Biodegradation **12:** 189–199, 2001.

Biotransformation kinetics of *Pseudomonas putida* for cometabolism of phenol and 4-chlorophenol in the presence of sodium glutamate

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Accepted 5 February 2001

Key words: phenol degradation, 4-cp transformation, modeling, cometabolism, ternary substrate system, inhibition, toxicity

Abstract

A kinetic model to describe the degradation of phenol and cometabolic transformation of 4-chlorophenol (4-cp) in the presence of sodium glutamate (SG) has been developed and validated experimentally. The integrated model accounts for cell growth, toxicity of 4-cp, cross-inhibitions among the three substrates, and the different roles of the specific growth substrate (phenol) and the conventional carbon source (SG) in the cometabolism of 4-cp. In this ternary substrate system, the overall phenol degradation and 4-cp transformation rates are greatly enhanced by the addition of SG since SG is able to attenuate the toxicity of 4-cp and therefore increase the cell growth rate. Model analysis indicates that the maximum specific degradation rate of phenol (0.819 mg (mg.h)$^{-1}$) is lowered by SG by up to 46% whereas the specific transformation rate of 4-cp is not directly affected by the presence of SG. The competitive inhibition coefficient of 4-cp to phenol degradation ($K_{i,cp}$) and that of phenol to 4-cp transformation ($K_{i,ph}$) were determined to be 6.49 mg l^{-1} and 0.193 mg l^{-1}, respectively, indicating that phenol imposes much larger competitive inhibition to 4-cp transformation than the converse. The model developed can simultaneously predict phenol degradation and 4-cp transformation, and is useful for dealing with cometabolism involving multiple substrates.

Introduction

Cometabolism, which refers to the transformation of a nongrowth substrate by cells growing on a growth substrate or by resting cells, is considered a special class of biological transformation. Such transformations have considerable environmental and ecological significance since a large number of organic compounds, especially the commonly used chlorinated solvents, are subject to cometabolism. Unfortunately, cometabolism often tends to be an unsustainable process. The presence of a nongrowth substrate can inhibit metabolism of the natural growth substrate because of its toxicity or recalcitrance, thereby decreasing cell growth and retarding biodegradation (Criddle 1993; Sáez & Rittmann 1993).

Much work has been devoted to the kinetics study of cometabolic processes, especially of the cometabolic transformation of trichloroethylene (TCE). Models have been proposed over the past few years to address important phenomena observed in cometabolism, such as competitive inhibition (Aziz et al. 1999), product toxicity (Alvarez-Cohen & McCarty 1991a; Zhong & Bajpai 2000), cell decay and death (Chang & Criddle 1997; Criddle 1993), enzyme inactivation and recovery (Ely et al. 1995; Zhong & Bajpai 2000), as well as cometabolic cofactor dependency (Chang & Alveraz-Cohen 1995; Chang & Criddle 1997; Sáez & Rittmann 1993). These phenomena may play quite different roles in different systems. For example, endogenous cell decay and inactivation due to product toxicity are major factors to be considered in the absence of a growth substrate. A concept of biomass transformation capacity has been introduced to quantify this effect (Alvarez-Cohen & McCarty 1991b). In the case of rapid oxidation of the

growth substrate, bacteria may show strong capacity to recover in response to inactivation, so that inactivation may not even be detected in some cases of TCE transformation (Ely et al. 1997). Generally speaking, no matter what approach is used in cometabolism modeling, it has been proposed that, for growing cells, the transformation rate is strongly linked to the oxidation of the growth substrate (Chang & Alvarez-Cohen 1995; Criddle 1993; Ely et al. 1995; Zhong & Bajpai 2000).

Biological treatment of wastewater and industrial effluents often involves utilization and degradation of mixed substrates. In cometabolic transformation of nongrowth substrates, different kinds of growth substrates are often also present, and they may have quite different roles in cometabolism because of the different types and functions of biodegradative enzymes involved. As a result, the extension of existing cometabolic models involving two substrates (one specific growth substrate and one nongrowth substrate) to a cometabolism system containing three substrates or for that matter, multiple substrates, is neither obvious nor straightforward. Toward this end, kinetics study of a ternary substrate system comprising one nongrowth substrate (4-cp) and two growth substrates (phenol as a specific growth substrate and SG as a conventional substrate) was investigated. In an earlier investigation, kinetics of cell growth was studied and a model was developed to understand the various substrate interactions on cell growth in this ternary substrate system (Wang & Loh 2000). The present work focuses on (i) the kinetics modeling of the degradation of phenol and the cometabolic transformation of 4-cp in the presence of SG, (ii) the effects of substrate interactions on phenol degradation and 4-cp transformation, and (iii) elucidating the roles of the two growth substrates, phenol and SG, in the cometabolism of 4-cp.

Description of models

Cell growth model

In our previous study, a mathematical model had been developed and validated for cell growth on the ternary substrate system (Wang & Loh 2000), such that

$$\mu = \mu_1 + \mu_2 - \mu_d \tag{1}$$

where μ represents the overall specific growth rate, μ_1 and μ_2 account for the contribution of growth on phenol and SG, respectively, and μ_d is the specific death rate resulting from the toxicity of 4-cp. The effects of the various substrate interactions among the three substrates on cell growth rate are integrated into the model equations for μ_1 and μ_2 (Wang & Loh 2000).

