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Kinetics of the biodegradation of phenol and 2-chlorophenol in a fixed biofilm reactor using a dewatered sludge–fly ash composite ceramic particle as a supporting medium

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ABSTRACT

The biodegradation kinetics of phenol, 2-chlorophenol (2-CP) and a mixture of phenol and 2-CP by acclimated activated sludge were investigated in aerobic batch reactors. The packed bed reactor filled with dewatered sludge–fly ash composite ceramic particle (DFCCP) was tested for continuous treatment of phenol plus 2-CP to validate the biofilm model. Batch experiments were conducted in a culture medium containing various initial concentrations of phenol (15.5–97.6 mg L^{-1}) and 2-CP (16.8–96.9 mg L^{-1}). The phenol was completely degraded within 10 h. Complete degradation of 2-CP occurred within 12 d for all levels of substrates. Based on the specific growth rate of the biomass vs. initial concentrations of phenol and 2-CP, the maximum specific growth rate using phenol was much greater than that of 2-CP. The magnitude of the K_s value revealed that the biomass had a much stronger affinity to phenol compared with 2-CP. The larger K_i value for 2-CP (127.6 mg L^{-1}) revealed that the biomass had a higher resistance to 2-CP inhibition compared with phenol (54.5 mg L^{-1}). A mixture of phenol and 2-CP in the batch experiments resulted in the complete removal of phenol within 4.2 d, however, the percentage of 2-CP removal was 55%–79% at this stage. Furthermore, the initial phenol and 2-CP concentrations were 25.2 and 19.3 mg L^{-1} corresponding to the removal efficiencies of 98% and 89%, respectively, while the packed bed biofilm reactor reached a steady-state condition. The experimental and modeling schemes proposed in this study could be employed to control and optimize the full-scale packed bed bioreactor performance of simultaneous biodegradation of mixed substrates in wastewater.

Keywords: Kinetics; Biodegradation; Phenol; 2-chlorophenol; Fixed biofilm reactor

1. Introduction

Phenol and chlorophenols produced from chemical industries such as petroleum refineries, pharmaceuticals, pesticides and plastic manufacturing have caused the serious ecological problem of environmental pollutants [1–3]. Concentrations of phenolic compounds in wastewater from oil refineries, coal gasification, coal liquefaction, coke orens, petrochemical and plastic as well as glass fiber manufactures are 10–70, 710, 6,800, 10–3,900, 50–600, 600–2,000 and 40–400 mg L^{-1}, respectively [4]. Chlorophenol concentrations of up to 190 mg L^{-1} have been reported in groundwater downstream of a sow mill or in the wastewater of a kraft mill [5]. In addition to being carcinogens and mutagens, phenols and chlorophenols are toxic to reduce the enzyme activity of living organisms and are lethal to fish at relatively low-level concentrations [6]. Conserving the quality of natural water resources is urgently needed to save aqueous environments. The removal of phenolic and chlorophenolic

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compounds from wastewater is required before safe disposal in water bodies [2,7]. Various treatment methods, such as activated carbon adsorption, chemical oxidation, and aerobic/anaerobic biodegradation, have been extensively applied to treat chlorophenolic compounds in wastewater [8,9]. Compared with physico-chemical methods, biodegradation has been widely used to address the issue of phenolic and chlorophenolic compounds in wastewater because of the low treatment and operational cost and low possibility of byproduct formation [10].

Aerobic microorganisms, including *Pseudomonas* sp., *Alcaligenes* sp., *Azotobacter* sp., *Rhodococcus* sp., and *Cryptococcus* sp., have been proven to be more efficient at degrading toxic aromatic compounds because they have a higher growth yield compared with anaerobic microorganisms. Moreover, aerobes have a high capacity in achieving complete mineralization of toxic aromatic compounds, rather than transformation, as in the case of anaerobic biodegradation [11]. Many studies have shown that mixed cultures have the ability to achieve complete mineralization of toxic organics to carbon dioxide [12–14]. For large-scale wastewater treatment, mixed consortia rather than pure cultures are preferred because they are easier to handle and contain diverse microorganisms capable of withstanding various unfavorable influent fluctuations. Additionally, the acclimation of inoculum can enhance the degradation capacities of a mixed culture [15].

The cost of treating sewage waste sludge and disposal is nearly 50% of the total cost incurred in municipal wastewater treatments. Therefore, identifying an economical method to reuse dewatered sludge is an important environmental issue [16]. Dewatered sludge is currently used in many applications including the production of bricks, an additive of cement, and cultivated soil [17]. Fly ash, produced from coal-fired power generation and other industrial sources, has been applied to cement and brick production as a filling in road works [18]. However, the utilization of both dewatered sludge and fly ash in civil engineering is limited. Thus, a mixture of dewatered sludge and fly ash to produce ceramic particles for water and wastewater treatment have attracted considerable attention because it not only reduces the impact of environmental pollution, but also solves the solid waste disposal problem, benefitting the environmental balance and the economy [18].

Biological processes used in wastewater treatment can be generally divided into two categories: suspended growth and attached growth processes [19]. Attached growth processes have many advantages over suspended growth processes, which include high surface area available for biofilm formation, high volumetric degradation rates of organics, high tolerance in concentration fluctuations of the toxic organics in the influent, and little residual sludge production [20]. Moreover, fixed attached-growth processes have also been successfully conducted to treat various organic pollutants in wastewater [21,22]. They are also easier to operate with an associated lower cost because it is not necessary to suspend the supporting media in the treatment processes.

Several studies have investigated the biodegradation kinetics of single phenol or 2-chlorophenol (2-CP) with pure or mixed cultures of microorganisms [7,13,14,23]. However, the simultaneous biodegradation kinetics of phenol and 2-CP using a mixed culture is yet to be demonstrated. Thus, this work was focused on developing a mathematical model system to describe the kinetics of simultaneous biodegradation of phenol and 2-CP in a biofilm process. In addition, the mathematical model system was verified by conducting a continuous flow packed bed biofilm reactor using a dewatered sludge–fly ash composite ceramic particle (DFCCP) as a supporting medium.

The objectives of this study were intended to (1) acclimate activated sludge by stepwise re-feeding of phenol and 2-CP as well as phenol plus 2-CP, respectively; (2) evaluate the biokinetic parameters (μ_{max}, K_s, K_i, $Y_{x/s}$, and k_d) and interaction parameters ($I_{CP,P}$ and $I_{P,CP}$) of acclimated activated sludge; and (3) operate a packed bed reactor to verify the kinetic model for the simultaneous biodegradation of phenol and 2-CP.

2. Materials and methods

2.1. Acclimation of activated sludge

The aerobic activated sludge was obtained from a municipal wastewater treatment plant located in central Taiwan. The acclimation of activated sludge was performed separately for phenol and 2-CP in two batch reactors with working volumes of 200 mL. The flask contains 25 mL of seed inoculum and 175 mL of nutrient medium with phenol, 2-CP and phenol plus 2-CP, respectively. The cultures were grown in 250 mL Erlenmeyer flasks and shaken at 120 rpm on a rotary shaker. They were incubated at 30°C with 4.0 mg L^{-1} of phenol, 5.0 mg L^{-1} of 2-CP, and 8.0 mg L^{-1} of phenol plus 4.4 mg L^{-1} of 2-CP, respectively. The concentrations of phenol and 2-CP were periodically monitored to observe biodegradation by cultures. After complete biodegradation, the concentrations of phenol, 2-CP and phenol plus 2-CP were spiked to enrich the phenol-degrading, 2-CP-degrading and phenol-2-CP-degrading organisms. Then the activated sludge or the turbid supernatant (containing the bacteria) was used for further experiments.

2.2. Culture medium

The batch and packed bed biofilm reactors were fed with a nutrient medium containing phenol, 2-CP, and phenol plus 2-CP, respectively. The nutrient medium contained the following ingredients (per liter) [18]: peptone, 0.12 g; yeast extract, 0.12 g; NaCl, 0.007 g; $MgSO_4{\cdot}7H_2O$, 0.002 g; $CaCl_2{\cdot}2H_2O$, 0.004 g; and trace metal solution, 200 μL. The trace metal solution contained the following ingredients (per liter): $FeSO_4{\cdot}7H_2O$, 1.36 g; $Na_2MoO_4{\cdot}2H_2O$, 0.24 g; $CuSO_4{\cdot}5H_2O$, 0.25 g; $ZnSO_4{\cdot}7H_2O$, 0.58 g; $NiSO_4{\cdot}6H_2O$, 0.11 g; $MnSO_4{\cdot}H_2O$, 1.01 g; and H_2SO_4, 1 mL. All of the inorganic chemicals were of analytical reagent grade, purchased from Merck Co. (Darmstadt, Germany). Phenol and 2-CP were obtained from Sigma-Aldrich (Darmstadt, Germany).

