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Kinetics of competitive cometabolism under aerobic conditions

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ABSTRACT

Commonly observed competitive substrate inhibition in cometabolism of organic contaminants is used as rate- and reducing-power-determining factors to develop a kinetic model of the competitive cometabolism. Analogous to the well-known theory of Leudeking-Piret kinetics where the product formation demands reducing power, cometabolism is modeled as a reducing power demanding process that also competes with microbial growth for the available reducing power from the degradation of energy-yielding primary substrate. The model further incorporates other growth-associated phenomena such as substrate inhibition and multiple growth/nongrowth substrate interactions that may occur during cometabolic transformation processes. The kinetic model is used successfully to predict a variety of degradation patterns of growth/nongrowth substrates, displayed by microbial cultures when exposed to different concentration ratios of growth to nongrowth substrate: a complete degradation of nongrowth substrates that coincides with the simultaneous depletion of a growth substrate and, in some other cases, an incomplete degradation of a nongrowth substrate following the complete depletion of a growth substrate. These distinct patterns of substrate degradation are attributed to intrinsic specificities of enzymes for cometabolism and lack of reducing power available from the growth substrate degradation. The efficacy of cometabolic capabilities of actively growing microbial cultures and pre-cultured resting cells is discussed in terms of reducing power available in such systems.

1. Introduction

Most microbial models used in environmental engineering have been applied for describing microbial growth on growth-substrates and nutrients under aerobic or anoxic conditions (Kim et al., 2018; Jun et al., 2019) or microbial conversion of organics to energy source like biogas under anaerobic condition (Lohani et al., 2018).

Many environmentally important xenobiotics are cometabolically degraded as a result of nonspecific enzyme activities of microorganisms in the presence (growing) and/or absence (resting) of a growth substrate. For instance, halogenated compounds such as trichloroethylene (TCE) and chlorophenols were degraded by methane (Li et al., 2014), methanol (Chheda and Sorial, 2017), toluene (Kim et al., 2014), and aromatic (Zhang and Tay, 2016) oxidizers. Several polychlorinated biphenyls were also cometabolically degraded under aerobic conditions (Nabavi et al., 2013). Besides the oxidative cometabolism under aerobic conditions, carbon tetrachloride or chlorinated alkanes and alkenes can be degraded by a reductive pathway in the absence of oxygen. However, the degradation mechanism and the specific enzymes involved are still not well-characterized (Nishino et al., 2013). Moreover, the reductive cometabolism of TCE would form highly toxic intermediates, such as vinyl chloride and dichloroethylene species, and thus may result in significant bioaccumulation (Shukla et al., 2014; Zalesak et al., 2017).

The significance and importance of quantitatively describing cometabolism has been long recognized, and a number of studies has been working on the development of cometabolism kinetic models. For instance, Goudar (2012) developed an explicit expression for determining cometabolism kinetics using progress curve analysis. Despite the recent advances, a competitive kinetic model aimed at evaluating oxidative cometabolism in the presence of both growth and nongrowth substrates under aerobic conditions is still limited. A growth substrate is defined as an electron donor that can support microbial growth, and the term nongrowth substrate denotes a variety of chemicals that are nonessential for cell growth but are transformed due to nonspecific enzyme activities.

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Nomenclature

Fundamental quantities

L	length
M_1	mass of growth substrate
M_2	mass of nongrowth substrate
M_b	mass of cell biomass
t	time

English symbols

b	first-order endogenous decay coefficient (t^{-1})
E	free enzyme in Table 1
ES_1	enzyme-substrate1 complexes in Table 1
ES_2	enzyme-substrate2 complexes in Table 1
ES_1S_1	enzyme-substrate1-substrate1 complexes in Table 1
ES_2S_2	enzyme-substrate2-substrate2 complexes in Table 1
ES_1S_2	enzyme-substrate1-substrate2 complexes in Table 1
ES_2S_1	enzyme-substrate2-substrate1 complexes in Table 1
f	fraction of reducing power diverted into cometabolism, either f_l or f_s (M_1/M_1)
f_l	fraction of reductant supply diverted to transformation of the nongrowth substrate under reducing power - limited conditions (M_1/M_1)
f_s	fraction of reducing power diverted to transformation of the nongrowth substrate under reducing power-sufficient conditions (M_1/M_1)
k_1	maximum specific rate of the growth substrate utilization ($M_1\ M_b^{-1}\ t^{-1}$)
k_2	maximum specific rate of the nongrowth substrate transformation in the absence of a growth substrate ($M_2\ M_b^{-1}\ t^{-1}$)
k_m	maximum reaction velocity of an enzyme
K_1	half-saturation constant of the growth substrate ($M_1\ L^{-3}$)
K_{11}	growth substrate inhibition constant by the growth substrate ($M_1\ L^{-3}$)
K_{12}	nongrowth transformation inhibition constant by the growth substrate ($M_1\ L^3$)
K_2	half-saturation constant of the nongrowth substrate ($M_2\ L^{-3}$)
K_{22}	nongrowth substrate inhibition constant by the non-growth substrate ($M_2\ L^{-3}$)
K_{21}	growth substrate utilization inhibition constant by the nongrowth substrate ($M_2\ L^{-3}$)
K_m	binding affinity of an enzyme for either growth or non-growth substrate
n	specificity ratio of growth substrate to nongrowth substrate = SP_1/SP_2 (dimensionless)
n_c	specific growth substrate utilization rate diverted to transformation of the nongrowth substrate ($M_1\ M_b^{-1}\ t^{-1}$)
P_1	intermediate that is further metabolized
P_2	intermediate that is not further metabolized
q_1	specific growth substrate utilization rate ($M_1\ M_b^{-1}\ t^{-1}$)
q_2	specific nongrowth transformation rate ($M_2\ M_b^{-1}\ t^{-1}$)
q_g	specific growth substrate utilization rate for cell growth ($M_1\ M_b^{-1}\ t^{-1}$)
S_1	growth substrate concentration ($M_1\ L^{-3}$)
S_2	nongrowth substrate concentration ($M_2\ L^{-3}$)
SP_1	specificity constant of the growth substrate = k_1/K_1 ($M_b^{-1}\ L^3\ t^{-1}$)
SP_2	specificityconstant of the nongrowth substrate=k_2/K_2 ($M_b^{-1}\ L^3\ t^{-1}$)
X	cell biomass concentration ($M_b\ L^{-3}$)
Y	observed yield ($M_b\ M_1^{-1}$)
Y_m	maximum yield ($M_b\ M_1^{-1}$)
W	growth substrate transformation yield, as defined in this paper ($M_2\ M_1^{-1}$)