According to the model, the specific growth rate can be calculated based on the initial substrate concentrations. However, the above equation (Equation (1)) only reflects the specific growth rate during the exponential growth phase when the specific growth rate is constant and independent of substrate concentration (Shuler & Kargi 1992). At the end of the exponential phase, cell growth in this ternary substrate system slows down near the depletion of phenol and/or SG due to depletion of nutrients and/or accumulation of by-products. In order to model the cell growth rate not only for the exponential growth phase but also the stationary and death phase, the following extension of Tessier equation (Shuler & Kargi 1992), is used:

$$\mu = \mu_1(1 - e^{-\alpha S_1}) + \mu_2(1 - e^{\beta S_2}) - \mu_d \tag{2}$$

The constant parameters, α and β, are obtained based on an empirical assumption that the respective growth rate (μ_1, μ_2) slows down by 10% at the point when the corresponding growth substrate has been consumed by 80%, i.e.

$$1 - e^{-\alpha S_1} = 0.9 \quad \text{when } S_1 = 0.2S_{10} \tag{2a}$$

$$1 - e^{-\beta S_2} = 0.9 \quad \text{when } S_2 = 0.2S_{20} \tag{2b}$$

Therefore, α and β can be determined based on initial substrate concentrations only. The validity of this assumption is assessed and discussed in detail in the first part of "Results and Discussion". Cell density is therefore modeled as:

$$X = X_0 e^{\int_0^t \mu \, \mathrm{d}t} \tag{5}$$

Phenol degradation

Phenol degradation has been modeled and verified over a wide range of initial phenol concentrations (25 $\sim$ 800 mg l^{-1}) (Wang & Loh 1999) as:

$$q_{ph} = -\frac{\mathrm{d}S_1}{X \, \mathrm{d}t} = \frac{R_m S_1}{K_S + S_1 + \frac{(S_{10} - S_1)^2}{K_p}} \tag{6}$$

To consider the competitive inhibition effect of 4-cp on phenol degradation, $K_{S,cp}$ replaces K_S in Equation (4) where:

$$K_{S,cp} = K_S \left(1 + \frac{S_3}{K_{i,cp}}\right) \quad (7)$$

When the cells were grown simultaneously on phenol and SG, the following empirical model was proposed to quantify the effect of SG on phenol degradation over a wide range of SG concentrations (0∼4000 mg l^{-1}):

$$\frac{R_{m,SG}}{R_m} = 1 - Q(1 - e^{-S_2/(P \cdot S_{10})}) \quad (8)$$

In this case, $R_{m,\mathrm{SG}}$ replaces R_m in Equation (4). So:

$$q_{ph,SG} = -\frac{\mathrm{d}S_1}{X\ \mathrm{d}t} = q_{ph}(1 - Q(1 - e^{-S_2/(P \cdot S_{10})}) \quad (9)$$

For the ternary substrate system, the specific degradation rate of phenol is described by:

$$q_{ph,cp,\mathrm{SG}} = -\frac{\mathrm{d}S_1}{X\ \mathrm{d}t} = \frac{R_{m,SG} S_1}{K_{S,cp} + S_1 + \frac{(S_{10} - S_1)^2}{K_p}} \quad (10)$$

4-cp transformation

Cometabolic transformation of a nongrowth substrate by resting cells is often described using Monod-like relationship. For 4-cp transformation in the absence of growth substrate:

$$q_{cp} = -\frac{\mathrm{d}S_3}{X\ \mathrm{d}t} = \frac{k_c S_3}{K_C + S_3} \quad (11)$$

In the presence of phenol (a specific growth substrate), the 4-cp transformation rate is linked to the utilization of phenol by a constant growth substrate transformation capacity, T_C^g, (Criddle 1993):

$$q_{cp} = (q_{ph} T_C^g + k_c) \frac{S_3}{K_{C,ph} + S_3} \quad (12)$$

where K_C in Equation (9) is replaced with $K_{C,ph}$ to account for competitive inhibition of phenol to 4-cp:

$$K_{C,ph} = K_C \left(1 + \frac{S_1}{K_{i,ph}}\right) \quad (13)$$

Unlike phenol, which is responsible for inducing key enzymes and providing reducing powers for cometabolism, the effect of the additional growth substrate, SG, in this ternary substrate system, is primarily responsible for generating more cells and attenuating the toxicity of 4-cp toward cell growth (Wang & Loh 2000). Nevertheless, the specific degradation rate of phenol is inhibited by SG as shown in Equation (7), so that the specific transformation rate of 4-cp in the presence of phenol and SG is described as:

$$q_{cp,ph,SG} = (q_{ph,cp,SG} T_C^g + k_c) \frac{S_3}{K_C \left(1 + \frac{S_1}{K_{i,ph}}\right) + S_3} \quad (14)$$

Since three different substrates are involved in this system and their consumption or transformation is strongly inter-related, simultaneous modeling of degradation profiles of all three substrates is quite laborious, and there may be propagation of errors during parameter estimation. To simplify the modeling process and minimize errors in estimation of model parameters, the utilization profile of SG is not modeled. Instead, the experimental measurements of SG utilization are correlated using a polynomial function and applied to the model equations shown above.

Materials and methods

Organism and culture conditions

The bacterium *Pseudomonas putida* ATCC 49451, which is able to grow on phenol and cometabolize 4-cp, was used throughout this work. In our previous study (Loh & Wang 1998), TOC analysis has confirmed that the TOC associated with 4-cp and its transformation products in the medium remains constant as 4-cp transformation proceeds. This ascertains that 4-cp is a nongrowth substrate in this ternary substrate system. The mineral salt medium was prepared as described elsewhere (Loh & Wang 1998). All media (except phenol and 4-cp), pipette tips, and Erlenmeyer flasks fitted with cotton plugs were autoclaved at 121 °C for 20 min for sterilization. Phenol and 4-cp (Merck, Darmstadt, Germany) were directly added in flasks as needed from stock solutions. Cells maintained on agar slants were activated in the mineral medium containing 200 mg l^{-1} phenol as the sole carbon substrate. The phenol-grown cells in the exponential growth phase were used as inoculum. All cultures were grown in 500 ml Erlenmeyer flasks containing 250 ml medium at 30 °C with shaking at 200 rpm on a New Brunswick rotary shaker.