2.3. Preparation of ceramic particles

The ceramic particles were composed of two waste materials (dewatered sludge and fly ash) with a mass ratio of 1:1; the dewatered sludge was obtained from a leachate treatment plant and fly ash was collected from coal-fired

power generation plant in Taichung, Taiwan. The waste raw materials were mixed in a muller and transported into a rotational disk; meanwhile tap water was injected to keep powdered materials slightly wet [24]. The powdered materials were then transported to a granulator to produce ceramic particles of similar diameter. The ceramic particles were desiccated using an oven with a temperature of 1,100°C. Finally, a DFCCP of diameter 0.5 cm and height 0.95 cm was obtained and cooled after screen separation.

2.4. Batch experiments

Three batch biodegradation experiments for phenol, 2-CP and phenol plus 2-CP were performed in 250 mL batch reactors with acclimated activated sludge. The initial mixed liquor volatile suspended solids (MLVSS) concentration in the batch reactors for the single substrate of phenol and 2-CP was 22.1 and 22.4 mg L^{-1}, respectively. The initial MLVSS for phenol plus 2-CP ranged between 52.0 and 63.4 mg L^{-1}. During the batch experiments, samples were periodically taken for the phenol and 2-CP concentrations and density (OD_{550}) analyses. The samples collected from each flask at predetermined time intervals were allowed to settle for 30 min, and optical density (OD_{550}) values of the supernatants were measured at 550 nm. The OD_{550} values were converted to MLVSS concentrations using a linear relationship [12].

2.5. Continuous flow bioreactor

A schematic diagram of the laboratory scale fixed biofilm process is shown in Fig. 1. A continuous fixed biofilm reactor was initiated by introducing the supernatant of the previous acclimated activated sludge after gravity sedimentation of the solids for 2 h as a seed inoculum. The fixed biofilm reactor (Fig. 1) consisted of a glass cylinder that was 70% packed with DFCCPs as the supporting media for biofilm attachment. The reactor porosity was 45.6%. The seed inoculum was placed in the reactor containing the DFCCPs. The liquid in the reactor was circulated by pump for 72 h to ensure the attachment of seed inoculum on the DFCCPs. A single plastic sieve was provided at the top of the packing DFCCPs to keep them in place and avoid initial floating. The effective working volume of biofilm reactor was 1.6 L, which yielded a hydraulic retention time (HRT) of 6 h. The reactor was maintained at 30 ± 0.2°C through a water jacket using a circulating water bath (Yih Der Inc., Taipei, Taiwan). The influent was provided at the bottom of the reactor using a digital peristaltic pump (Model HT100IJ, Longer Pump, China). The influent feed was a synthetic wastewater containing phenol and 2-CP as binary substrates. The pH was buffered at 7.1 ± 0.2 by $HPO_4^{2-}/H_2PO_4^-$ in the feed solution. The influent flow rate was 2.11 mL min^{-1}. The effluent and recirculation loop were separated with a trigeminal tube to connect the tubing. The flow rate of the recirculation loop was maintained at 31.65 mL min^{-1} to ensure a recycle ratio (Q_r/Q) of 15 and the reactor reached an almost completely mixed condition.

2.6. Analytical techniques

HPLC-UV with an Alliance 2695 liquid chromatography (Waters Co., Milford, MA, USA) was used for the measurement of phenol and 2-CP. The apparatus consisted of a Waters 2707 autosampler and Waters 2487 UV/Vis detector, and was equipped with a Symmetry® C18 column (particle size, 5 μm). The diameter of the column size was 3.9 mm and a height of 150 mm. The samples were filtrated through a Millipore filter (0.22 μm) before the analysis of phenol and 2-CP. A mobile phase composed of 50 mM potassium phosphate/acetonitrile (70/30, v/v) was used to elute the samples at a flow rate of 0.7 mL min^{-1}. The UV/Vis spectrophotometric detector was set to 254 nm. The injection volume in each sample was 6 μL. The average retention times for phenol and 2-CP was approximately 5.69 and 8.96 min, respectively. Three calibration plots were obtained and used to estimate the concentrations of unknown phenol, 2-CP and biomass. The concentrations of phenol and 2-CP were expressed by the following regression equation: Phenol (mg L^{-1}) = 0.0957 + 2.0 × 10^{-4} × (Area), R^2 = 1.0000; 2-CP (mg L^{-1}) = 0.1597 + 4.0 × 10^{-4} × (Area), R^2 = 0.9999, respectively. The MLVSS were monitored spectrophotometrically by measuring the absorbance at a wavelength of 550 nm using an UV/Vis spectrophotometer (Shimadzu, model UV-1700, Kyoto, Japan). The MLVSS concentration was detected using standard methods [25]. The observed biomass concentration followed the following regression equation: MLVSS (mg L^{-1}) = –1.480 8 + 327.72 × OD_{550}, R^2 = 0.9927. All of the measurements were performed in duplicate and arithmetic averages were taken throughout the data analysis and calculations [13].

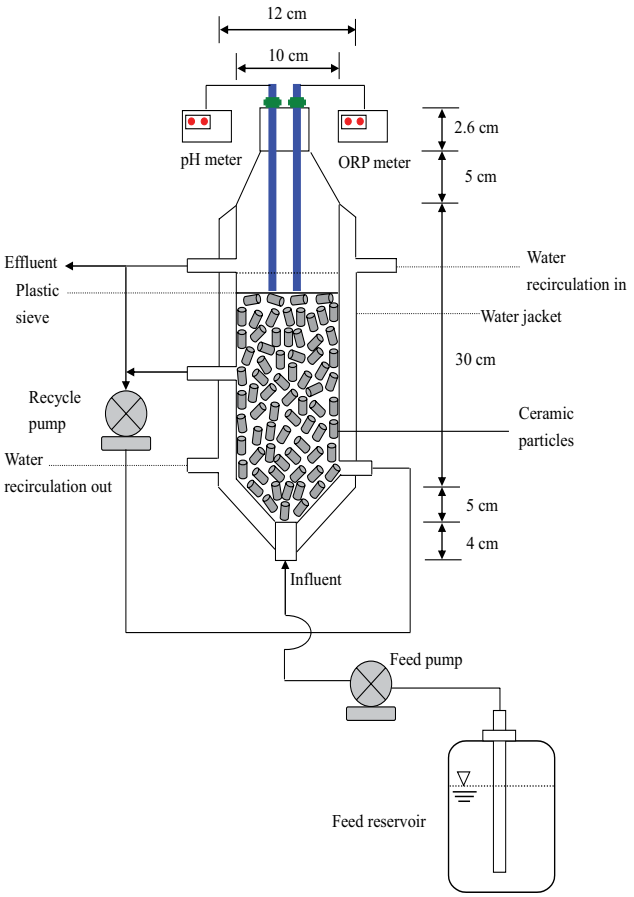

Fig. 1. Laboratory scale fixed biofilm process.

3. Model descriptions

3.1. Batch growth kinetics

The specific growth rate in the exponential phase of the batch reactor is calculated by:

$$\mu = \frac{\ln\left(X_2/X_1\right)}{\left(t_2 - t_1\right)} \tag{1}$$

where X_1 and X_2 are the biomass concentrations (MLVSS) at times t_1 and t_2, respectively. The value of μ can be computed from the linearized plot of ln$X$ vs. time in the exponential phase of the growth of activated sludge. Haldane kinetics was used to describe the specific growth rate for phenol and 2-CP, respectively:

$$\mu = \frac{d\ln X}{dt} = \frac{\mu_{\max} S}{K_s + S + \frac{S^2}{K_i}} \tag{2}$$

where $\mu_{\max}$ is the maximum specific growth rate (d^{-1}); S is the substrate concentration (mg L^{-1}); K_s is the Monod half-saturation constant (mg L^{-1}) and K_i is the substrate inhibition constant (mg L^{-1}).

The yield of biomass (MLVSS) on phenol and 2-CP was determined by the following equation:

$$Y_{x/s} = \frac{\left(X_M - X_0\right)}{\left(C_S - C_0\right)} \tag{3}$$

where X_M and X_0 are the maximum and initial biomass concentrations (MLVSS), respectively; and C_s and C_0 are the substrate concentrations at the maximum biomass and initial substrate concentration, respectively (mg L^{-1}).

A typical growth curve for the biomass includes an endogenous phase after the complete consumption of the substrate. During the endogenous phase, some part of the biomass becomes food for the rest of the biomass. The declining phase in the batch system can be modeled by the following equation:

$$\frac{dX}{dt} = -k_d X \tag{4}$$

where X is the biomass concentration (MLVSS) and k_d is the decay coefficient (h^{-1} or d^{-1}). After integration and arrangement of Eq. (4), the value of k_d can be expressed as follows:

$$k_d = -\frac{\ln\left(X_2/X_1\right)}{t_2 - t_1} \tag{5}$$

where X_1 and X_2 are the biomass concentrations at t_1 and t_2, respectively.