Greek Symbol

μ	specific growth rate (t^{-1})

In oxidative cometabolism, initial activation reactions of substrates are generally catalyzed by nonspecific oxygenases, e.g., methane monooxygenase (MMO) in methanotrophs (Zhang et al., 2017), ammonia monooxygenase (AMO) in ammonia oxidizers (Alves et al., 2018), and toluene dioxygenase (TDO) in toluene oxidizers (Nitisakulkan et al., 2014). These oxygenase-catalyzed reactions require molecular O_2 as the terminal electron acceptor and NADH as the reducing power for the oxidation of both growth and non-growth substrates. Consequently, pre-cultured "resting" cells in the absence of a suitable source of reducing power exhibited a finite transformation capacity for nongrowth substrates (Rostkowski et al., 2013). The reducing power-limited nature of resting cell cultures has been further demonstrated (Pieja et al., 2011); methanotrophic strains and mixed methane-oxidizing cultures containing PHB (poly-β-hydroxybutyrate, i.e., a source for NADH) exhibited better sustained TCE transformation rates than those strains without PHB inclusions.

One possible way to overcome the limited reducing power in resting cell transformation systems is to provide a suitable growth substrate so that a continuous source of reducing power can be provided. However, since a common enzyme activity is responsible for the oxidation of both growth and nongrowth substrates, one of substrates can inhibit the oxidation rate of the other. Therefore, addition of a growth substrate to degrade nongrowth substrates by actively growing cultures can also be limited due to competitive substrate inhibition. Nevertheless, previous researchers have recognized the need for a continuous source of reducing power for cometabolism and proposed kinetic models for actively growing cell culture systems (Liu, 2009; Liu et al., 2015). In these models, the oxidation of a growth substrate generates reducing power for cell growth and cometabolism. However, different approaches were taken to quantify how much reducing power that may be channeled into cometabolism from cell growth that incidentally also requires reducing power. For instance, several models assumed an unlimited amount of reducing power for cometabolism, i.e., the transformation rate of a nongrowth substrate is only dependent on substrate inhibition but not on the availability of reducing power (Broholm et al., 1990; Ely et al., 1995). Some models assumed that the oxidation rate of a nongrowth substrate (S_2) should be always linked to the oxidation rate of a growth substrate (S_1) in a proportional manner (i.e., $dS_2/dt = \alpha\ dS_1/dt$ or $dS_2/dt = \alpha\ dS_1/dt + \beta$, where α and β are constants) (Saéz and Rittmann, 1993). However, assumptions in these models may not always be valid under different concentrations of growth and nongrowth substrates due to the intrinsic substrate inhibition. Substrate inhibition is a concentration-dependent process that will affect the availability of reducing power for cometabolism. As results of simplifications in these models, the previous models reported in literature can be used to simulate only one of two possible substrate degradation patterns. For instance, the models proposed by Broholm et al. (1992) and Ely et al. (1995) could be used to simulate the synchronous degradation pattern (Fig. 1(a) and 1(b)) but