Analytical Methods

Cell mass density was measured with UV spectrophotometer at 600 nm. The OD value was converted to dry cell mass using a dry weight calibration curve obtained for this bacterial strain (Wang & Loh 1999). The dry cell mass density was determined by centrifuging a known volume of suspension culture and drying in a pre-weighed ceramic dish at 80 °C until the weight remained constant.

Phenol and 4-cp concentrations were measured by gas chromatography, the detailed procedures of which have been described previously (Loh & Wang 1998). SG was determined by HPLC using a pre-column OPA (ortho-phthaldehyde) derivatization technique (Wang & Loh 2000).

Estimation of the model parameters

The forward Euler method was used to solve the differential equations numerically. Model parameters were estimated by curve-fitting using nonlinear least squares technique (Wang & Loh 2000).

In Equation (3), X_0 represents the initial biomass concentration. Under the condition of very low inoculum size, estimation of X_0 becomes necessary because of the inherent inaccuracy of OD measurement at time zero and the existence of a lag phase. In addition, the cell growth profile described by Equation (3) is very sensitive to X_0 (Chang et al. 1993). To account for these factors, X_0 was chosen as an adjustable parameter obtained by curve-fitting the experimental data to Eqs. (2) and (3).

Model parameters for degradation of phenol alone (R_m, K_S, K_p) have been determined previously (Wang & Loh 1999). A series of independent batch cultures with phenol and SG as dual growth substrates were conducted to obtain Q and P of Equaiton (6). The competitive inhibition parameter of 4-cp to phenol degradation was determined by curve-fitting experimental data of phenol degradation profiles in the presence of 4-cp, to Eqs. (4) and (5).

To determine the maximum specific transformation rate (k_c) and the half-saturation coefficient (K_C), 4-cp transformation in the absence (or at very low concentration) of growth substrate was monitored over short-time intervals for a range of initial 4-cp concentrations. The transformation capacity of phenol (T_C^g), and the competitive inhibition coefficient ($K_{i,ph}$) were determined based on experimental data of 4-cp transformation in the presence of phenol.

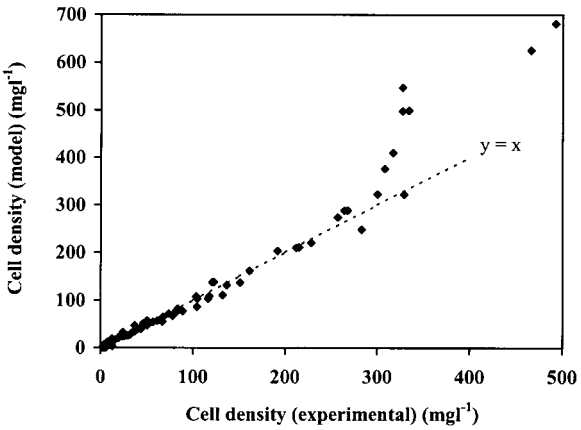

Figure 1. Comparison of cell density between experimental data and model simulations.

Results and Discussion

Cell growth

Eqs. (2) and (3) were used to model cell growth on individual growth substrates as well as various combinations of the three substrates. Table 1 shows the correlation coefficient (R^2) matched to Eqs. (2) and (3), over the entire growth curve in each experiment. For all of them (except tests 3, 8 and 12), R^2 was higher than 0.98. For these experiments, the concentrations of the growth substrates were not very high and the maximum cell mass density for each of these tests never exceeded 300 mg l^{-1}. The lower R^2 for experiments 3, 8, and 12 resulted from the poorer fit at high cell mass density near the end of each experiment. Nevertheless, if the correlation was applied to the period during which 0∼90% of the initial phenol and 4-cp were removed, the R^2 for tests 3, 8 and 12 were respectively, 0.967, 0.995, and 0.967. We therefore conclude that Eqs. (2), and (3) are suitable for modeling our cell growth profiles.

Figure 1 plots experimental data versus model simulations for the cell densities obtained for all tests included in Table 1; good agreement was obtained for cell mass density up to about 300 mgl^{-1}. A greater deceleration than 10% in the specific growth rate was observed experimentally when the cell mass density was larger than 300 mg l^{-1}. Nevertheless, our assumption for determining α and β is sufficient for modeling phenol and 4-cp degradation kinetics since in all the experiments, most of the phenol and 4-cp were removed prior to achieving 300 mg l^{-1} cell mass density.