For the substrate mixtures, the specific growth rate on the binary inhibitory substrates is described by the sum kinetics model [26]:

$$\mu_{\text{mix}} = \frac{\mu_{\max,P} S_P}{K_{s,P} + S_P + \frac{S_P^2}{K_{i,P}} + I_{\text{CP},P} S_{\text{CP}}} + \frac{\mu_{\max,\text{CP}} S_{\text{CP}}}{K_{s,\text{CP}} + S_{\text{CP}} + \frac{S_{\text{CP}}^2}{K_{i,\text{CP}}} + I_{P,\text{CP}} S_P} \tag{6}$$

where the subscripts P and CP represent phenol and 2-CP, respectively; $\mu_{\max,P}$ and $\mu_{\max,\text{CP}}$ are the maximum specific growth rate of biomass on phenol and 2-CP, respectively; S_P and S_{CP} are the phenol and 2-CP concentrations in the reactor (mg L^{-1}), respectively; $K_{s,P}$ and $K_{s,\text{CP}}$ are the Monod half-saturation constants of phenol and 2-CP (mg L^{-1}), respectively; $K_{i,P}$ and $K_{i,\text{CP}}$ are the inhibition constants of phenol and 2-CP (mg L^{-1}), respectively; $I_{\text{CP},P}$ is the inhibition constant of 2-CP to biomass growth on phenol (dimensionless); and $I_{P,\text{CP}}$ is the inhibition constant of phenol to biomass growth on 2-CP (dimensionless).

3.2. Continuous flow biofilm kinetics

To derive the mathematical expressions for the utilization of the binary substrates in the aerobic biofilm process, various assumptions are made: (1) there are substrate interactions between phenol and 2-CP [3,26,27]; (2) the biofilm and suspended biomass are homogeneous; (3) the biofilm density is constant; (4) a stagnant layer covers the biofilm [28]; (5) concentrations of phenol and 2-CP within the biofilm vary only in the direction normal to the biofilm surface; (6) synchronous phenol and 2-CP utilization by acclimated activated sludge [29]; (7) the growth of the biofilm does not affect the flow pattern of liquid in the reactor; (8) phenol and 2-CP are transported from the bulk liquid to biofilm phase through the stagnant-liquid layer by molecular diffusion; (9) Fick's law governs diffusion within the biofilm; and (10) the shear loss of the biofilm increases the amount of suspended biomass in the bulk liquid phase.

A kinetic model used to predict phenol and 2-CP within the biofilm must be able to provide estimations of phenol and 2-CP biodegradation by biofilm simultaneously. The unsteady state form of mass transfer diffusion across the liquid-biofilm interface and biodegradation of phenol and 2-CP in the biofilm can be described by Fick's law and the Haldane kinetics on the binary inhibitory substrates [30]:

$$\frac{\partial S_{f,P}}{\partial t} = D_{f,P}\frac{\partial^2 S_{f,p}}{\partial z_f^2} - \frac{\mu_{\max,P} S_{f,P} X_f}{Y_P\left(K_{s,P} + S_{f,P} + \frac{S_{f,P}^2}{K_{i,P}} + I_{\text{CP},P} S_{f,\text{CP}}\right)} \tag{7}$$

$$\frac{\partial S_{f,\text{CP}}}{\partial t} = D_{f,\text{CP}}\frac{\partial^2 S_{f,\text{CP}}}{\partial z_f^2} - \frac{\mu_{\max,\text{CP}} S_{f,\text{CP}} X_f}{Y_{\text{CP}}\left(K_{s,\text{CP}} + S_{f,\text{CP}} + \frac{S_{f,\text{CP}}^2}{K_{i,\text{CP}}} + I_{P,\text{CP}} S_{f,P}\right)} \tag{8}$$

$$\text{IC1}: S_{f,P}\left(z_f, 0\right) = 0 \tag{9}$$

$$\text{IC2}: S_{f,\text{CP}}\left(z_f, 0\right) = 0 \tag{10}$$

where $S_{f,P}$ and $S_{f,\text{CP}}$ are concentrations of phenol and 2-CP in the biofilm (mg L^{-1}), respectively; $D_{f,P}$ and $D_{f,\text{CP}}$ are the diffusivities of phenol and 2-CP, respectively, in the biofilm (cm^2 d^{-1}), respectively; $\mu_{\max,P}$ and $\mu_{\max,\text{CP}}$ are the maximum growth rates by phenol and 2-CP (d^{-1}), respectively; Y_P and Y_{CP} are the growth yields on phenol and 2-CP (mg VSS mg substrate^{-1}), respectively; $K_{s,P}$ and $K_{s,\text{CP}}$ are the Monod half-saturation constants of phenol and 2-CP (mg L^{-1}), respectively; $K_{i,P}$ and

$K_{i,CP}$ are the inhibition constants of phenol and 2-CP (mg L^{-1}), respectively; $I_{CP,P}$ is the inhibition constant of 2-CP to biomass growth on phenol (dimensionless); $I_{P,CP}$ is the inhibition constant of phenol to biomass growth on 2-CP (dimensionless); X_f is the density of the biofilm (mg VSS mL^{-1}); z_f is the axial coordinate in the biofilm (μm). Eqs. (7) and (8) require two boundary conditions. The fluxes of the phenol and 2-CP at the wall of the inert medium were zero; thus, the concentration gradients of phenol and 2-CP at the wall must be zero:

$$\frac{\partial S_{f,P}}{\partial z_f} = 0 \tag{11}$$

$$\frac{\partial S_{f,CP}}{\partial z_f} = 0 \tag{12}$$

At the liquid/biofilm interface, the flux diffusing into the interface from the liquid film is equal to the flux diffusing out of the interface into the biofilm:

$$J_P = D_{f,P}\frac{\partial S_{f,P}}{\partial z_f}\left(L_f, t\right) = k_{f,P}\left(S_{b,P} - S_{s,P}\right) \tag{13}$$

$$J_{CP} = D_{f,CP}\frac{\partial S_{f,CP}}{\partial z_f}\left(L_f, t\right) = k_{f,CP}\left(S_{b,CP} - S_{s,CP}\right) \tag{14}$$

where $k_{f,P}$ and $k_{f,CP}$ are the liquid-film mass transfer coefficients of phenol and 2-CP (cm d^{-1}), respectively; and $S_{s,P}$ and $S_{s,CP}$ are the concentrations of phenol and 2-CP at the liquid/biofilm interface (mg L^{-1}), respectively.

Since the density of the biofilm is assumed constant, the volume and thickness of the biofilm must increase with time as the biofilm grows. The growth kinetics of the biofilm can be described by the following equation:

$$\frac{dL_f}{dt} = \int_0^{L_f} \frac{\mu_{max,P} S_{f,P}}{\left(K_{s,P} + S_{f,P} + \frac{S_{f,P}^2}{K_{i,P}} + I_{CP,P} S_{f,CP}\right)} + \frac{\mu_{max,CP} S_{f,CP}}{\left(K_{s,CP} + S_{f,CP} + \frac{S_{f,CP}^2}{K_{i,CP}} + I_{P,CP} S_{f,P}\right)} X_f dz_f \tag{15}$$

$$\text{IC}: L_f\left(0\right) = L_{f0} \tag{16}$$

In the above equation, L_f is the biofilm thickness (μm); $k_{d,P}$ and $k_{d,CP}$ are the decay coefficients of the biomass on phenol and 2-CP (d^{-1}), respectively; and f_s is the specific shear-loss coefficient of biomass (d^{-1}). The change of the phenol and 2-CP concentrations, as well as the suspended biomass concentration with time in the liquid phase of the biofilm reactor can be described using the following unsteady state mass balances [30]:

$$\frac{dS_{b,P}}{dt} = \frac{Q}{V\varepsilon}\left(S_{b,P0} - S_{b,P}\right) - \left(\frac{A}{V\varepsilon}\right) J_P - \frac{\mu_{max,P} S_{b,P} X_b}{Y_P\left(K_{s,P} + S_{b,P} + \frac{S_{b,P}^2}{K_{i,P}} + I_{CP,P} S_{b,CP}\right)} \tag{17}$$

$$\frac{dS_{b,CP}}{dt} = \frac{Q}{V\varepsilon}\left(S_{b,CP0} - S_{b,CP}\right) - \left(\frac{A}{V\varepsilon}\right) J_{CP} - \frac{\mu_{max,CP} S_{b,CP} X_b}{Y_{CP}\left(K_{s,CP} + S_{b,CP} + \frac{S_{b,CP}^2}{K_{i,CP}} + I_{P,CP} S_{b,P}\right)} \tag{18}$$

$$\frac{dX_b}{dt} = \left(\frac{\mu_{max,P} S_{b,P}}{\left(K_{s,P} + S_{b,P} + \frac{S_{b,P}^2}{K_{i,P}} + I_{CP,P} S_{b,CP}\right)} + \frac{\mu_{max,CP} S_{b,CP}}{\left(K_{s,CP} + S_{b,CP} + \frac{S_{b,CP}^2}{K_{i,CP}} + I_{P,CP} S_{b,P}\right)} - \left(k_{d,P} + k_{d,CP}\right) - \frac{Q}{V\varepsilon}\right) \times X_b + \frac{A}{V\varepsilon} f_s L_f X_f \tag{19}$$

$$\text{IC1}: S_{b,P}\left(0\right) = S_{b,P0} \tag{20}$$

$$\text{IC2}: S_{b,CP}(0) = S_{b,CP0} \tag{21}$$

$$\text{IC3}: X_b\left(0\right) = X_{b0} \tag{22}$$

where $S_{b,P0}$ and $S_{b,CP0}$ are the concentrations of phenol and 2-CP in the feed (mg L^{-1}), respectively; $S_{b,P}$ and $S_{b,CP}$ are the concentrations of phenol and 2-CP in the bulk liquid (mg L^{-1}), respectively; X_{b0} is the initial suspended biomass concentration in the bulk liquid (mg VSS L^{-1}); X_b is the suspended biomass concentration in the bulk liquid (mg VSS L^{-1}); Q is the influent flow rate (mL d^{-1}); V is the working volume of the reactor (mL); ε is the reactor porosity (dimensionless); and A is the total surface area of the medium (cm^2).