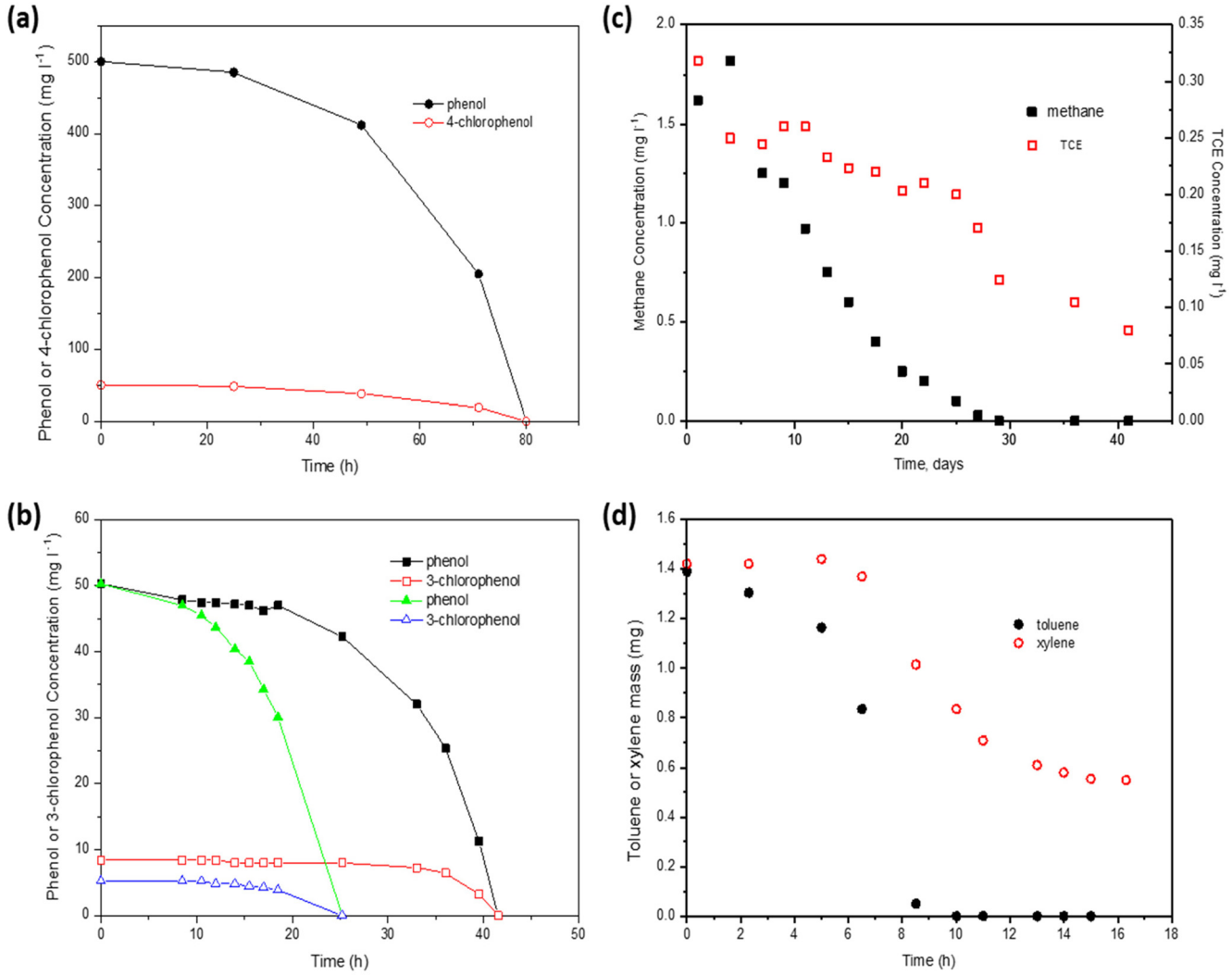

Fig. 1. Synchronous degradation patterns of both the growth and nongrowth substrates, where the value of (a) and (b) was gathered from Polnisch et al. (1992) and Menke and Rehm (1992), respectively. Incomplete degradation patterns of the nongrowth substrate, where the value of (c) and (d) was obtained from Broholm et al. (1992) and Charng et al. (1993), respectively.

not the incomplete transformation pattern (Fig. 1(c) and 1(d)). Similarly, the models proposed by Saéz and Rittmann (1993) and Criddle (1993) could be used for the incomplete transformation pattern but not the synchronous degradation pattern.

As aforementioned, despite a few studies has been done on cometabolic degradation kinetics, little research on distinguishing the kinetics of growing and non-growing substrates coexisted in the complex cometabolic degradation system was found. In this study, a kinetic model of competitive cometabolism that can predict both patterns of substrate degradation are proposed to overcome shortcomings of the previous kinetic models. Specifically, the availability of reducing power for cometabolism is deduced by a growth substrate mass balance from specificity values of microbial cultures/substrates involved and concentrations of growth/nongrowth substrates in the culture media.

2. Methodology

2.1. Theoretical basis and assumptions

Relevant kinetic properties of cometabolic reactions catalyzed by purified enzymes and cell cultures that have been reported in literature are briefly reviewed to provide a theoretical basis for the model development. Cometabolic kinetic studies of oxygenases have shown that both the maximum reaction velocity (v_{max}) and binding affinity (K_m) for substrate are significantly influenced by substituent groups present in the nongrowth substrate, or by the molecular size of nongrowth substrates (Hao et al., 2002). For instance, Dorn and Knackmuss (1978) reported that the 1,2 dioxygenase from *Pseudomonas* species exhibited a reduced v_{max}, but a decreased K_m (i.e., higher affinity) in the presence of electron-withdrawing groups such as in halogenated catechols. Opposite effects of increased v_{max} and increased K_m values were reported for electron-donating groups (e.g., methyl- and methoxy-catechols). On the same token, competitive microbial cometabolism could be described by intrinsic specificities (v_{max}/K_m) for growth and nongrowth substrates.

