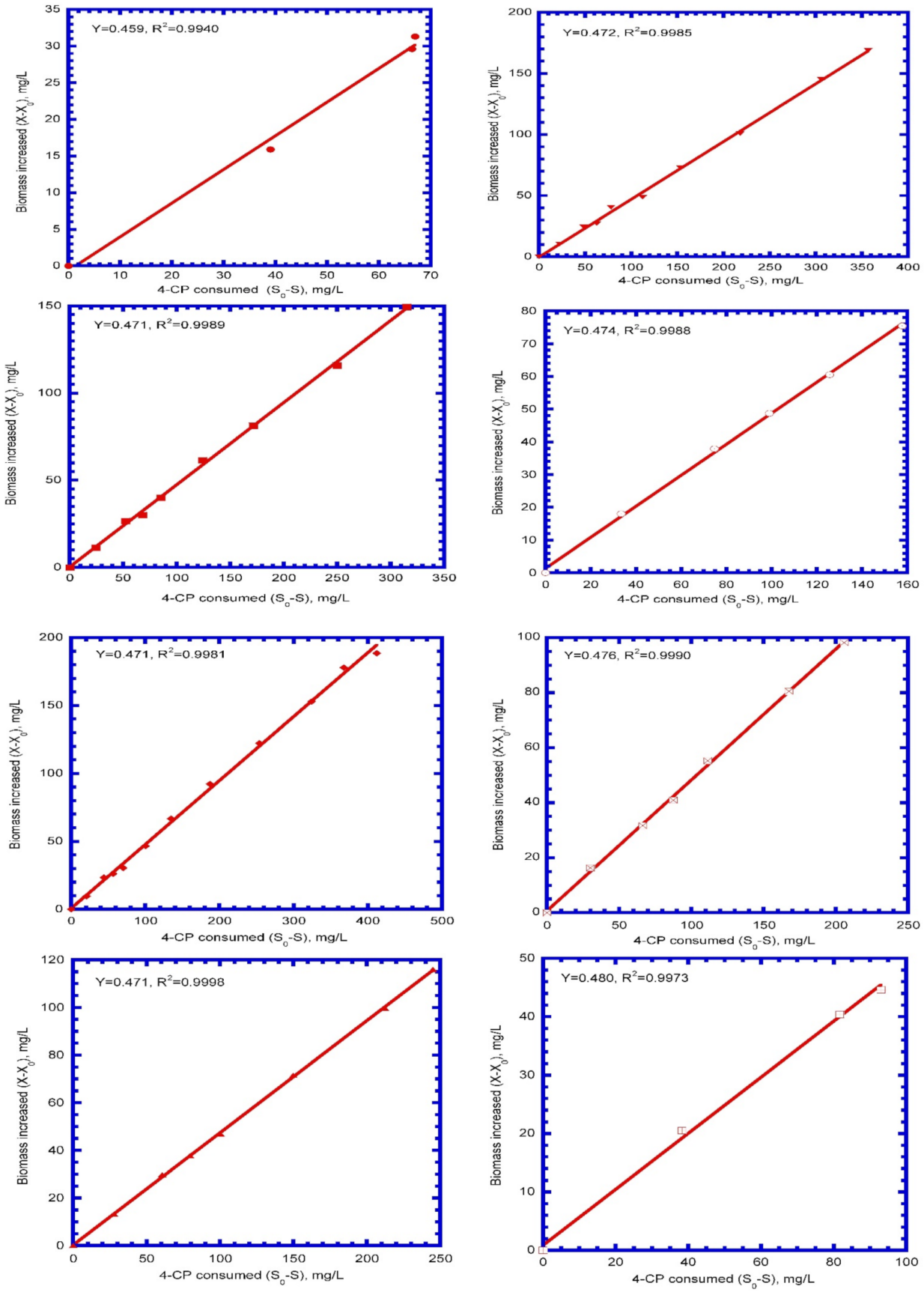


Figure 4. Batch experiments to determine the growth yield (*Y*) at various 4-CP initial concentrations.

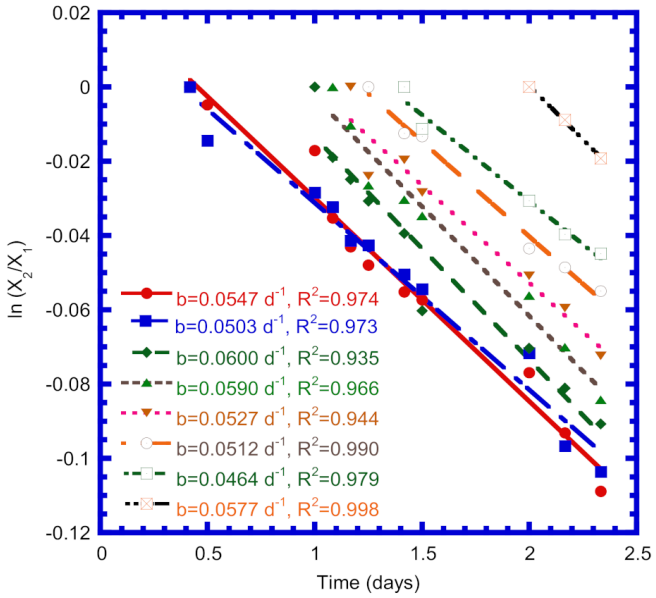


Figure 5. Batch experiments to determine the decay constants (*b*).