3.3. Continuous flow biofilm model solution

The biofilm model system can be solved as a transient problem from the unsteady-state to steady-state conditions. The model system contains two partial differential equations and four ordinary differential equations, including several boundary and initial conditions. All of the differential equations can be simplified by defining dimensionless variables leading to dimensionless forms. Applying the orthogonal collocation method to the dimensionless forms of the partial differential equations yields ordinary differential equations, which can then be solved using Gear's method. Legendre polynomials in planar geometry were applied to approximate the concentration profiles of the phenol and 2-CP in the biofilm. The computer programs were coded in Fortran and executed with a Macintosh computer with an accelerator located at the Central Taiwan University of Science and Technology (Taichung, Taiwan).

4. Results and discussion

4.1. Acclimation of activated sludge to phenol and 2-CP

Aerobic sewage sludge was inoculated into the culture medium (200 mL) with phenol and 2-CP in 250 mL Erlenmeyer flasks to obtain phenol and 2-CP acclimated cultures. Fig. 2a shows the acclimation of aerobic activated sludge to phenol. The time required for the complete degradation of 4 mg L^{-1} of

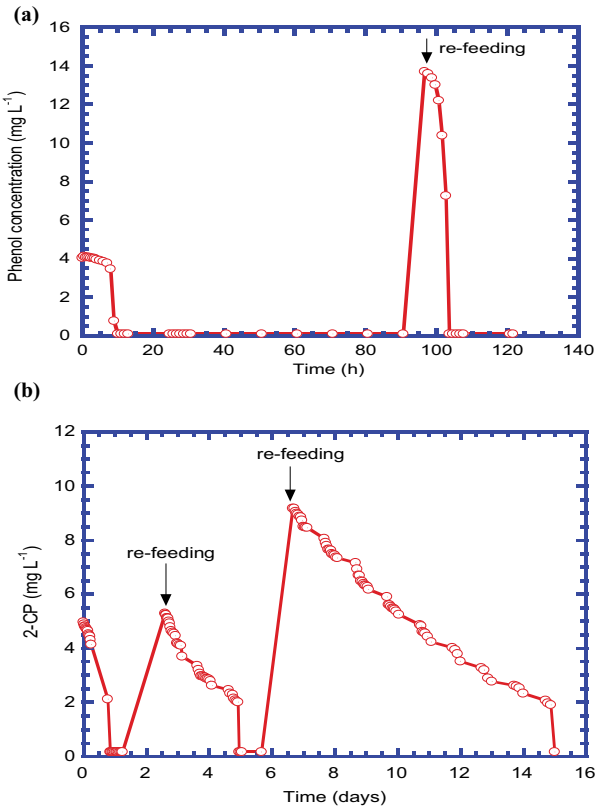

Fig. 2. Acclimation of aerobic activated sludge to: (a) phenol and (b) 2-CP.

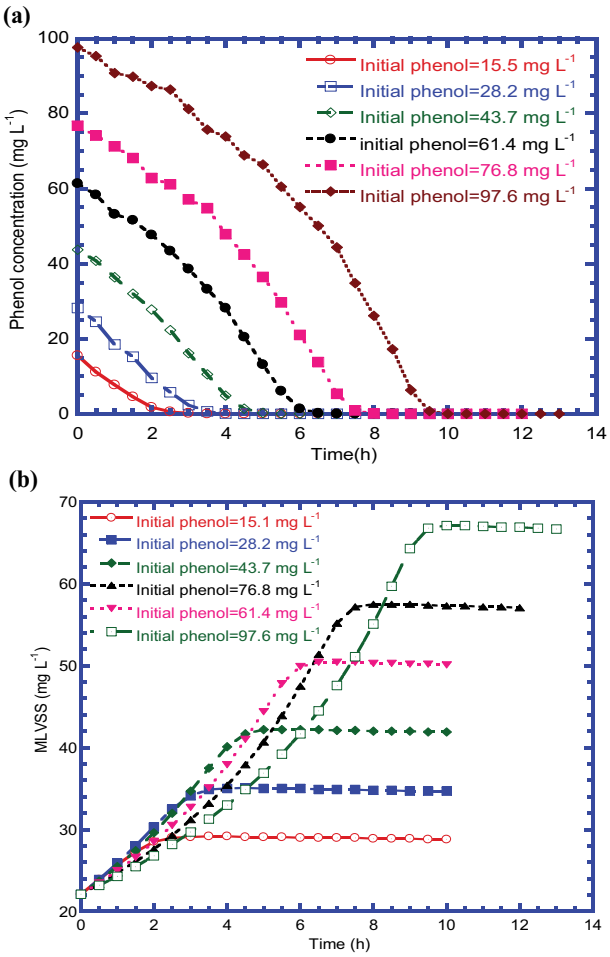

Fig. 3. Time course of the change in concentration for different initial phenol contents with acclimated activated sludge: (a) phenol and (b) MLVSS.

phenol by non-acclimated activated sludge sludge was 10 h. When the activated sludge was spiked a second time with phenol, the complete removal of 13.7 mg L^{-1} took only 17 h. The second feeding of phenol upon complete substrate depletion significantly reduced the lag time before the biodegradation started. This experimental result is consistent with that obtained by Bajaj et al. [20] who used quick successive feeding of 2-CP to observe the lag time of biodegradation. The 2-CP degradation by non-acclimated activated sludge is depicted in Fig. 2b. When the initial concentration of 2-CP was 5.0 mg L^{-1}, the time required for complete degradation was only 0.85 d, which increased to 2.4 and 8.3 d as the initial 2-CP concentration was increased to 5.3 and 9.2 mg L^{-1}, respectively. A decreased degradation rate with increasing initial 2-CP concentration was also observed, which implied that the 2-CP acted as an inhibitor. Acclimation and addition of the growth substrates are two methods to enhance the biodegradation efficiency of 2-CP. Majumder and Gupta [31] reported that the adaptation of microorganisms was found to improve the extent of biodegradation of chlorophenols even though chlorophenols are inhibitory to microorganisms. Moreover, addition of growth substrates to the medium has shown to improve the extent of biodegradation of chlorinated aromatic compounds [32].

4.2. Degradation of single substrate by acclimated activated sludge

The degradation of single substrate and biomass growth using acclimated activated sludge are shown in Figs. 3 and 4. Batch experiments were conducted in a culture medium containing various initial concentrations of phenol (15.5–97.6 mg L^{-1}) and 2-CP (16.8–96.9 mgL^{-1}). Samples drawn from the batch reactors at different time were analyzed for phenol and 2-CP as well as MLVSS. The degradation of phenol and 2-CP started with no lag phase. This result was similar to that reported by Sahinkaya and Dilek [33] who found that the lag phase for non-acclimated culture disappeared after acclimation. It is evident that the time required for complete degradation of phenol and 2-CP is significantly different at each substrate level. The time needed for the complete removal of phenol is significantly shorter than that of 2-CP. Our experiments indicated that the phenol was completely degraded in a range of 2.5–9.5 h for all substrate levels, however, the time required for complete degradation of 2-CP was 7–12 d for all substrate levels. Buitrón et al. [23] reported a time of 10 h for the complete transformation of approximately 25 mg L^{-1} phenol in a mixture of phenolic compounds, which is comparable with our study because the acclimated activated sludge was also used in their studies. Sivarajan et al. [34] conducted a batch reactor experiment and found that the time needed for the maximum 2-CP removal efficiency was achieved after 15 d at a mixing ratio of 80/20

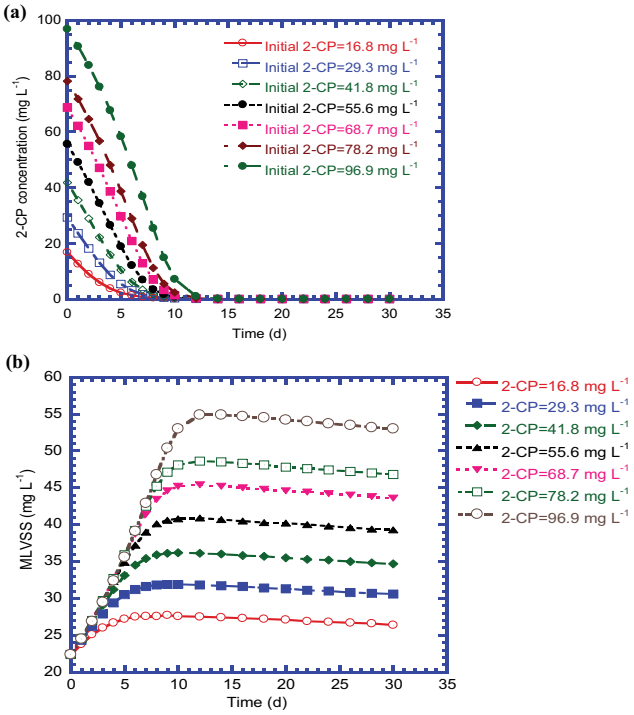

Fig. 4. Time course of the change in concentration for different initial 2-CP contents with acclimated sludge: (a) 2-CP and (b) MLVSS.

for a synthetic 2-CP-starch solution, which was close to the time required in this study.