The specificities exhibited by *Methylosinus trichosporium* OB3b (Rostkowski et al., 2013) or *Pseudomonas putida* F1 (Juang and Tsai, 2006) were equally useful in describing degradation kinetics of various nongrowth substrates. Hence, the relative specificities of growth/nongrowth substrates by actively growing cultures (e.g., nitrifiers with AMO, aromatic oxidizers with TDO or methane oxidizers with MMO) could provide an insight into the inherent substrate competitive inhibition when both growth and nongrowth are present. In addition, since the reducing power required

for cometabolism is obtained from the utilization of growth substrate, the available amount of reducing power for cometabolism is expected to be dependent on the substrate inhibition between growth and nongrowth substrates.

2.2. Development of competitive cometabolic kinetic model

Table 1 presents the equilibria and rate-limiting reaction steps incorporated in the model. Potential interactions between growth and nongrowth substrates, including substrate inhibitions [Eqs. (3) and (4)] and cross-inhibitions [Eqs. (5) and (6)], are incorporated in the model. In Eqs. (7) and (8), P_1 is further oxidized to provide necessary metabolites for microbial growth and reducing power, whereas P_2 (formed by a reducing power-dependent cometabolic transformation of a nongrowth substrate) cannot be further metabolized for the benefit for cell growth, resulting in the accumulation of partially oxidized intermediates, as typically shown in pure culture studies. However, P_2 (e.g., TCE transformation product) may be further oxidized completely to CO_2 in a mixed culture system.

The oxidation rate of each class substrate depends on its respective rate constant and concentration of equilibrium-determined enzyme-substrate complexes as follows:

$$-\frac{dS_1}{dt} = k_1[ES_1] \tag{9}$$

$$-\frac{dS_2}{dt} = k_2[ES_2] \tag{10}$$

Based on the equilibrium analysis, the other equations in Table 1 can be incorporated into Eqs. (9) and (10) as follows:

$$-\frac{dS_1}{dt} = \frac{k_1 X[S_1]/K_1}{1 + [S_1]/K_1 + [S_2]/K_2 + [S_1]^2/K_1K_{11} + [S_2]^2/K_2K_{22} + [S_1][S_2]/K_1K_{12} + [S_1][S_2]/K_2K_{21}} \tag{11}$$

$$-\frac{dS_2}{dt} = \frac{k_2 X[S_2]/K_2}{1 + [S_1]/K_1 + [S_2]/K_2 + [S_1]^2/K_1K_{11} + [S_2]^2/K_2K_{22} + [S_1][S_2]/K_1K_{12} + [S_1][S_2]/K_2K_{21}} \tag{12}$$

To relate the consumption of a growth substrate to cell growth, cometabolism and cell maintenance, a specific growth substrate utilization rate (q_1) can be divided as follows (Criddle, 1993):

$$q_1 = q_g + n_c + \frac{b}{Y_m} \tag{13}$$

where q_g, n_c, and b/Y_m are the specific utilization rate of a growth substrate used for cell growth, degradation of a nongrowth substrate, and cell maintenance, respectively.

The term n_c is further defined in this paper as a ratio of the specific transformation rate of a nongrowth substrate (q_2) to a maximum nongrowth substrate transformation yield (W) as follows:

$$n_c = \frac{q_2}{W} \tag{14}$$

where W is the mass of nongrowth substrate transformed per unit mass of growth substrate utilized solely for cometabolism. Hence, the definition of W is analogous to the maximum growth substrate yield and the product yield coefficient of the Luedeking-Piret kinetics for product formation. The W value may depend on both the requirement of reducing power for cometabolism and the overall energy metabolism of microbial cultures.

By substituting the term for cometabolic requirement [Eq. (14)] and expressions for specific utilization rate of the growth substrate [$q_1 = -(dS_1/dt)/X$], specific substrate utilization rate for cell growth [$q_g = (dX/dt)/(X \cdot Y_m)$], and specific transformation rate of the nongrowth substrate [$q_2 = -(dS_2/dt)/X$] into Eq. (13), the following expression is obtained:

$$-\frac{dS_1}{dt} = \frac{1}{Y_m}\frac{dX}{dt} - \frac{1}{W}\frac{dS_2}{dt} + \frac{b}{Y_m}X \tag{15}$$

Eq. (15) can be rearranged to give the rate of cell growth in terms of the overall growth substrate utilization rate, nongrowth substrate transformation rate, and cell maintenance as:

$$\frac{dX}{dt} = -Y_m\left(\frac{dS_1}{dt} - \frac{1}{W}\frac{dS_2}{dt}\right) - bX \tag{16}$$

Three coupled equations [e.g., Eqs. (11), (12) and (16)] can now be used to describe competitive substrate transformation kinetics and cell growth, assuming that the competition-dependent utilization rate of a growth substrate is sufficient to provide all the cellular need of reducing power for growth, cometabolism, and maintenance. However, under some culture conditions at which S_2 becomes far greater than S_1, substrate concentration-dependent rate expression for dS_1/dt may not provide all the cellular needs; one good example is that of resting cell transformation systems where $S_1 = 0$ such that only a finite transformation of S_2 is obtained in the absence of a suitable electron donor. The expression for dS_1/dt as given in Eq. (11) will always be valid since the utilization of a growth substrate will not only benefit cell growth but also can provide some reducing power for cometabolism and maintenance. As for the case of cometabolism, transformation of a nongrowth substrate by cell cultures not only consumes reducing power but it also does not produce any reducing power to sustain its own transformation process. As a result, the expression for dS_2/dt (e.g., Eq. (12)) as derived without any consideration of reducing power limitation may not always be correct under a possible limitation of reducing power, e.g., $S_2 \gg S_1$.