4.3. Mass Transfer Coefficients

The diffusion coefficient in biofilm (D_f) is reported to be 0.2–1.0 times as that in water (D_w) [42]. The molecular diffusion coefficient of 4-CP in water was estimated by the empirical formula developed by Wilke and Chang [43]. A ratio of 0.8 (D_f/D_w) was used to estimate the diffusion coefficient of 4-CP in biofilm [44]. The value of D_f for 4-CP in biofilm was 0.684 cm^2/d. The 4-CP mass transport from the bulk liquid across the diffusion layer to the biofilm surface can be defined by

$$k_f = \frac{j_D u}{S_C^{2/3}} \tag{9}$$

where u is the flow velocity, and S_c is the Schmidt number. The j_D-factor can be computed by the following equation [45]:

$$j_D = \frac{0.765}{\varepsilon(R_e{}^{0.82})} + \frac{0.365}{\varepsilon(R_e{}^{0.386})} \tag{10}$$

where R_e is Reynolds number. The above formula is applied to a *Re* range of 0.01–15,000. The k_f value was obtained as 282.5 cm/d.

4.4. 4-CP Degradation in PBR

Table 1 presents the kinetic and operational conditions as the input parameters for the kinetic model system of the PBR. Figure 6a illustrates the 4-CP effluent concentration varied against operation time over 50 days. At the initial stage, the 4-CP effluent concentration peaked at 234.1 mg/L (S_b/S_{b0} = 0.949) due to the diluted characteristics of the 4-CP feed. At the subsequent stage, the effluent curve leveled off and deviated from the peak of the curve during the transient period. At the third stage, the 4-CP degradation ran from 20 to 50 days. During this period, a steady-state condition in PBR was achieved. The 4-CP effluent concentration at the steady state was approximately 9.70 mg/L (S_b/S_{b0} = 0.0393). The attached and suspended biomasses grew rapidly during this period. It was observed that the model simulation fitted the experimental results well during the entire operation time with a correlation coefficient of 0.939. Eker and Kargi [46] used a rotating tubes biofilm reactor (RTBR) to observe the treatment of 4-CP containing wastewater at a high A/Q (biofilm surface/flow rate) ratio and feed COD concentration. The finding of their study

reported that more than 95% 4-CP and toxicity removals were achieved with an A/Q ratio of 186 m^2 d m^{-3}, and feed COD of 6000 mg/L with the feed 4-CP of 1000 mg/L.

Table 1. Input parameters for kinetic model system of packed bed reactor.

Symbol	Kinetic Parameters (Unit)	Value
S_{b0}	Influent concentration of 4-CP (mg/L)	246.7
μ_m	Maximum specific growth rate (d^{-1})	1.30
K_S	Half-saturation constant (mg/L)	8.38
K_I	Inhibition constant (mg/L)	279.4
Y	Biomass yield (mg SS/mg 4-CP)	0.472
b	Decay constant (d^{-1})	0.054
b_s	Shear-loss coefficient (d^{-1})	0.223
D_f	Diffusion coefficient (cm^2/d)	0.684
k_f	Mass transfer coefficient (cm/d)	282.5
L_{f0}	Initial biofilm thickness (μm)	0.75
X_f	Biofilm density (mg/mL)	8.86
X_{b0}	Initial suspended biomass (mg SS/L)	8.9
Q	Influent flow rate (mL/d)	3038–15,192
V	Working volume (mL)	1568
ε	Reactor porosity (dimensionless)	0.46