Table 1. R-square values for the fit of the cell growth model to experimental data

Test No.	Substrate concentration (mg l^{-1})			α	β	R^2 value
	S_{10}	S_{20}	S_{30}	(mg l^{-1})$^{-1}$	(mg l^{-1})$^{-1}$	
1	106	0	0	0.109	–	0.987
2	507	0	0	0.0227	–	0.996
3	800	0	0	0.0144	–	0.834
4	0	195	0	–	0.0592	0.991
5	0	506	0	–	0.0228	0.989
6	0	1014	0	–	0.0114	0.995
7	104	51	0	0.111	0.226	0.999
8	207	998	0	0.0557	0.0116	0.881
9	210	0	194	0.0549	–	0.993
10	202	0	105	0.0571	–	0.980
11	202	197	200	0.0571	0.0585	0.994
12	193	1049	94	0.0597	0.0110	0.816

A good simulation of cell growth profile is crucial to modeling substrate degradation profiles as the error in degradation model would be increased or masked when using a poor growth kinetics model. An alternate common approach for cell growth modeling is to couple cell growth rate directly with the substrate removal rate. This approach, however, is inaccurate when the cell mass yield coefficient varies (which is often observed) (Allsop et al. 1993; Wang & Loh 1999), especially over a wide range of substrate concentrations. Furthermore, the presence of a nongrowth substrate can also substantially reduce the cell mass yield (Loh & Wang 1998; Oh et al. 1994).

Phenol degradation

In our previous study on biodegradation of phenol, we have reported the use of Equation (4) to describe phenol degradation profiles over a wide range of initial phenol concentrations. This is possible due to the inclusion of the inhibition effect of intermediates of phenol degradation as well as the consideration of a variable cell mass yield (Wang and Loh 1999). This model serves as the basis to study the effect of SG and 4-cp on phenol degradation in this work.

To obtain the kinetics for the effect of SG on phenol degradation, Q in Equation (6) was determined for cases when the second term, $\exp(-S_2/(PS_{10}))$, was negligible. This was achieved at low initial concentration of phenol and high initial concentration of SG. Aimed at obtaining Q, two experiments containing phenol/SG at 50/500 mg l^{-1} and 100/1000 mg l^{-1}, respectively, were performed. Figure 2 shows

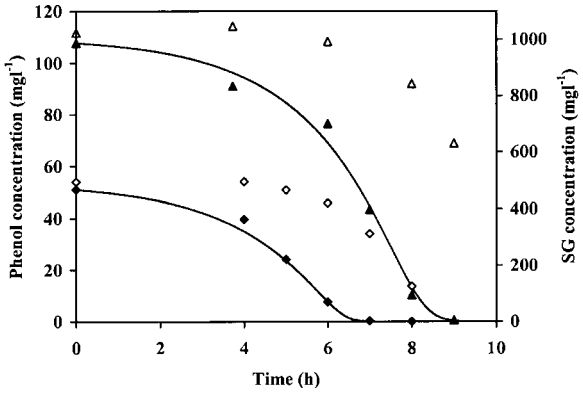

Figure 2. Phenol removal in the presence of a relatively high SG concentrations. Symbols: ▲ and ♦ represent phenol concentrations while the corresponding open symbols represent SG concentrations. Lines represent model fit.

that for these experiments, only less than 40% of the initial SG added was consumed when phenol was depleted. During the phenol degradation period, therefore, $\exp(-S_2/(PS_{10}) \approx 0$ unless P is much larger than unity, which we will show otherwise. Based on the experimental data, Q was found to be 0.464 (R^2 = 0.998). In contrast to Q, Equation (6) is more sensitive to P at low SG concentrations. P was determined to be 0.750 based on experiments with initial SG concentrations of 20 mg l^{-1}, 50 mg l^{-1}, and 100 mg l^{-1}, each with a fixed initial phenol concentration of 50 mg l^{-1}.

According to Equation (6), Q represents the maximum inhibition effect of SG on phenol degradation. Despite the fact that the presence of SG reduced the specific phenol degradation rate by up to 46%, even

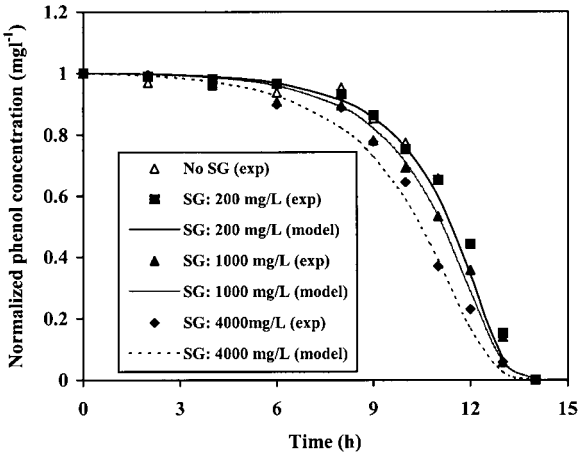

Figure 3. Effect of SG on phenol degradation at initial nominal phenol concentration of 200 mg l^{-1}.

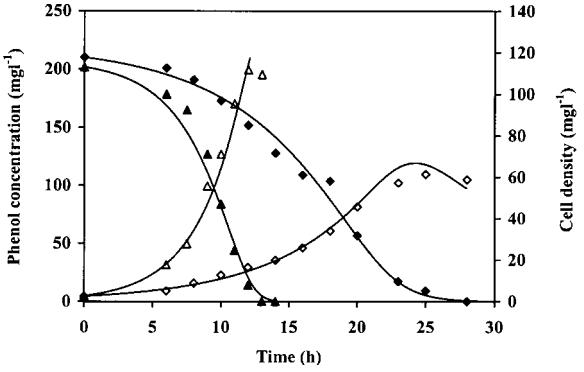

Figure 4. Model simulation for effect of 4-cp on phenol degradation. Symbols: ▲ and ♦ represent phenol concentrations in the presence of initial 4-cp concentrations of 100 mg l^{-1} and 200 mg l^{-1}, respectively. The corresponding symbols represent cell density. Lines represent model fit.