To define precisely under what conditions a possible reducing power limitation may occur, an alternate growth substrate mass balance to that of Eq. (13) is invoked as follows:

$$\frac{dS_1}{dt} = \frac{1}{fW}\frac{dS_2}{dt} \tag{17}$$

Here, $(dS_2/dt)/W$ represents the amount of a growth substrate being consumed solely for cometabolism of a nongrowth substrate. If f is denoted as a fraction of a growth substrate being consumed for cometabolism at any time, a total growth substrate mass balance can be obtained by Eq. (17) as compared to Eq. (13). Under conditions such that $(dS_1/dt) \gg (dS_2/dt)$, i.e., no reducing power limitation on dS_2/dt, f can directly be calculated from Eq. (17) with the substitution of rate expressions given by Eqs. (11) and (12) as follows:

$$f_S = \frac{\frac{1}{W}\left(\frac{dS_2}{dt}\right)}{\frac{dS_1}{dt}} = \frac{1}{nW}\frac{S_2}{S_1} \tag{18}$$

Here, n is the specificity ratio of (k_1/K_1) to (k_2/K_2), and the subscript s indicates that the f value is under no reducing power limitation. Since both n and W are constant parameters, the f_s value is expected to vary depending on the concentrations of S_1 and S_2.

Table 1
Equilibrium relationships and rate-limiting steps used in the proposed model of competitive cometabolism in the presence of growth substrate.

Equilibrium relationships	Eq.	Rate-limiting reaction steps	Eq.
$E + S_1 \rightarrow ES_1$; K_1	(1)	$ES_1 \rightarrow E + P_1$	(7)
$E + S_2 \rightarrow ES_2$; K_2	(2)	$ES_2 \rightarrow E + P_2$	(8)
$ES_1 + S_1 \rightarrow ES_1S_1$; K_{11}	(3)		
$ES_2 + S_2 \rightarrow ES_2S_2$; K_{22}	(4)		
$ES_1 + S_2 \rightarrow ES_1S_2$; K_{12}	(5)		
$ES_2 + S_1 \rightarrow ES_2S_1$; K_{21}	(6)		

However, when $S_2 \gg S_1$, the f value can no longer be calculated from Eq. (18) due to previously mentioned invalidity of (dS_2/dt) expression (e.g., Eq. (12)), but the f value is assumed to asymptotically approach a constant value. Thus, when $S_2 \gg S_1$, the degradation rate of nongrowth substrate [as opposed to the f_s value that can calculated from Eq. (18)] becomes proportional to the utilization rate of growth substrate as follows:

$$\left(\frac{dS_2}{dt}\right)_l = f_l W \frac{dS_1}{dt} \tag{19}$$

where the subscript "l" indicates the limited reducing power (f_l) available for cometabolism. Hence, in order for cometabolism of a nongrowth substrate to occur without the limitation of reducing power, the competition-dependent utilization rate of the growth substrate should be greater than the competition-dependent as well as reducing power-dependent degradation rate of nongrowth substrate as follows:

$$\frac{dS_1}{dt} > \frac{1}{f_l W}\frac{dS_2}{dt} \tag{20}$$

By substituting Eqs. (11) and (12) into Eq. (20) and rearranging, the following condition (hereafter, referred as the conditional equation of reducing power for cometabolism) must be satisfied at each point in batch cell cultures:

$$S_1 > \frac{S_2}{nf_l W} \tag{21}$$

If satisfied, Eqs. (11), (12) and (16) are applicable to describe substrate degradation kinetics and cell growth without any reducing power limitation. Otherwise, Eqs. (11), (16) and (19) are required as summarized in Table 2.

2.3. Model validation with degradation data

Table 3 summarizes the values of model parameters used in the simulation of competitive cometabolic kinetics. Experimental data from our laboratory and other data reported in literature for degradation of various nongrowth substrates in the presence of a growth substrate were numerically simulated using the proposed kinetic model. The numerical values of the model parameters for our data have been numerically determined that yield the best fit of the data. As for literature data, similar values of kinetic parameters such as k_1, K_1, Y_m, and b as reported by respective investigators were used [e.g., in Fig. 2(b) simulation, values of k_1, K_1, Y_m and b were within one standard deviation reported in the literature (Charng et al., 1993)].

Table 2
Summary of proposed cometabolic model equations. [a]

Kinetic parameters	No reducing power limiting conditions	Reducing power limiting conditions
Utilization rate of a growth substrate	Eq. (11)	Eq. (11)
Degradation rate of a nongrowth substrate	Eq. (12)	Eq. (19)
Rate of cell growth	Eq. (16)	Eq. (16)

[a] The reducing power sufficient and limiting conditions are defined by the reducing power limitation equation [Eq. (21)]. These conditions apply to any n value system.