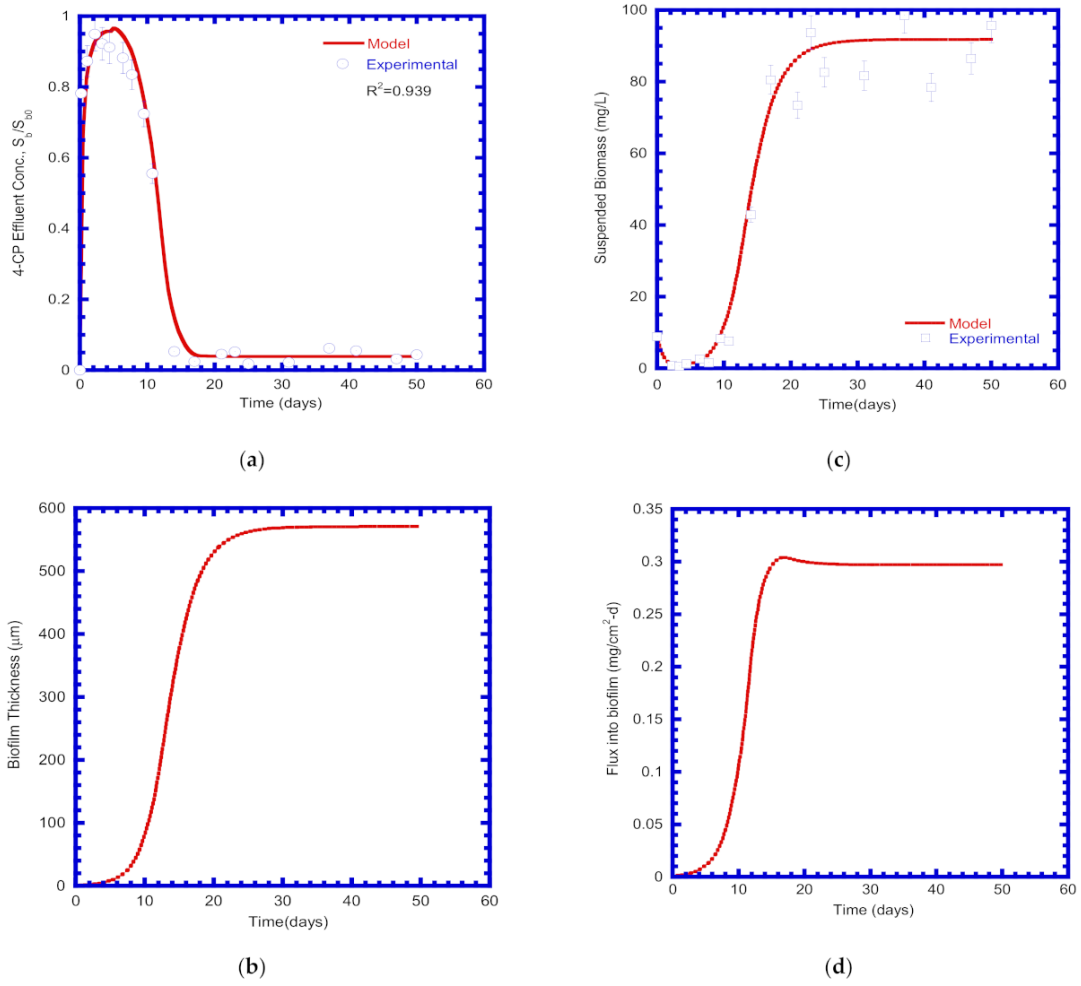


Figure 6. Comparison of experimental results and model prediction: (**a**) 4-CP effluent, (**b**) biofilm growth, (**c**) suspended biomass growth and (**d**) Flux diffused into biofilm.

4.5. Biomass Growth

Figure 6b shows the biofilm growth during PBR operation at an HRT of 12.4 h. After completing the startup lag phase, the vigorous growth of biofilm was observed during the non-steady-state period of 8–20 days. The biofilm thickness reached a maximal value of 570.8 μm at the steady-state performance.

The suspended solids (SS) concentration was detected to evaluate the growth of suspended biomass during the PBR operation (Figure 6c). The effluent curve of SS followed the lag phase, log-growth phase, and steady-state growth phase. The lag stage lasted about 8 days before the suspended biomass started to grow. The abrupt increase in SS content during the transient period (8–20 days) indicated the vigorous growth of suspended biomass. The maximum value of SS content at the steady state period (20–50 days) was approximately 92 mg SS/L. Model-simulated and experimental data showed a satisfactory agreement with each other.

4.6. Flux Diffused into Biofilm Layer

The variation of model-simulated flux against operational time in the PBR performance is plotted in Figure 6d. At the onset of biodegradation, the flux value was very small, indicating that 4-CP degradation by attached biomass was negligible during the lag phase. In sequence, the flux increased abruptly with time, which represented the attached biomass actively mineralizing 4-CP to lower its influent concentration. The flux of 4-CP was then maintained at a constant value because the PBR performance reached a steady-state condition. The flux diffused into biofilm was about 0.297 mg/cm^2-d at the steady-state condition.