Table 2. Summary of model parameters for simulating phenol degradation in the ternary substrate system

Model parameters	Values
Degradation of phenol alone*	
R_m	0.819 (± 0.056) mg(mg.h)$^{-1}$
K_S	2.19 (± 0.68) mg l^{-1}
K_p	810 (± 210) mg l^{-1}
Effect of SG	
P	0.750 (± 0.127) (dimensionless)
Q	0.464 (± 0.024) (dimensionless)
Competitive inhibition of 4-cp	
$K_{i,cp}$	6.49 (±0.93) mg l^{-1}

*Obtained previously (Wang and Loh 1999). Parameters estimated are shown ± 1 standard deviation in parentheses.

at high SG concentration, phenol was completely consumed in all the experiments. Figure 3 shows that the effect of SG, over a concentration range of 0∼4000 mg l^{-1}, on the overall phenol degradation kinetics was not significant. The comparison between experimental data and model predictions also shows very good agreement. Similar results were also obtained for other initial concentrations of phenol (400 mg l^{-1}, 600 mg l^{-1}, and 800 mg l^{-1}). Although the specific degradation rate of phenol is lower in the presence of SG, more cells are generated with simultaneous utilization of phenol and SG.

The competitive inhibition constant of 4-cp to phenol degradation was estimated based on an experiment performed at 200 mg l^{-1} 4-cp. Another experiment conducted at 100 mg l^{-1} 4-cp was used to validate the model prediction. The initial nominal concentration of phenol for these two tests was 200 mg l^{-1}. Figure 4 shows that the model simulated phenol degradation kinetics very well for both sets of experimental data. The toxicity of 4-cp has resulted in reduced cell growth rate and cell mass yield on phenol. It can be reasoned based on the validated model that the presence of 4-cp also decreased the specific degradation rate of phenol. On the other hand, phenol also exerted strong competitive inhibition on 4-cp transformation. It was observed that rapid removal of 4-cp occurred only near the depletion of phenol (data shown later). The effect of phenol on 4-cp transformation will be quantified and discussed in detail subsequently.

Table 2 summarizes the model parameters obtained for phenol degradation in the ternary substrate system. Figure 5 plots a comparison of model predictions against experimental data for 200 mg l^{-1} each of phenol, 4-cp and SG. The corroboration is excellent with a coefficient of correlation of $R^2 = 0.997$.

Although there was no significant effect of SG on the overall phenol degradation rate in the absence of 4-cp (Figure 3), a comparison of Figures 4 and 5 indicates that the addition of SG markedly enhanced phenol degradation in the presence of 4-cp. With 200 mg l^{-1} 4-cp, the same amount of phenol was removed in 28 hours without SG (Figure 4) compared to less than 17 hours with the addition of 200 mg l^{-1} SG (Figure 5).

This difference in the effect of SG on phenol degradation in the presence and absence of 4-cp is attributed to the effect of SG on cell growth. In the absence of 4-cp, from Eqs. (4), (6), and (7), the direct effect of SG on the specific phenol degradation rate is obtained as:

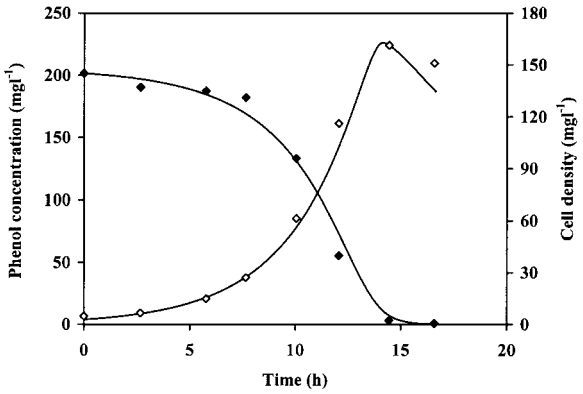

Figure 5. Model prediction for phenol degradation in the ternary substrate system of 200 mg l^{-1} each of phenol, 4-cp and SG. Symbols: ♦ represents experimental phenol concentration and ◊ represents experimental cell density. Lines represent model simulations.

$$\frac{q_{ph,SG}}{q_{ph}} = \frac{R_{m,SG}}{R_m} = 1 - Q(1 - e^{-S_2/(P \cdot S_{10})}) \quad (15)$$

In the presence of 4-cp, based on Eqs. (4–6) and (8), the direct effect of SG on the specific phenol degradation rate is described by:

$$\frac{q_{ph,cp,SG}}{q_{ph,cp}} = \frac{R_{m,SG}}{R_m} = 1 - Q(1 - e^{-S_2/(P \cdot S_{10})}) \quad (16)$$

From Eqs. (13) and (14), it can be seen that the direct effect of SG on specific phenol degradation rate is the same regardless of the presence of 4-cp.

In the absence of 4-cp, according to the cell growth model developed previously (Wang & Loh 2000), while addition of SG to phenol-containing medium may not necessarily increase the specific growth rate, the addition of SG shortens the lag phase slightly (represented by a higher estimated X_0 (data not shown), resulting in a higher cell density (Equation (3)). However, this effect is negated by the inhibition effect of SG on the specific phenol degradation rate as given by Equation (13). The net result of these is the observed insignificant effect of added SG on the overall phenol degradation.