3. Results and discussion

3.1. Cometabolic transformation with the specificity ratio (n) > 1

The simulation results for TCE, xylene and Aroclor 1254 in the presence of methane, toluene and biphenyl, respectively, are shown in Fig. 2(a), 2(b) and 2(c), respectively. These three sets of experimental data represented cometabolic transformation systems in which the ratio (n) of specificity value of the nongrowth to the growth substrate is greater than 1 (as shown in Table 3). In those cases, cell culture systems always show an incomplete degradation pattern of the nongrowth substrate after the depletion of the growth substrate. Apparently, this particular degradation pattern is due to the fact that the nongrowth substrates (TCE, xylene and Aroclor 1254) cannot effectively compete with their respective growth substrates at the steps of oxygenase-catalyzed reactions that are required for the degradation of these substrates. Initially, the growth substrates in these cases ($n > 1$) was preferentially utilized; as a result, the initial degradation rate of each respective nongrowth substrate was insignificant. As the utilization rates of growth substrates increased due to cell growth, the degradation rates of nongrowth substrates also increased due to a lesser competition. However, as time progressed further, nongrowth substrates became preferred substrates for a binding by virtue of the mass action (i.e., S_2 remained relatively high). At the same time, the growth substrates were being nearly depleted, making the reducing power become a limiting factor toward the end of a batch growth period. At this stage, the actual degradation rate of nongrowth substrate could only be proportional (i.e., f_l value) to the competitively-inhibited utilization rate of nearly-depleted growth substrates. Furthermore, the conditional equation of reducing power for cometabolism precludes any further degradation of nongrowth substrates when the growth substrates are completely depleted, as implied in Eq. (19).

The hypothesis for the conditional equation of reducing power for cometabolism is clearly supported in Fig. 2(c), where no degradation of Aroclor 1254 is evident after the growth substrate (biphenyl) is completely depleted. Also, no significant degradation of TCE or xylene after the depletion of methane or toluene was evident in Fig. 2(a) and Fig. 2(b), respectively. So far, it has been generally accepted that a continued degradation of nongrowth substrates after the depletion of growth substrates occurs since some of reducing power can be supplied through an endogenous decay. Therefore, if the endogenous cell decay were responsible for reducing power necessary for cometabolism, a continued degradation of nongrowth substrates would have been clearly evident in Fig. 2. The cessation of nongrowth substrate degradation after the depletion of growth substrates shown in Fig. 2 raises a question as to the origin of reducing power in resting cell transformation systems where pre-cultured cells are used. Possibly, a remnant of reducing power from previous growth-associated activities on a growth substrate is responsible for the degradation of a nongrowth substrate in such systems. Incidentally, the previous finite transformation capacity model did not contain an endogenous cell decay term (Alvarez-Cohen and Mccarty, 1991), even though the authors imply that the reducing power is from endogenous cell decay. Additionally, the insignificant endogenous degradation of a nongrowth substrate as implied in the reducing power limitation equation can qualitatively be surmised by an intuition that there are many more enzymes requiring the reducing power for maintenance activities than those responsible for cometabolism.

Table 3
Summary of kinetic parameters used in model simulations.[a]

Kinetic parameters	Unit	Fig. No. 2(a)	2(b)	2(c)	3(a)	3(b) and 4
k_1	mg S_1/mg cell·hr	0.072	0.65	0.1	0.89	1.2
k_2	mg S_2/mg cell·hr	0.019	0.43	0.006	0.08	0.15
K_1	mg S_1/L	0.2	2	15	1	1.5
K_{11}	mg S_1/L	1	-[b]	-	10	250
K_{12}	mg S_1/L	-	-	-	-	233
K_2	mg S_2/L	0.25	4	10	0.065	0.15
K_{22}	mg S_2/L	1	-	-	10	60
K_{21}	mg S_2/L	-	-	-	-	194
Y_m	mg cells/ mg S_1	0.2	1.3	1.0	1.2	0.72
b	hr^{-1}	0.005	0.02	0.02	0.02	0.002
W	mg S_2/mg S_1	1	2	1	1	1
n	-	6	3	11	0.7	0.8
f_l	-	0.4	0.4	0.33	0.4	0.4

[a] In these simulations, dS_2/dt is equal to $f_l W(dS_1/dt)$ if the reducing power limitation equation is not satisfied at each simulation step, i.e., when $S_1 \leq S_2/(f_l \text{ n W})$.
[b] Denotes no substrate- or cross- inhibition terms.