4.7. 4-CP Concentration Profiles in Biofilm and Liquid Film

The kinetic model for 4-CP concentration profiles in biofilm and liquid film layers was described in Equation (3). Equation (3) was solved by numerical methods to obtain the model-simulated values. Figure 7 shows the model simulated with the variation of 4-CP concentration profiles against the biofilm and liquid film layers at different operation times. The 4-CP concentration of 184.3 mg/L (S_f/S_{b0} = 0.7470) in bulk liquid was dropped to 183.9 mg/L (S_f/S_{b0} = 0.7454) at a transient period of 10.58 d (Figure 7a). A concentration profile curve was observed in the biofilm layer, indicating that diffusion resistance occurred in the biofilm layer. At the surface of the supporting medium, the 4-CP concentration was 183.4 mg/L (S_f/S_{b0} = 0.7435). It was observed that the 4-CP concentration decreased from 14.06 (S_f/S_{b0} = 0.057) to 12.93 mg/L (S_f/S_{b0} = 0.0524) in the liquid film at an operation time of 13 d (Figure 7b). A curve of concentration profile was observed from the interface of the liquid film and biofilm to the surface of the supporting medium. The 4-CP concentration at the surface of the ceramic particle was 7.28 mg/L (S_f/S_{b0} = 0.0295). At a steady-state period of 40 days, the 4-CP concentration of 9.62 mg/L (S_f/S_{b0} = 0.039) in the bulk liquid phase was decreased to 8.61 mg/L (S_f/S_{b0} = 0.0349) at the interface of liquid film and biofilm phases (Figure 7c). Moreover, a typical concentration profile curve was observed in the biofilm layer with a low 4-CP concentration of 0.59 mg/L (S_f/S_{b0} = 0.0024) at the wall of the supporting medium.

4.8. The Effect of Operation Parameters on 4-CP Removal Efficiency

The effect of HRTs and inlet organic loading rates on 4-CP removal efficiency is shown in Figure 8. The PBR was operated until the steady-state behavior was achieved. At the steady state, the influent flow rate was adjusted to obtain the different HRT at 12.4, 6.2, 4.2, 3.1, and 2.5 h with an initial 4-CP concentration of 246.7 mg/L. The corresponding 4-CP removal efficiencies varied with HRT, as shown in Figure 8a. The 4-CP removal efficiency increased as the HRT increased, indicating that higher HRT increased 4-CP degradation in PBR. The corresponding removal efficiency of 4-CP ranged from 70.8 to 96.1% as the HRT was maintained at 2.5–12.4 h. The high removal efficiency of 4-CP was attributed to sufficient retention time for effective biomass growth to degrade 4-CP [47]. Figure 8b

presents the effect of 4-CP inlet loading rates on 4-CP effluent concentration and removal efficiencies. The increase in inlet loading rates increased the 4-CP effluent concentration. Further, the corresponding removal efficiency of 4-CP was 94.6–96.1%, while the inlet 4-CP loading rate was 0.11–0.51 kg/m^3-d. The change in 4-CP inlet loading rates did not affect the removal efficiencies significantly because the steady-state condition in the PBR system was achieved. Swain et al. [37] operated a moving bed and packed bed bioreactor to investigate the removal of 4-CP by the Bacillus consortium isolated from the petroleum refinery. Their experimental results revealed that the maximum removal efficiencies were obtained as 94.34 and 90.62% at an inlet 4-CP loading rate of 0.025 kg/m^3-d in a moving bed bioreactor (MBBR) and packed bed bioreactor (PBBR), respectively. However, the removal efficiencies of 4-CP were decreased to 50.83 and 43.67% for MBBR and PBBR, respectively, while the inlet 4-CP loading rate was increased up to 0.167 kg/m^3-d.