In contrast, in the presence of 4-cp, SG addition greatly increases the cell growth rate. For example, we have found that at 200 mg l^{-1} each of phenol and 4-cp, the specific growth rate was increased substantially from 0.13 h^{-1} to 0.30 h^{-1} by the addition of 200 mg l^{-1} SG; while in the absence of 4-cp, the specific growth rate decreased from 0.52 h^{-1} (at 200 mg l^{-1} phenol alone) to 0.44 h^{-1} (200 mg l^{-1} each of phenol and SG) due to the cross-inhibition between phenol and SG (Wang & Loh 2000). This indicates that SG could effectively attenuate the toxicity of 4-cp toward cell growth. The contribution of SG to the higher specific growth rate with a concomitant production of more cell mass outweighs the inhibition effect of SG on the specific phenol degradation rate. Our observations are consistent with that reported by Papanastasiou and Maier (1982) that enhancement of overall 2,4-D degradation rate in the presence of glucose was observed because cell growth on 2,4-D alone was much lower than that on a mixture of glucose and 2,4-D.

4-cp transformation

To study 4-cp transformation in the ternary substrate system, the model parameters for 4-cp transformation in the absence of growth substrate were determined first. Cells were initially grown on 300 mg l^{-1} phenol as the sole growth substrate. The medium was spiked with 4-cp when phenol was almost depleted in the medium (less than 1 mg l^{-1}). As shown in Figure 6a, it took about 4 and 7 hours respectively to completely transform the 55 and 80 mg l^{-1} 4-cp spiked. This slow 4-cp transformation rate contrasted with our earlier observation that rapid removal of 4-cp occurred near phenol depletion due to removal of competitive inhibition by phenol. We hypothesized that cells may have been inactivated by the shock loading of 4-cp because of its toxicity to the cells. To better understand the effect of shock loading of 4-cp, 4-cp was added at a range of concentrations before phenol depletion. These experiments were performed with the same initial phenol concentrations of 300 mg l^{-1}. The cultures were spiked with 4-cp during the period of rapid phenol utilization. As shown in Figure 6b, the addition of 4-cp at time zero immediately stopped phenol degradation. The time required for re-utilization of phenol increases with the concentration of 4-cp spiked. With 92 mg l^{-1} 4-cp, phenol utilization stopped for only about 10 minutes; this time was prolonged to 50 minutes and 170 minutes for 144 mg l^{-1} and 193 mg l^{-1} 4-cp addition, respectively. These results show that cells were inactivated by shock loading of 4-cp. Since no 4-cp transformation was observed during the period that phenol utilization halted, the inactivation could only be due to the toxicity of 4-cp itself rather than intermediates or products arising from

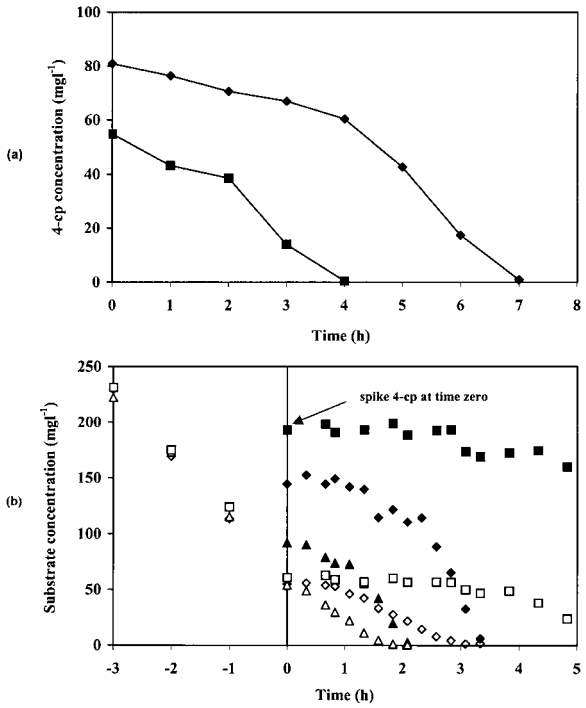

Figure 6. (a) 4-cp transformation profile when 55 mg l^{-1} (■) or 80 mg l^{-1} (♦) of 4-cp was added upon depletion of phenol; (b) 4-cp transformation profile when 4-cp at 92 mg l^{-1} (▲), 144 mg l^{-1} (♦), or 193 mg l^{-1} (■) was added prior to depletion of phenol. The corresponding open symbols represent phenol concentrations.

the 4-cp transformation process. This is in agreement with observations reported by Kim and Hao (1999) that in the cometabolic degradation of chlorophenols by phenol-induced cells of *Acinetobacter* species, no product toxicity was observed in 4-cp transformation. Figure 6b also shows that the inactivated cells could recover and continue to grow on phenol and transform 4-cp after some time. With exposure to shock loading of higher 4-cp concentrations, a longer time was required for recovery.

In intensive studies of cometabolism of TCE, it has been observed that transformation of TCE led to cell inactivation caused by damage to cellular constituents (Oldenhuis et al. 1991; Rasche et al. 1991). Unlike TCE, in our research, 4-cp itself is primarily responsible for the inactivation of cells. The addition of 4-cp after depletion of phenol would require much longer time for the cells to recover, and only partial recovery achieved in the absence of growth substrate.

In order to determine the model parameters k_c and K_C, two conditions should therefore be satisfied. Firstly, cells should have fully recovered after shock loading of 4-cp and secondly, data of 4-cp transformation should be taken at low phenol concentration in order to avoid competitive inhibition of phenol. Under such circumstances, and for the case of K_C being negligible compared to the 4-cp concentration at higher than 30 mg l^{-1}, K_C was determined to be 0.633 mg (mg.h)$^{-1}$. K_C was then obtained as 5.55 mg l^{-1} by fitting the whole range of the 4-cp transformation data to Equation (9). According to the analyses on parameter sensitivity performed by Alvarez-Cohen and McCarty (1991c), the maximum transformation rate (k_c) should be measured at concentrations well above the K_C value. It is clear that our experimental data satisfied this condition. Three sets of experimental data were used for estimation of the two parameters.