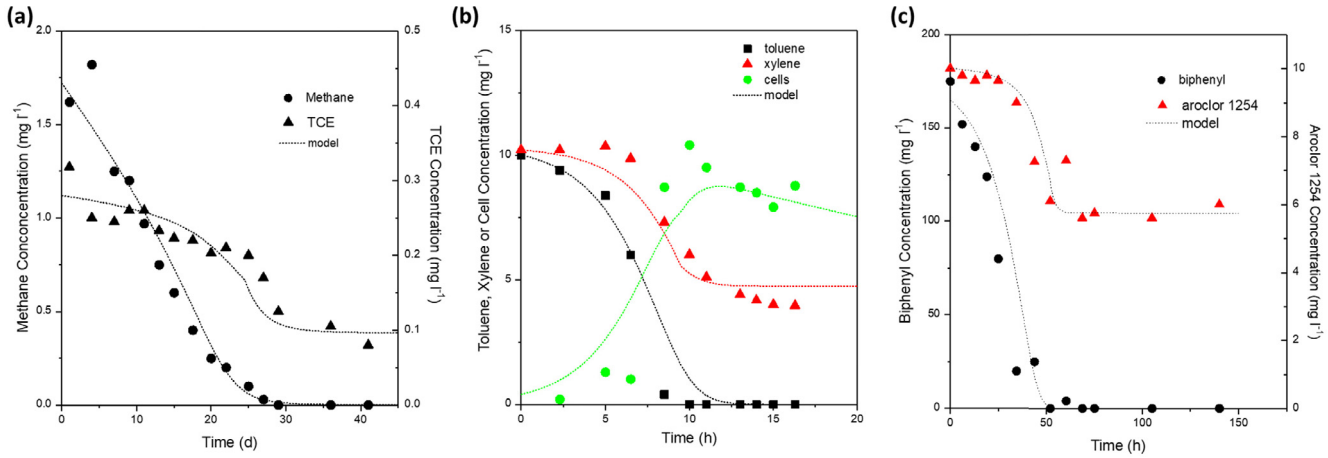

Fig. 2. Model simulation results: (a) TCE removal in the presence of methane, where the input data was gathered from the literature (Broholm et al., 1992). (b) xylene removal in the presence of toluene, where the input data was gathered from the literature (Charng et al., 1993). (c) Aroclor 1254 removal in the presence of biphenyl, where the input data was gathered from the literature (Kohler et al., 1988).

3.2. Cometabolic transformation with the specificity ratio (n) < 1

Fig. 3(a) and (b) illustrate the simulation results of the cases with $n < 1$ for 3-chlorophenol and 4-chlorophenol, respectively, in the presence of phenol. Here, the nongrowth substrates (3-chlorophenol and 4-chlorophenol) are preferred by the virtue of their higher specificity values at the rate-limiting step where aromatic ring is cleaved by a catechol dioxygenase. The experimental runs with a high initial concentration of 3-chlorophenol or 4-chlorophenol required a longer time to degrade both growth and nongrowth substrates but still resulted in the synchronous degradation of both substrates. Therefore, the initial ratios of phenol to chlorophenols were sufficient to provide all the necessary reducing power for substrate inhibition-dependent utilization/degradation rates of phenol and chlorophenols throughout the batch period, i.e., Eq. (21), was satisfied throughout the batch culture period to the complete degradation of chlorophenols.

A possible pattern of the incomplete degradation of nongrowth substrate, e.g., 4-chlorophenol, even with $n < 1$ is demonstrated in Fig. 4. For $n < 1$, based on the conditional equation of reducing power for cometabolism, the incomplete transformation of nongrowth substrate may occur if S_1 (growth substrate) is not enough to provide all the necessary reducing power for cell growth, maintenance and cometabolism. However, the model prediction using the same set of model parameters used for Fig. 3(b) was not good as in the cases of high phenol/4-chlorophenol concentration ratios. In cell culture systems with $n > 1$, the conditional equation of reducing power for cometabolism is only violated toward the end of a batch period where the growth substrate is nearly depleted. However, the conditional equation of reducing power for cometabolism in cell culture systems with $n < 1$ can be violated immediately from the start of a batch growth if the initial concentration ratio of S_1 to S_2 is low as in Fig. 4. This might have caused an innate cellular adaptation to relieve the immediate burden of a severe reducing power limitation. In fact, the actual degradation rate of 4-chlorophenol was greater than the model-predicted value, and, at the same time, an unusual decrease in the specific growth rate was evident (arrow in Fig. 4). The sudden decrease in the specific growth rate was attributed to microbial stringent responses under carbon and energy limitations. This might have shunted more of the reducing power into 4-chlorophenol degradation at the expense of less cell growth than dictated by f_l in the proposed model. Further research is needed to fully characterize systems $n < 1$ under low S_1/S_2 conditions.

3.3. Engineering significance

Since many experimental findings indicate that a variety of contaminants can be degraded by either pre-cultured resting or growing microbial cultures, the merit of each option needs further