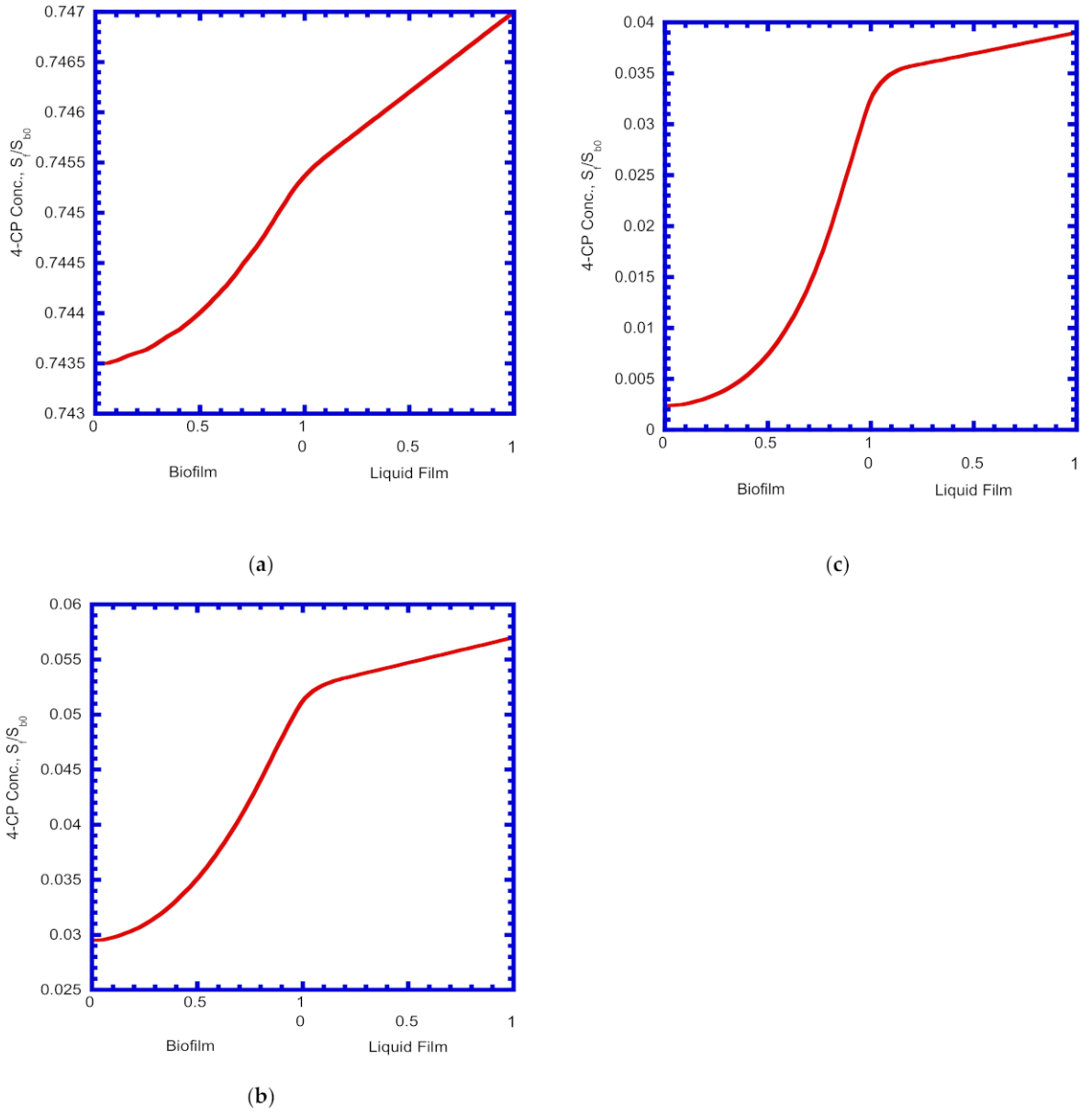
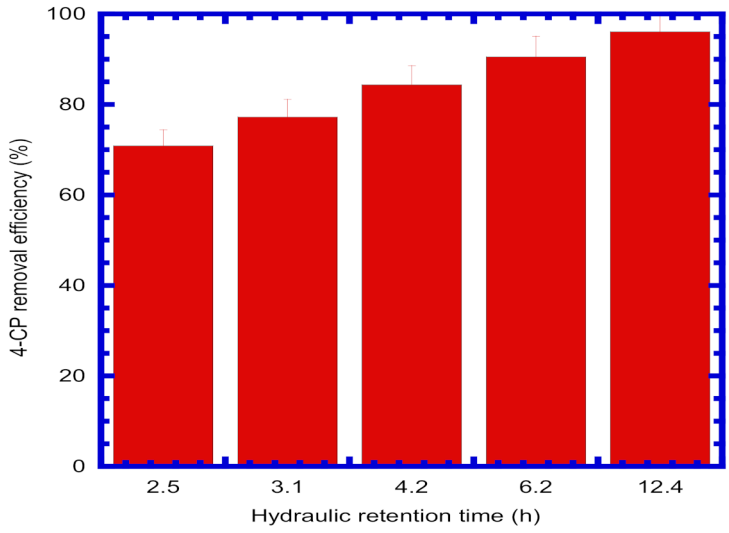
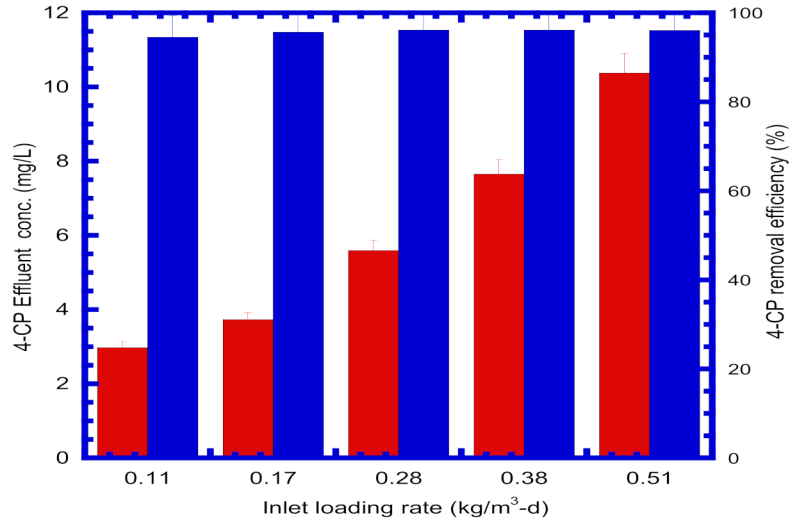


Figure 7. 4-CP concentration profile in the liquid film and biofilm at (**a**) 10.58 days, (**b**) 13 days, and (**c**) 40 days.