4.3. Biomass growth on single substrate

Fig. 5 shows the specific growth rates of the biomass on phenol and 2-CP, respectively. The maximum specific growth rates, μ_{max}; the half saturation constant, K_s; and inhibition constant, K_i were obtained using the non-linear least-squares regression method and fitting the experimental data with correlation coefficients (R^2) of 0.948 for phenol and 0.958 for 2-CP, respectively. The experimental results indicated that the maximum growth rates of the phenol-degrading and 2-CP-degrading enrichments were obtained at approximately 28 mg L^{-1} phenol and 69 mg L^{-1} 2-CP, respectively. Below the concentrations of 28 mg L^{-1} phenol and 69 mg L^{-1} 2-CP, respectively, growth seemed to be suboptimal due to substrate limitation and above these concentrations growth was inhibited increasingly due to substrate inhibition [14]. The best-fit values of the parameters were obtained in the following model equations for phenol and 2-CP:

$$\text{Phenol:}\quad \mu_P = \frac{6.552 S_P}{6.3 + S_P + \frac{S_P^2}{54.5}} \tag{23}$$

$$\text{2-CP:}\quad \mu_{CP} = \frac{0.185 S_{CP}}{25.8 + S_{CP} + \frac{S_{CP}^2}{127.6}} \tag{24}$$

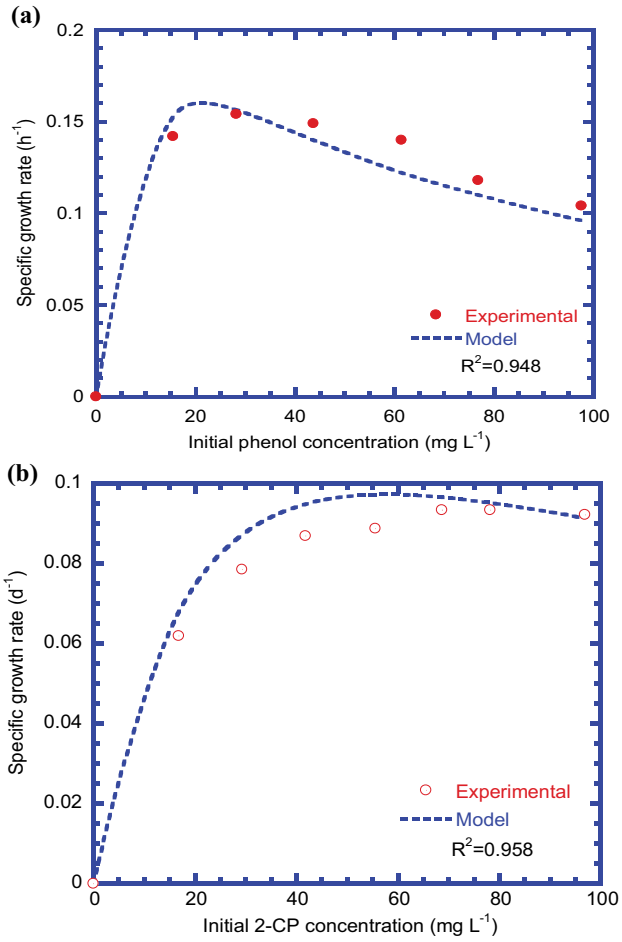

Fig. 5. Specific growth rate (μ) as a function of initial concentrations: (a) phenol and (b) 2-CP.

where μ_P and μ_{CP} are the specific growth rates of the phenol-degrading and 2-CP-degrading enrichment cultures (d^{-1}), respectively. The μ_{max}, K_s, and K_i values for phenol obtained from this study was 6.552 d^{-1}, 6.3 mg L^{-1}, and 54.5 mg L^{-1}, respectively, which fall within the ranges in the literature of 3.144–10.512 d^{-1}, 3.9–74.65 mg L^{-1} and 72.4–648.13 mg L^{-1}, respectively [12]. In the case of 2-CP biodegradation, the Haldane biokinetic parameters, μ_{max}, K_s, and K_i, obtained in this study were 0.185 d^{-1}, 25.8 mg L^{-1}, and 127.6 mg L^{-1}, respectively. This indicated that the biomass degraded phenol more rapidly than for 2-CP, since the maximum specific growth rate on phenol was much greater than that on 2-CP. In addition, the magnitude of the K_s value revealed that the biomass had a much stronger affinity to phenol than to 2-CP. The larger K_i value on 2-CP (127.6 mg L^{-1}) obtained from this study revealed that the biomass had a higher resistance to 2-CP inhibition than to phenol (54.5 mg L^{-1}). Moreover, the growth of the biomass was inhibited more significantly by phenol than by 2-CP at an initial concentration greater than 69 mg L^{-1}.

The growth yield and decay coefficient of the biomass on phenol and 2-CP were determined from experimental data using Eqs. (3) and (4). The estimated biokinetic parameters ($Y_{x/s}$ and k_d) on phenol and 2-CP obtained in the batch

growth experiments are listed in Tables 1 and 2, respectively. The growth yields on phenol ranged from 0.461 to 0.467 mg mg^{-1} for initial phenol concentrations of 15.5–97.6 mg L^{-1}. The obtained decay coefficients of the biomass ranged from 4.87×10^{-2} – 5.19×10^{-2} d^{-1} for initial phenol concentrations of 15.5–97.6 mg L^{-1}. The average growth yield on single phenol ($Y_{x/s,P}$) was 0.464 mg mg^{-1} with a standard deviation value of 2.324×10^{-3}. The decay coefficient on single phenol was $5.01 \times 10^{-2} \pm 1.235 \times 10^{-3}$. The growth yield on 2-CP alone varied from 0.320 to 0.339 mg mg^{-1} with a mean value of 0.334 and a standard deviation value of 6.868×10^{-3}. The obtained decay coefficient on single 2-CP measured from various batch experiments was $2.22 \times 10^{-3} \pm 2.944 \times 10^{-5}$.

4.4. Acclimation of activated sludge to phenol plus 2-CP

The acclimation of aerobic activated sludge to phenol plus 2-CP by the re-feeding of a fresh medium with substrates is shown in Fig. 6. The data points represent the mean of three replicate cultures. After two feedings of the medium, as well as with phenol and 2-CP, and an incubation period of 18.8 d, the degradation rate of phenol was enhanced from 10.2 to 13.2 mg L^{-1} d^{-1} at the first re-feeding. At the second re-feeding, the degradation rate was approximately 11.5 mg L^{-1} d^{-1}. The results suggest that acclimation and growth of the phenol-degrading microbial population were apparent. The degradation rates of 2-CP was 5.11, 1.78 and 1.96 mg L^{-1} d^{-1}. The values of the degradation rates for 2-CP were much lower than that of phenol. The experimental results also indicated that the time required for complete degradation of phenol was much shorter than that of 2-CP, which revealed that the 2-CP produced more inhibition to non-acclimated activated sludge than that of phenol. However, the time required for complete removal of 2-CP at the second re-feeding was shorter than that at the first re-feeding indicating that activated sludge was gradually adapted to 2-CP.