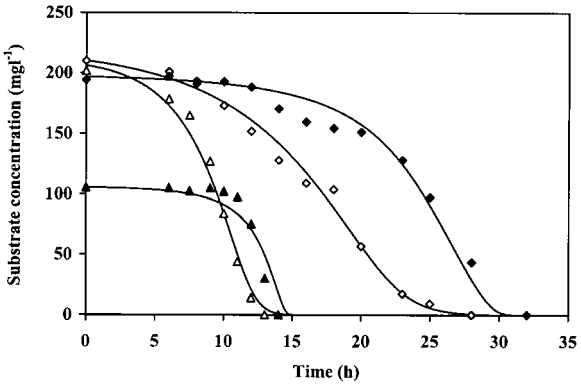

Figure 7. Simultaneous modeling of phenol degradation and 4-cp transformation in the absence of SG. Filled symbols represent 4-cp concentrations while the corresponding open symbols represent phenol concentrations.

Specific degradation rate of phenol was calculated from Equation (4) using the parameters obtained earlier. Together with experimental data for 4-cp transformation in the presence of phenol, the constant transformation capacity of phenol (T_C^g) and the competitive inhibition coefficient of phenol to 4-cp transformation ($K_{i,ph}$) were determined. Figure 7 illustrates the comparison between experimental data and the simultaneous simulation of phenol degradation and 4-cp transformation. The fit between the experimental data and model simulations for both experiments is good.

Comparing the two competitive inhibition coefficients, $K_{i,cp}$ = 6.49 mg l^{-1} (4-cp to phenol) and $K_{i,ph}$ = 0.193 mg l^{-1} (phenol to 4-cp), it is clear that the inhibition of phenol to 4-cp transformation is much greater than the converse. Similar results were obtained in cometabolism of TCE with methane as the growth substrate (Chang & Criddle 1997).

Table 3. Summary of model parameters for simulating 4-cp transformation in the ternary substrate system

Model parameters	Values
No growth substrate	
k_c	0.633 ($\pm$ 0.017) mg(mg.h)$^{-1}$
K_C	5.55 ($\pm$ 1.15) mg l^{-1}
Presence of phenol	
T_C^g	3.88 ($\pm$ 1.66) mg 4-cp/mg phenol
$K_{i,ph}$	0.193 ($\pm$ 0.053) mg l^{-1}

Parameters estimated are shown $\pm$ 1 standard deviation in parentheses.

Table 3 summarizes the model parameters obtained for modeling 4-cp transformation. Combined with the model parameters in Table 2 for phenol degradation, the models for cometabolism of phenol and 4-cp can be solved numerically to predict simultaneously phenol degradation and 4-cp transformation in the presence of SG. Two independent experiments were performed to assess the applicability of the models developed: in one, the system comprised 200 mg l^{-1} each of phenol, 4-cp and SG; in the other, the system comprised 200 mg l^{-1} phenol, 100 mg l^{-1} 4-cp and 1000 mg l^{-1} SG. Figure 8a shows a comparison between model prediction and experimental data for the first experiment. The profiles of both phenol and 4-cp removal were well predicted with concomitant simulation of cell growth. The second experiment serves to represent the case of a maximum cell density higher than 300 mg l^{-1}. Figure 8b shows that the agreement between experimental results and model predictions for phenol degradation and 4-cp transformation is still very good. As mentioned earlier, the cell growth model overestimated the cell mass density when the maximum cell density was higher than about 300 mg l^{-1}. Nevertheless, this overestimation did not affect simulations of phenol and 4-cp degradation since much of phenol and 4-cp have been removed before the cell density increased to 300 mg l^{-1}.

Comparing 4-cp transformation with and without SG, it was found that 4-cp transformation has been enhanced significantly with SG addition. Similar to the effect of SG on phenol degradation in the presence of 4-cp, the enhancement of 4-cp transformation is also attributed to the production of higher levels of active cell mass through simultaneous utilization of SG. Despite the similarity, however, there is no direct effect of SG on the specific 4-cp transformation rate according to Equation (12) in contrast to the inhibition effect that

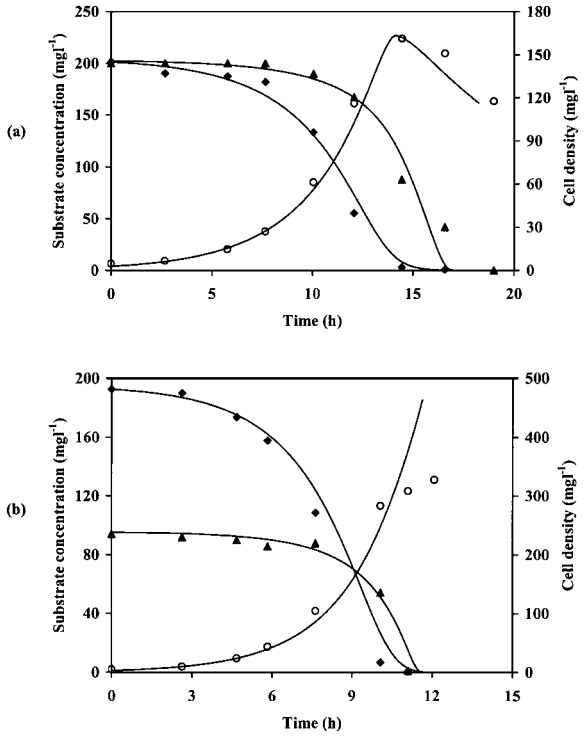

Figure 8. Simultaneous modeling of phenol degradation and 4-cp transformation in the ternary substrate system. (a) 200 mg l^{-1} each of phenol, 4-cp and SG; (b) 200 mg l^{-1} phenol, 100 mg l^{-1} 4-cp and 1000 mg l^{-1} SG. Symbols: ♦, phenol concentration; ▲, 4-cp concentration; ○, cell density.