(**a**)



(**b**)

Figure 8. The effect of operation parameters on 4-CP removal efficiency at the steady state (**a**) hydraulic retention times (**b**) inlet loading rates.

5. Conclusions

The Monod and Haldane kinetics were used to evaluate the growth of 4-CP degrading biomass and biodegradation. The compared results of batch experiments and kinetic models indicated that the Haldane kinetics had a better fit to the experimental data than the Monod kinetics did. In the batch experiments, the best-fitted kinetic parameters obtained from the comparison of the Haldane model and experimental data were applied as the input parameters of the kinetic model system of PBRs. The performance of PBRs packed with ceramic particles as supporting media was estimated for 4-CP removal and validated the kinetic model system of PBRs. The kinetic model system fitted with the experimental results of 4-CP effluent concentrations from unsteady- to steady-state stages well with a high correlation coefficient of 0.939. With the increase in HRT, the removal efficiency of 4-CP was increased with increasing the HRT. Further, the removal efficiency of 4-CP at the steady-state stage remained at 94.6–96.1% as the inlet 4-CP loading rate was increased from 0.11 to 0.51 kg/m^3-d. The PBR filled with ceramic particles as supporting media for biofilm attachment could be employed to treat 4-CP effluent from the discharge of various industries.

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References

1. Movahedyan, H.; Assadi, A.; Amin, M. Effects of 4-chlorophenol loadings on acclimation of biomass with optimized fixed time sequencing batch reactor. *J. Environ. Health Sci. Eng.* **2008**, *5*, 225–234.
2. Dey, A.; Sarkar, P.; Das, A. View of studies on biodegradation of 4-chlorophenol and 4-nitrophenol by isolated pure cultures. *Eur. J. Sustain. Dev.* **2019**, *8*, 281. [CrossRef]
3. Azizi, E.; Abbasi, F.; Baghapour, M.A.; Shirdareh, M.R.; Shooshtarian, M.R. 4-chlorophenol removal by air lift packed bed bioreactor and its modeling by kinetics and numerical model (artifical neural network). *Sci. Rep.* **2021**, *11*, 670. [CrossRef] [PubMed]
4. Arora, P.K.; Bae, H. Bacterial degradation of chlorophenols and their derivatives. *Microb. Cell Factories* **2014**, *13*, 31. [CrossRef]
5. Gupta, P.; Sreekrishnan, T.R.; Shaikh, Z.A. Evaluating the effects on performance and biomass of hybrid anaerobic reactor while treating effluents having glucose with increasing concentrations of 4-chlorophenols. *J. Environ. Chem. Eng.* **2018**, *6*, 2643–2650. [CrossRef]
6. Patel, N.; Shahane, S.; Bhunia, B.; Mishra, U.; Chaudhary, V.K.; Srivastav, A.L. Biodegradation of 4-chlorophenol in batch and continuous packed bed reactor by isolated Bacillus subtilis. *J. Environ. Manag.* **2022**, *301*, 113851. [CrossRef]
7. Sandhibigraha, S.; Mandal, S.; Awasthi, M.; Bandyopadhyay, T.; Bhunia, B. Optimization of various process parameters for biodegradation of 4-chlorophenol using Taguchi methodology. *Biocatal. Agric. Biotechnol.* **2020**, *24*, 101568–101577. [CrossRef]
8. Farah, M.A.; Ateeq, B.; Ali, M.N.; Sabir, R.; Ahmad, W. Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms. *Chemosphere* **2004**, *55*, 257–265. [CrossRef]