Table 1
Summary of biokinetic parameters for single phenol degradation

Run number	Initial phenol concentration (mg L^{-1})	Biokinetic parameters	
		$Y_{x/s,P}$ (mg mg^{-1})	$k_{d,P}$ (d^{-1})
1	15.5	0.464	5.04×10^{-2}
2	28.2	0.467	4.94×10^{-2}
3	43.7	0.461	4.90×10^{-2}
4	61.4	0.466	5.19×10^{-2}
5	76.8	0.462	5.09×10^{-2}
6	97.6	0.465	4.87×10^{-2}
Mean	–	0.464	5.01×10^{-2}
Standard deviation	–	2.324×10^{-3}	1.235×10^{-3}

Table 2
Summary of biokinetic parameters for single 2-CP degradation

Run number	Initial phenol concentration (mg L^{-1})	Biokinetic parameters	
		$Y_{x/s,CP}$ (mg mg^{-1})	$k_{d,CP}$ (d^{-1})
1	16.8	0.320	2.19×10^{-3}
2	29.3	0.330	2.22×10^{-3}
3	41.8	0.335	2.18×10^{-3}
4	55.6	0.336	2.25×10^{-3}
5	68.7	0.338	2.23×10^{-3}
6	78.2	0.339	2.21×10^{-3}
7	96.9	0.339	2.26×10^{-3}
Mean	–	0.334	2.22×10^{-3}
Standard deviation	–	6.868×10^{-3}	2.944×10^{-5}

4.5. Simultaneous degradation of phenol plus 2-CP in batch experiments

The ability of the acclimated activated sludge to simultaneously degrade phenol and 2-CP was evaluated by monitoring the disappearance of phenol and 2-CP at their different initial concentrations in the batch culture over a 23 d period. Fig. 7 shows the variations in the phenol and 2-CP concentrations with time by acclimated activated sludge with different initial phenol (17.2–30.4 mg L^{-1}) and 2-CP (14.3–29.1 mg L^{-1}) contents. The phenol and 2-CP concentrations in the batch reactors decreased steeply with time at lower initial phenol and 2-CP concentrations, as compared with those containing higher levels of phenol and 2-CP. It is noted that the phenol was completely removed within 4.2 d, however, the percentage of 2-CP removal was 79%, 59%, 60%, and 55% at initial 2-CP concentrations of 14.3, 18.6, 23.4, and 29.1 mg L^{-1}, respectively. Based on the data presented in Fig. 7, the degradation of 2-CP started without lag period. The highest degradation rates of 2-CP were observed before 2.6 d, and consequently prolonged the intermediate lag length of 12–18 d for an initial 2-CP concentration of 18.6–29.1 mg L^{-1}. The acclimated biomass was able to achieve complete biodegradation within 9–23 d for an initial 2-CP concentration of 14.3–29.1 mg L^{-1}.

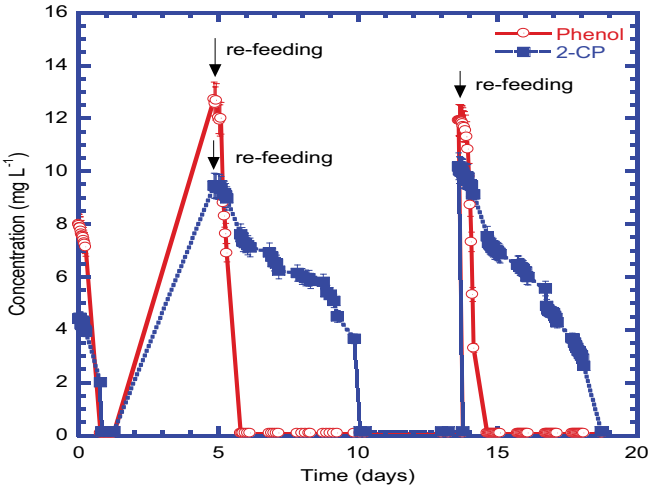

Fig. 6. Acclimation of aerobic activated sludge by both phenol and 2-CP.

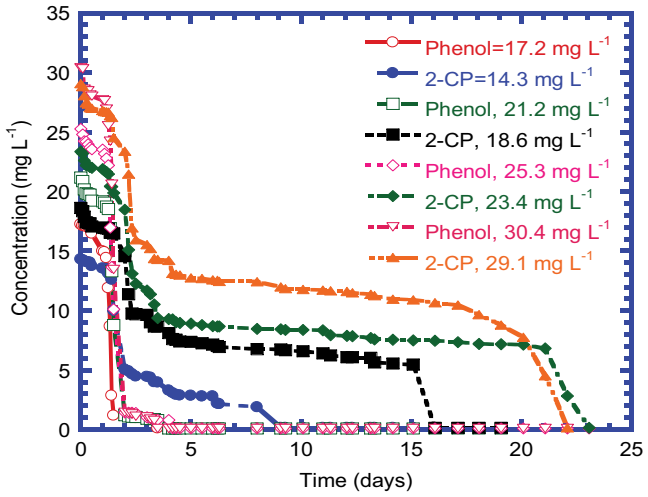

Fig. 7. Time course of the change in phenol and 2-CP concentrations in the batch reactors.

An interesting finding of these experiments was that the 2-CP as cometabolic compound was completely biodegraded successfully by the acclimated biomass after depletion of phenol under resting cell conditions [33]. A similar result was also reported in Aktaş [35] in which the 2-CP and 2-NP were biodegraded successfully only after the phenol was completely biodegraded.

Changes in the final phenol and 2-CP concentrations and phenol and 2-CP removal efficiencies at different initial phenol and 2-CP concentrations after 15 d batch experiments are illustrated in Fig. 8. The results revealed that the removal percentage decreased and final 2-CP concentration was proportionally higher with increasing initial 2-CP concentration. The 2-CP percentage removal was 100% at an initial 2-CP concentration of 14.3 mg L^{-1}, dropping to 70% at an initial concentration of 18.6 mg L^{-1} and to only 63% at an initial concentration of 29.1 mg L^{-1}, with final 2-CP concentrations of 0, 5.5, and 10.9 mg L^{-1}, respectively. The removal efficiencies of phenol were 100% at the initial phenol concentrations of 17.2–30.4 mg L^{-1} after 15 d batch tests.

The specific growth rates estimated from the slope of the linearized plots of ln*X* vs. time in the log growth phrase are shown in Fig. 9. The specific growth rates for all batch experiments on binary substrates performed at 30°C ranged from 0.185 to 0.204 d^{-1} at different initial phenol and 2-CP contents. As mentioned earlier, the interaction parameters of $I_{CP,P}$ and $I_{P,CP}$ indicate the degree to which 2-CP affects the biodegradation of phenol and vice versa for $I_{P,CP}$. Larger values indicate stronger inhibition [36]. Fig. 10 presents the specific growth rate of the biomass on phenol plus 2-CP. As in the case of single substrate systems, the biokinetic parameters of $I_{CP,P}$ and $I_{P,CP}$ were determined using the non-linear least-squares regression method. The kinetic parameters (μ_m, K_s, and K_i) in the equation are the same as for the phenol and 2-CP experiments [26]. Based on the experimental and modeling results, the best fit of Eq. (6) is:

$$\mu_{mix} = \frac{6.552S_P}{6.3 + S_P + \frac{S_P^2}{54.5} + 35.8S_{CP}} + \frac{0.185S_{CP}}{25.8 + S_{CP} + \frac{S_{CP}^2}{127.6} + 77.6S_P} \quad (25)$$

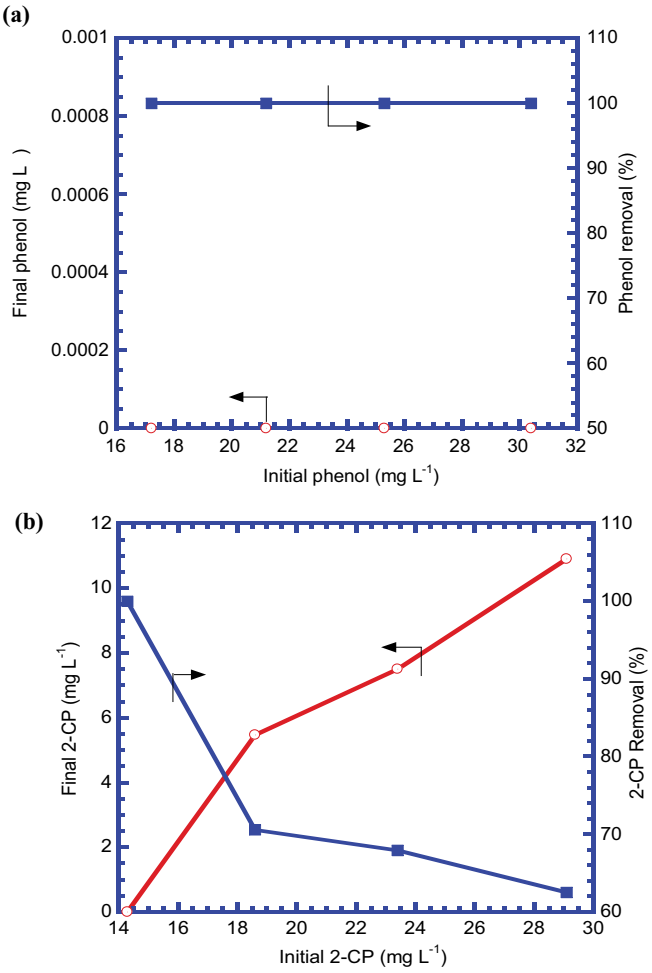

Fig. 8. Variation in the final concentration and removal efficiency after a 15 d batch operation with initial concentrations of (a) phenol and (b) 2-CP.

The obtained value of the correlation coefficient (R^2) was 0.998. The values of $I_{CP,P}$ and $I_{P,CP}$ were 35.8 and 77.6, respectively.