SG exerts on the specific phenol degradation rate as given by Equation (6).

By now, it is clear that the capacity of a cell to cometabolically transform a nongrowth substrate (i.e. the specific transformation rate of nongrowth substrate) can be enhanced by the presence of growth and energy substrates. This enhancement may be attributed to elevated levels of catabolic enzyme activity due to substrate induction, faster generation of reducing power such as NADH or NADPH, and/or higher rate of synthesis of new enzymes in response to inactivation (Chang & Alvarez-Cohen 1995; Criddle 1993; Ely et al. 1995). Therefore, the specific transformation rate of nongrowth substrate is linked to the oxidation of growth substrate. The model analysis in this work suggests that this linkage occurs primarily between the nongrowth substrate and the specific growth substrate; their interactions are directly responsible for the degradation rate enhancement. Based on the kinetics models developed, for the cometabolism of 4-cp, phenol and SG play quite different roles although both of them are growth substrates and are responsible for

production of new cells. As a specific growth substrate, phenol initiates the key enzymes for its own degradation and 4-cp transformation. Phenol oxidation efficiently regenerates the reducing power and continuous phenol degradation sustains the cells' ability to cometabolically transform 4-cp. On the other hand, the major contribution of SG is to increase the specific growth rate and generate more active cells through its metabolism.

Conclusion

The developed kinetics models for phenol degradation and cometabolic transformation of 4-cp in the presence of an additional carbon substrate, SG, have been validated experimentally. The model is not only able to describe the degradation of phenol and cometabolic transformation of 4-cp in the ternary substrate system, it is also capable of quantifying the different roles of the specific growth substrate and the additional carbon source. Model analysis suggests that there is no direct effect of SG on the specific transformation of 4-cp despite its significant contribution to the overall 4-cp transformation rate through the enhancement of cell growth. The experimental results and the developed models can be very useful for dealing with cometabolism involving multiple substrates. For example, the recognition and quantification of different behaviors and functions of different growth substrates may be helpful to simplify the kinetics modeling process, and for application to the design of engineered processes in wastewater treatment.

Acknowledgements

The authors wish to acknowledge the research funding provided by the Environmental Technology Enterprise with grant numbers RP 3602037 and RP 960710A.

Nomenclature

k_c	maximum specific transformation rate of 4-cp (mg l^{-1})
K_C	half-saturation constant for 4-cp transformation (mg l^{-1})
$K_{cp,ph}$	half-saturation constant for 4-cp transformation in presence of phenol (mg l^{-1})
$K_{i,cp}$	inhibition coefficient of 4-cp to phenol degradation (mg l^{-1})
$K_{i,ph}$	inhibition coefficient of phenol to 4-cp transformation (mg l^{-1})
K_p	proportionality constant (mg l^{-1})
K_S	half-saturation constant for phenol degradation (mg l^{-1})
$K_{S,cp}$	half-saturation constant for phenol degradation in presence of 4-cp (mg l^{-1})
P	model parameter for SG inhibition on phenol degradation (dimensionless)
Q	coefficient of SG inhibition on phenol degradation (dimensionless)
q_{cp}	specific transformation rate of 4-cp (mg(mg.h)$^{-1}$)
$q_{cp,ph}$	specific transformation rate of 4-cp in presence of phenol (mg(mg.h)$^{-1}$)
$q_{cp,ph,SG}$	specific transformation rate of 4-cp in the ternary substrate system (mg(mg.h)$^{-1}$)
q_{ph}	Specific degradation rate of phenol (mg(mg.h)$^{-1}$)
$q_{ph,SG}$	Specific degradation rate of phenol in presence of SG (mg(mg.h)$^{-1}$)
$q_{ph,cp,SG}$	Specific degradation rate of phenol in the ternary substrate system (mg(mg.h)$^{-1}$)
R_m	Maximum specific degradation rate of phenol (mg(mg.h)$^{-1}$)
$R_{m,SG}$	Maximum specific degradation rate of phenol in presence of SG (mg(mg.h)$^{-1}$)
S_i	concentration of substrate i (i =1, 2, or 3) (mg l^{-1})
S_{10}	initial substrate concentration of phenol (mg l^{-1})
S_{20}	initial substrate concentration of SG (mg l^{-1})
S_{30}	initial substrate concentration of 4-cp (mg l^{-1})
t	time (h)
T_C^g	Growth substrate transformation capacity (mg 4-cp/mg phenol)
X	biomass concentration (mg l^{-1})
X_0	initial biomass concentration (mg l^{-1})
α	model constant for cell growth on phenol ((mg l^{-1})$^{-1}$)
β	model constant for cell growth on SG ((mg l^{-1})$^{-1}$)
μ	overall specific growth rate (h^{-1})
μ_1	specific growth rate on phenol (h^{-1})
μ_2	specific growth rate on SG (h^{-1})
μ_d	cell decay rate (h^{-1})

Numerical subscripts

1	Growth substrate, phenol
2	Growth substrate, SG
3	Nongrowth substrate, 4-cp

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