9. Dizicheh, A.A.; Bayat, M.; Alimohmmadi, M.; Hashemi, J. Survey bioremediation of 4-chlorophenol by yeast and mold isolated from industrial and petroleum wastewaters (Imam Khomeini seaport, Mahshahr). *Int. J. Mol. Clin. Microbiol.* **2018**, *8*, 957–966.
10. Kwean, O.S.; Cho, S.Y.; Yang, J.W.; Cho, W.; Park, S.; Lim, Y.; Shin, M.C.; Kim, H.S.; Park, J.; Kim, H.S. 4-chlorophenol biodegradation facilitator composed of recombinant multi-biocatalysis immobilized onto montmorillonite. *Bioresour. Technol.* **2018**, *259*, 268–275. [CrossRef]
11. Swain, G.; Sonwani, R.K.; Singh, R.S.; Jaiswal, R.P.; Rai, B.N. Removal of 4-chlorophenol by Bacillus flexus as free and immobilized system: Effect of process variables and kinetic study. *Environ. Technol. Innov.* **2021**, *21*, 101356. [CrossRef]
12. Kurniawan, T.A.; Lo, W.H.; Sillanpää, M.E. Treatment of contaminated water laden with 4-chlorophenol using coconut shell waste-based activated carbon modified with chemical agents. *Sci. Sep. Technol.* **2011**, *46*, 460–472. [CrossRef]
13. Yang, H.; Ye, S.; Zeng, Z.; Zeng, G.; Tan, X.; Xiao, R.; Wang, J.; Song, B.; Du, L.; Qin, M.; et al. Utilization of biochar for resource recovery from water: A review. *Chem. Eng. J.* **2020**, *397*, 125502. [CrossRef]
14. Xie, S.; Li, M.; Liao, Y.; Qin, Q.; Sun, S.; Tan, Y. In-situ preparation of biochar loaded particle electrode and its application in the electrochemical degradation of 4-chlorophenol in wastewater. *Chemosphere* **2021**, *273*, 128506. [CrossRef] [PubMed]
15. Erol Nalbur, B.; Alkan, U. The inhibitory effects of 2-CP and 2,4 DCP containing effluents on sequencing batch reactors. *Int. Biodeterior. Biodegrad.* **2007**, *60*, 178–188. [CrossRef]
16. Sahinkaya, E.; Dilek, F.B. Biodegradation kinetics of 2,4-dichlorophenol by acclimated mixed culture. *J. Biotechnol.* **2007**, *127*, 716–726. [CrossRef]
17. Wang, Q.; Li, Y.; Li, J.; Wang, Y.; Wang, C.; Wang, P. Experimental and kinetic study on the cometabolic biodegradation of phenol and 4-chlorophenol by psychrotrophic *Pseudomonas putida* LY1. *Environ. Sci. Pollut. Res.* **2015**, *22*, 565–573. [CrossRef]
18. Li, S.; Ma, B.; She, Z.; Guo, L.; Zhao, Y.; Jin, C.; Gao, M. Effect of norfloxacin on performance, microbial enzymatic activity and microbial community of a sequencing batch reactor. *Environ. Technol. Innov.* **2020**, *18*, 100726. [CrossRef]
19. Loh, K.C.; Yu, Y.G. Kinetics of carbazole degradation by Pseudomonas putida in presence of sodium salicylate. *Water Res.* **2000**, *34*, 4131–4138. [CrossRef]
20. Wang, S.J.; Loh, K.C. Growth kinetics of *Pseudomonas putida* in cometabolism of phenol and 4-chlorophenol in the presence of a conventional carbon source. *Biotechnol. Bioeng.* **2000**, *68*, 437–447. [CrossRef]
21. Hao, O.J.; Kim, M.H.; Seagren, E.A.; Kim, H. Kinetics of phenol and chlorophenol utilization by *Acinetobacter* species. *Chemosphere* **2002**, *46*, 797–807. [CrossRef]
22. Gąszczak, A.; Bartelmus, G.; Rotkegel, A.; Greñ, I.; Janecki, D. Kinetics of cometabolic biodegradation of 4-chlorophenol and phenol by *stenotrophomonas maltophilia* KB2. *Chem. Process Eng.* **2018**, *39*, 395–410.
23. Sahinkaya, E.; Dilek, F.B. Biodegradation of 4-chlorophenol by acclimated and unacclimated activated sludge-Evaluation of biokinetic coefficients. *Environ. Res.* **2005**, *99*, 243–252. [CrossRef] [PubMed]