4.6. Determination of mass transfer coefficients

The diffusivities of phenol and 2-CP in water were determined using the Wilke–Chang correlation [37]. The diffusivities of phenol and 2-CP in water (D_w) calculated at 30°C were 0.949 and 0.861 $cm^2\ d^{-1}$, respectively. A ratio of D_f/D_w = 0.8, applied by Jih and Huang [38] for model calculations of an anaerobic filter, was used to determine the diffusivity in biofilm in this study. Thus, the D_f values for phenol and 2-CP were equivalent to 0.759 and 0.689 $cm^2\ d^{-1}$, respectively. The liquid-film mass transfer coefficient (k_f) was determined using a formula suitable for packed bed reactors [39]. The computed k_f values for phenol and 2-CP were 478.7 and 448.4 cm d^{-1}, respectively. An empirical formula derived by Speitel and DiGiano [40] was used to determine the specific shear-loss coefficient (f_s) of the biofilm on DFCCPs. The value of f_s for the acclimated biomass was equal to 0.29 d^{-1}.

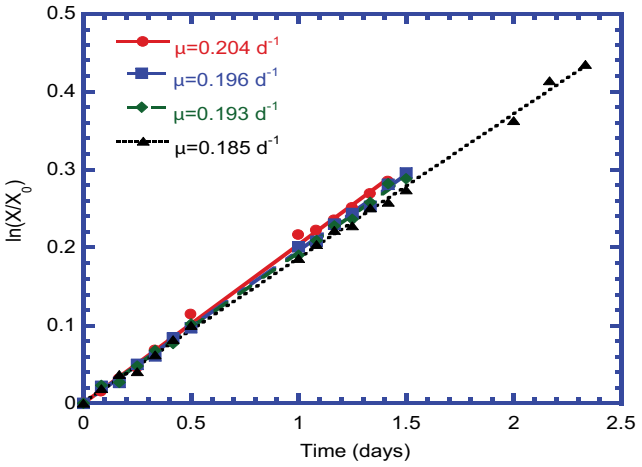

Fig. 9. Specific growth rate estimation for different initial substrate concentrations (●) phenol = 17.2 mg L^{-1}, 2-CP = 14.3 mg L^{-1}; (■) phenol = 21.2 mg L^{-1}, 2-CP = 18.6 mg L^{-1}; (♦) phenol = 25.3 mg L^{-1}, 2-CP = 23.4 mg L^{-1}; and (▲) phenol = 30.4 mg L^{-1}, 2-CP = 29.1 mg L^{-1}.

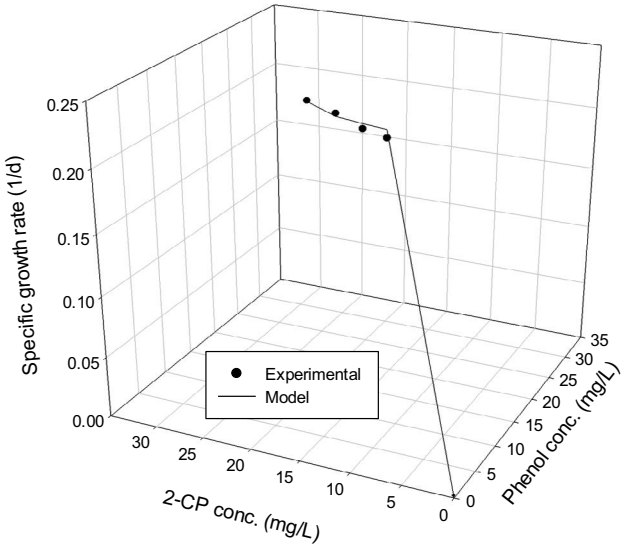

Fig. 10. Kinetic fit of the specific growth rate of acclimated activated sludge on the binary substrates of phenol and 2-CP with a correlation coefficient R^2 = 0.998.

4.7. Determination of biofilm density

At the end of continuous flow experiment, 50 DFCCPs were transferred, one by one with tweezer to preclude inclusion of interstitial water, to a tared aluminum pan. The DFCCPs containing biofilm were weighed before and after drying in the oven. Since water comprises about 99% of the total biofilm mass [41], the weight loss upon drying is assumed to be the summation of biofilm and adsorbed water. Clean DFCCPs were immersed in clean water and picked out to measure the amount of adsorbed water by the same method. The biofilm volume was obtained by the difference of the two measurements. The biofilm density thus computed by dividing the biofilm mass by biofilm volume was equal to 8.52 mg MLVSS mL^{-1}.

4.8. Simultaneous degradation of phenol plus 2-CP in a packed bed reactor

Table 3 summarizes the biokinetic and reactor parameters of acclimated activated sludge as well as the operating conditions of the reactor for the model predictions. An interesting finding of the continuous-flow experiment that 2-CP could be cometabolically biodegraded with acclimated suspended and attached biomasses in the presence of phenol as growth substrate. Phenol-degrading bacteria could induce the necessary enzymes for cometabolic transformation of 2-CP [35]. The degrees of phenol and 2-CP removal obtained under continuous flow conditions are illustrated in Figs. 11a and b. The biodegradation of phenol and 2-CP was observed from non-steady-state to steady-state conditions. The average effluent concentrations of phenol and 2-CP reached 0.52 and 2.14 mg L^{-1}, respectively, at the steady-state condition. The initial phenol and 2-CP concentrations were 25.2 and 19.3 mg L^{-1} corresponding removal efficiencies of 98% and 89%, respectively, while the system was stable. Satisfactory agreements between the model predictions and actual experimental data for phenol and 2-CP were obtained with correlation coefficients (R^2) of 0.922 and 0.967, respectively. Large differences in the model predictions and actual experimental data for the steady-state period can be attributed to the difficulties encountered in analysis at low effluent concentrations of phenol and 2-CP.

4.9. Biomass growth on phenol plus 2-CP

The acclimated activated sludge with 8.36 mg L^{-1} of MLVSS was inoculated into the fixed biofilm reactor. The MLVSS of the activated sludge increased from a transient period to the steady-state condition as shown in Fig. 11c. No significant lag phase occurred from the start of the suspended biomass growth. A period of exponential growth within 10 d was observed when the phenol and 2-CP were degraded at a faster rate. The suspended biomass growth reached a maximum of 38.9 mg L^{-1} in the steady-state condition due to biodegradation. The model prediction was in good agreement with the experimental result with a correlation coefficient (R^2) of 0.970. The model curve for the biofilm growth as a function of time is shown in Fig. 11d. By comparing Figs. 11a and b, the variation of suspended and attached biomass (biofilm) with respect to time showed the same growth trend, indicating that the suspended biomass and biofilm simultaneously biodegraded the phenol and 2-CP in the bioreactor. The growth of the biofilm abruptly increased with time during the period of 10 d. The biofilm then achieved a steady-state condition during the period of 10–30 d. The growth of the biofilm reached a maximum value of around 60 μm at the steady state.

4.10. Fluxes into the biofilm on phenol plus 2-CP

Fig. 11e illustrates that the model-predicted fluxes of phenol and 2-CP into the biofilm varied with time. At the start of the experiment, there were significant fluxes of phenol and 2-CP diffusing into the biofilm as the biofilm growth was vigorous. It is noted that the phenol flux increased much faster than that of the 2-CP because phenol has a larger

Table 3
Summary of the biokinetic and reactor parameters, as well as the operation conditions for the model predictions

Symbol	Parameters description, unit	Value	Remarks
ε	Reactor porosity, dimensionless	0.456	Measured
A	Total surface area of ceramic particles, cm^2	4.039×10^3	Calculated
$k_{d,P}$	Decay coefficient of biomass on phenol, d^{-1}	5.01×10^{-2}	Measured
$k_{d,CP}$	Decay coefficient of biomass on 2-CP, d^{-1}	2.22×10^{-3}	Measured
f_s	Specific shear-loss coefficient of biomass, d^{-1}	0.29	Calculated
$D_{f,P}$	Diffusivity of phenol in the biofilm, $cm^2\ d^{-1}$	0.759	Calculated
$D_{f,CP}$	Diffusivity of 2-CP in the biofilm, $cm^2\ d^{-1}$	0.689	Calculated
$I_{CP,P}$	Inhibition constant of 2-CP to biomass growth on phenol, dimensionless	35.8	Measured
$I_{P,CP}$	Inhibition constant of phenol to biomass growth on 2-CP, dimensionless	77.6	Measured
$k_{f,P}$	Mass transfer coefficient of phenol, cm d^{-1}	478.7	Calculated
$k_{f,CP}$	Mass transfer coefficient of 2-CP, cm d^{-1}	448.4	Calculated
$K_{i,P}$	Inhibition constant of phenol, mg L^{-1}	54.5	Measured
$K_{i,CP}$	Inhibition constant of 2-CP, mg L^{-1}	127.6	Measured
$K_{s,P}$	Saturation constant of phenol, mg L^{-1}	6.3	Measured
$K_{s,CP}$	Saturation constant of 2-CP, mg L^{-1}	25.8	Measured
L_{f0}	Initial biofilm thickness, μm	7.5	Assumed
Q	Influent flow rate, mL d^{-1}	6.4×10^3	Measured
S_{P0}	Concentration of phenol in feed, mg L^{-1}	25.2	Measured
S_{CP0}	Concentration of 2-CP in feed, mg L^{-1}	19.3	Measured
V	Effective working volume, mL	1.6×10^3	Measured
X_{b0}	Initial biomass concentration in bulk liquid, mg MLVSS L^{-1}	8.36	Measured
X_f	Biofilm density, mg MLVSS mL^{-1}	8.52	Measured
Y_P	Growth yield of biomass on phenol, mg MLVSS (mg phenol)$^{-1}$	0.464	Measured
Y_{CP}	Growth yield of biomass on 2-CP, mg MLVSS (mg 2-CP)$^{-1}$	0.334	Measured
$\mu_{max,P}$	Maximum specific growth rate of biomass on phenol, d^{-1}	6.552	Measured
$\mu_{max,CP}$	Maximum specific growth rate of biomass on 2-CP, d^{-1}	0.185	Measured

maximum specific utilization rate (k). The time required to attain a maximum value of the phenol and 2-CP fluxes was 1 and 10 d, respectively. The biofilm vigorously degraded the phenol and 2-CP and became thicker during the transient period. Thus, the concentration gradient of phenol and 2-CP between the bulk liquid and biofilm/liquid interface increased, significantly increasing the phenol and 2-CP fluxes into the biofilm due to the vigorous biodegradation by the biofilm. After transient periods of 1 and 10 d for phenol and 2-CP, the fluxes reached maximal constant values of 0.037 and 0.026 mg $cm^{-2}\ d^{-1}$, respectively.