24. Buitron, G.; Gonzalez, A. Characterization of the microorganisms from an acclimated activated sludge degrading phenolic compounds. *Water Sci. Technol.* **1996**, *34*, 289–294. [CrossRef]
25. Assadi, A.; Alimoradzadeh, R.; Movahedyan, H.; Amin, M. Intensified 4-chlorophenol biodegradation in an aerobic sequencing batch reactor: Microbial and kinetic properties evaluation. *Environ. Technol. Innov.* **2021**, *21*, 101243. [CrossRef]
26. Kim, J.H.; Oh, K.K.; Lee, S.T.; Kim, S.W.; Hong, S.I. Biodegradation of phenol and chlorophenols with defined mixed culture in shake-flasks and a packed bed reactor. *Process Biochem.* **2002**, *37*, 1367–1373. [CrossRef]
27. Buitron, G.; Gonzalez, A.; Lopez-Marin, L.M. Biodegradation of phenolic compounds by an activated sludge and isolated bacteria. *Water Sci. Technol.* **1998**, *37*, 371–378. [CrossRef]
28. Chung, J.; Choi, J.; Chung, S. Pilot study of specific microbe immobilization cells (SMICs) technology in removing tetramethyl ammonium hydroxide for reuse of low strength electronics wastewater. *J. Hazard. Mater.* **2020**, *384*, 120829. [CrossRef]
29. Turan, V. Calcite in combination with olive pulp biochar reduces Ni mobility in soil and its distribution in chili plant. *Int. J. Phytoremediat.* **2022**, *24*, 166–176. [CrossRef]
30. Patel, B.P.; Kumar, A. Biodegradation of 4-chlorophenol in an airlift inner loop bioreactor with mixed consortium: Effect of HRT, loading rate and biogenic substrate. *Biotech* **2016**, *6*, 117. [CrossRef]
31. Wang, G.; Liu, Y.; Wu, M.; Zong, W.; Yi, X.; Zhan, J.; Liu, L.; Zhou, H. Coupling the phenolic oxidation capacities of a bacterial consortium and in situ-generated manganese oxides in a moving bed biofilm reactor (MBBR). *Water Res.* **2019**, *166*, 115047. [CrossRef] [PubMed]
32. Fu, D.; Singh, R.P.; Yang, X.; Ojha, C.S.P.; Surampalli, R.Y.; Kumar, A.J. Sediment in-situ bioremediation by immobilized microbial activated bawds: Pilot-scale study. *J. Environ. Manag.* **2018**, *226*, 62–69. [CrossRef] [PubMed]
33. Kurade, M.B.; Waghmode, T.R.; Xiong, J.Q.; Govindwar, S.P.; Jeon, B.H. Decolorization of textile industry effluent using immobilized consortium cells in upflow fixed bed reactor. *J. Clean. Prod.* **2019**, *213*, 884–891. [CrossRef]
34. Geed, S.R.; Kureel, M.K.; Giri, B.S.; Singh, R.S.; Rai, B.N. Performance evaluation of malathion biodegradation in batch and continuous packed bed bioreactor (PBBR). *Bioresour. Technol.* **2017**, *227*, 56–65. [CrossRef]
35. Saravanan, V.; Sreekrishnan, T.R. Modelling anaerobic biofilm reactors—A review. *J. Environ. Manag.* **2006**, *81*, 1–18. [CrossRef]
36. Wang, S.; Boyjoo, Y.; Choueib, A.; Zhu, Z.H. Removal of dyes from aqueous solution using fly ash and red mud. *Water Res.* **2005**, *39*, 129–138. [CrossRef]
37. Swain, G.; Sonwani, R.K.; Singh, R.S.; Jaiswal, R.P.; Rai, B.N. A comparative study of 4-chlorophenol biodegradation in a packed bed and moving bed bioreactor: Performance evaluation and toxicity analysis. *Environ. Technol. Innov.* **2021**, *24*, 101820. [CrossRef]
38. Zhao, J.; Chen, X.; Wang, L.; Xu, Y.; Li, J.; Li, Y. Effects of elevated 4-chlorophenol loads on components of polysaccharides and proteins and toxicity in an activated sludge process. *Chem. Eng. J.* **2017**, *330*, 236–244. [CrossRef]
39. APHA. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association: Washington, DC, USA, 2005.
40. Kumar, A.; Kumar, A.; Kumar, S. Biodegradation kinetics of phenol and catechol using *Pseudomonas putida* MTCC 1194. *Biochem. Eng. J.* **2005**, *22*, 151–159. [CrossRef]
41. Konya, I.; Eker, S.; Kargi, F. Mathematical modelling of 4-chlorophenol inhibition on COD and 4-chlorophenol removals in an activated sludge unit. *J. Hazard. Mater.* **2007**, *143*, 233–239. [CrossRef]
42. Arvin, E.; Kristensen, G.H. Effect of denitrification on the pH in biofilms. *Water Sci. Technol.* **1982**, *14*, 833–848. [CrossRef]
43. Wilke, C.E.; Chang, P. Correlation of diffusion coefficients in dilute solutions. *AICHE J.* **1955**, *1*, 264–270. [CrossRef]
44. Williamson, K.; McCarty, P.L. Verification studies of the biofilm model for bacterial substrate utilization. *J. Water Pollut. Control Fed.* **1976**, *48*, 281–296.
45. Dwivedi, P.N.; Upadhyay, S.N. Particle-fluid mass transfer in fixed and fluidized bed. Ind. Eng. *Chem. Proc. Des. Dev.* **1977**, *16*, 157–165. [CrossRef]
46. Eker, S.; Kargi, F. COD, para-chlorophenol and toxicity removal from synthetic wastewater using rotating tubes biofilm reactor (RTBR). *Bioresour. Technol.* **2010**, *101*, 9020–9024. [CrossRef]
47. Kureel, M.K.; Geed, S.R.; Rai, B.N.; Singh, R.S. Novel investigation of the performance of continuous packed bed bioreactor (CPBBR) by isolated *Bacillus* sp. M4 and proteomic study. *Bioresour. Technol.* **2018**, *266*, 335–342. [CrossRef] [PubMed]