4.11. Concentration profiles of phenol plus 2-CP

Fig. 11f shows the concentration profiles of phenol and 2-CP in the biofilm, liquid film, and bulk liquid obtained at days 4.4 and 30. The phenol and 2-CP in the bulk liquid diffused into the biofilm through the liquid film to form concentration profiles due to diffusional resistance. On day 4.4, the thickness of the biofilm was approximately 47 μm and the biofilm significantly utilized the phenol and 2-CP. The biofilms for phenol and 2-CP at this stage are classified as shallow and penetrated biofilm, respectively [42]. On day 30, the fixed biofilm reactor approached a steady-state condition, in which the concentrations of phenol and 2-CP in the biofilm decreased abruptly near the surface of the ceramic particles. It was noted that the 2-CP encountered a smaller diffusional resistance compared with phenol due to a low maximum specific growth rate on 2-CP. In this stage, the biofilms for phenol and 2-CP are called shallow and penetrated biofilms, respectively.

4.12. Proposed practical application

Many physico-chemical and biological methods were employed for phenol removal. In physio-chemical methods, the efficiency of phenol by polymerization, electrocoagulation, photodecomposition, electro-Fenton and adsorption was 90%, 97%, 90%, 96%, and 95%, respectively [43]. In biological methods, the removal efficiency of phenol was 90% and 98% by stabilization pond process and moving bed biofilm process, respectively [43]. Zilouei et al. [5] performed

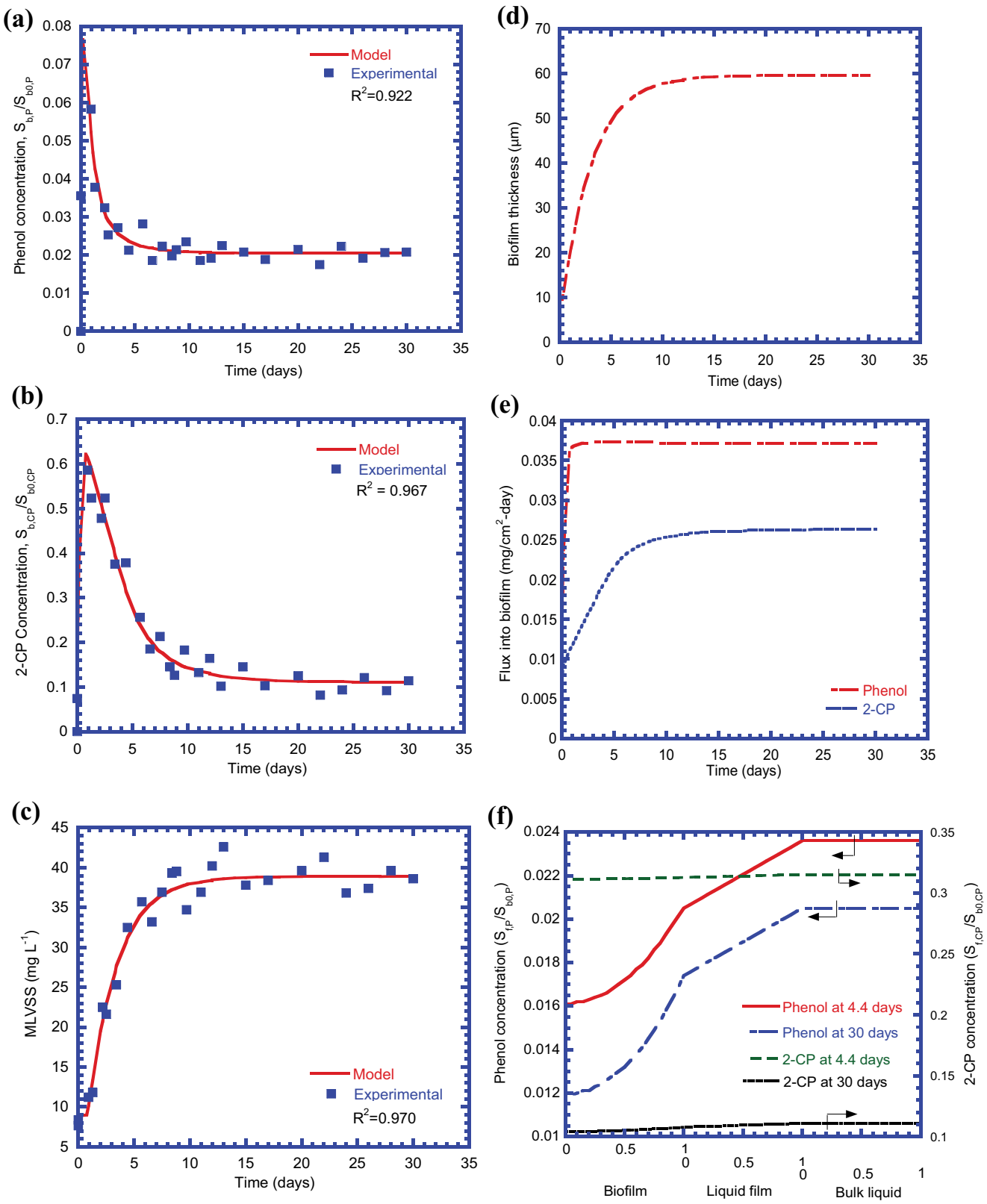

Fig. 11. Experimental data and model predictions in the continuous flow system: (a) phenol effluent, initial phenol concentration = 25.2 mg L^{-1}; (b) 2-CP effluent, initial 2-CP concentration = 19.3 mg L^{-1}; (c) suspended biomass effluent; (d) biofilm growth; (e) flux into the biofilm; and (f) concentration profiles of phenol and 2-CP.

two packed bed bioreactors for continuous treatment of a mixtures of 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol at two different temperatures. They found that the removal efficiencies of all chlorophenolic compounds were more than 99%. Furthermore, Bajaj et al. [44] conducted a continuous treatment of 2-CP at increasing concentrations up to 2,600 mg L^{-1} in an anaerobic fixed bed reactor and found that the removal efficiency of 2-CP was 81%. In this study, the removal efficiency of phenol and 2-CP was 98% and 89% by a packed bed bioreactor at a steady-state condition, respectively. The approaches of experiments and kinetic model presented in this study could be employed to design a pilot-scale or full-scale packed bed bioreactor system for synchronous removal of phenol and chlorophenols by acclimated activated sludge in wastewater.

5. Conclusions

This study demonstrated that non-acclimated and acclimated activated sludge has a higher capacity for the biodegradation of phenol than that of 2-CP. The biodegradation time of 2-CP by a non-acclimated activated sludge in binary substrates via re-feeding was improved by 4 d upon the second re-feeding of the same amount of 2-CP. Batch biodegradation experiments were performed in order to investigate phenol and 2-CP, as well as the growth of acclimated activated sludge. Our experiments indicated that the phenol was completely degraded within 10 h, however, the time required for complete degradation of 2-CP was within 12 d for all levels of substrates. In addition, the Haldane model was applied to fit the results of the batch growth experimental data of phenol and 2-CP to determine the biokinetic parameters (μ_{max}, K_s, and K_i). The growth yield ($Y_{x/s}$) and decay coefficient (k_d) were determined by the batch growth results. The sum kinetic model was used to fit the experimental data of phenol plus 2-CP to determine the interactions parameters ($I_{CP,P}$ and $I_{P,CP}$). Furthermore, a continuous-flow packed bed reactor was conducted to evaluate the biodegradation of phenol plus 2-CP. The results of the kinetic experiments indicated that the removal efficiencies of phenol and 2-CP were 98% and 89%, respectively. The experimental results of the effluent concentrations for phenol plus 2-CP, as well as the suspended biomass, agreed well with the results of the model predictions.

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