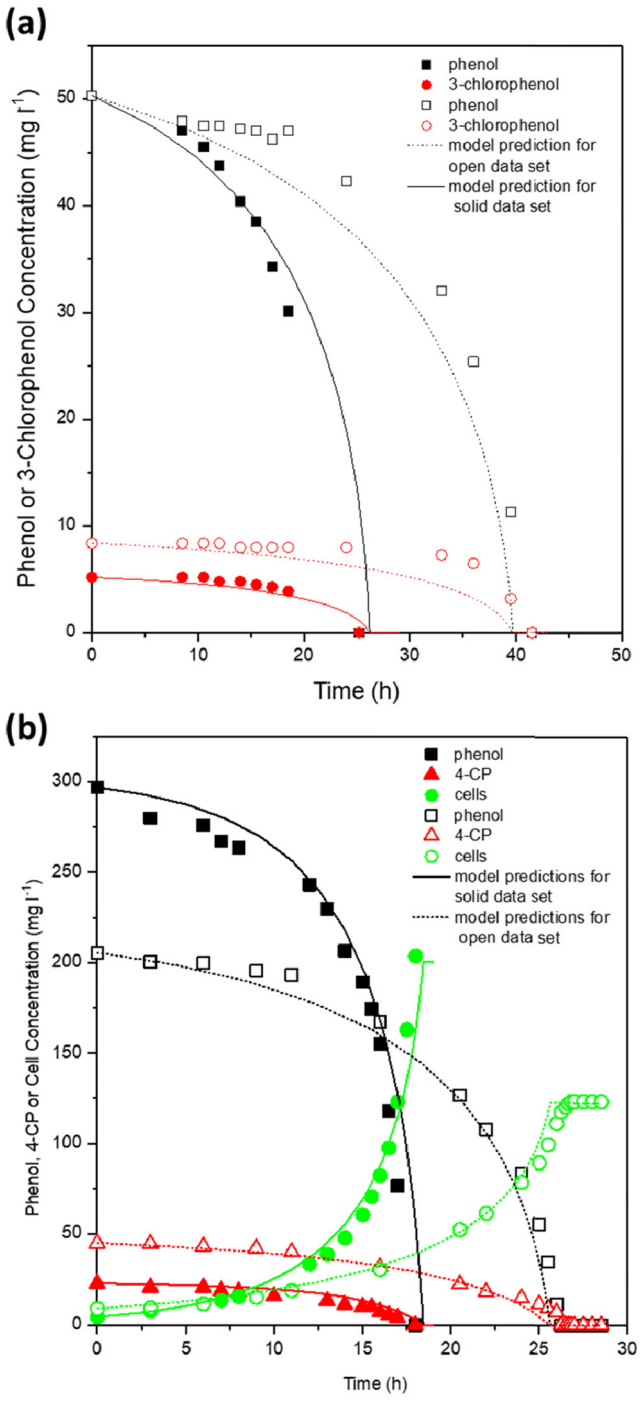

Fig. 3. Model simulation results with high concentration ratios of (a) phenol/3-chlorophenol, where the input data was gathered from the literature (Menke and Rehm, 1992) and (b) phenol/4- chlorophenol, where the input data was gathered from the literature (Kim, 1995).

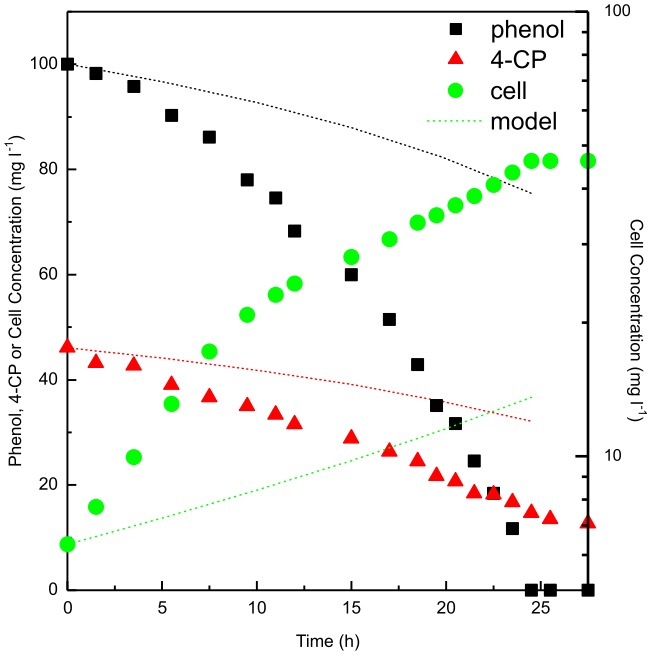

Fig. 4. Model simulation results with a low concentration ratio of phenol/4-CP. The input data was gathered from the literature (Kim, 1995).

evaluation for an effective application of microbial cometabolism. In cometabolic transformation by pre-cultured resting cells, the main disadvantages appear to be limited reducing power and inability to cope with possible effects of product toxicity. Cometabolic transformation in the presence of growth substrate may provide a continuous reducing power and possible recovery from the effect of product toxicity, but the substrate inhibition between growth and nongrowth substrates seems to be the main obstacle, especially for systems with $n \geq 1$.

For cometabolic transformation cases where $n > 1$, a two-stage reactor system, in which microbial cells are cultured in the first reactor with a suitable growth substrate in the absence of nongrowth substrate and the second reactor is used for the cometabolic transformation to bypass the effect of substrate inhibition, could be used. At the expense of the first reactor (e.g., higher growth substrate requirement and larger reactor size), a sufficient transformation capacity of resting cells could be obtained to degrade the nongrowth substrate in the second reactor. However, a process optimization of such systems should ultimately require an increased specific value of the biomass transformation capacity of resting cells in the first reactor (Alvarez-Cohen & Speitel, 2001). The biomass transformation capacity of resting cells reflects the amount of available reducing power, which clearly depends on the growth history of a particular culture. For example, the PHB accumulation as well as the type of MMO in methanotrophs depends on conditions under which cells are cultured (i.e., Cu limitation for type I MMO that exhibits higher transformation rate of the nongrowth substrate). Furthermore, due to invariant strategies of microbial cells to optimize the energy-requiring growth process, the amount of reducing power may be severely limited by the time that cells reach the resting stage (or the stationary phase in batch cultures). Although the PHB storage and its subsequent utilization as a source of NADH in the TCE oxidation may be envisaged as an alternative way to increase the transformation capacity of pre-cultured cells, factors determining the available amount of reducing power at the end of the growth period are not well understood.

The use of cometabolic capability of microbial cells in the presence of growth substrate, however, exploits optimal characteristics of microbial growth processes. The theory that a substrate which exhibits a higher specific growth rate will be utilized preferentially in the presence of multiple substrates is essential in predicting diauxic growth of cells. Since the competitive cometabolism is not based on internal regulatory processes, such as induction/derepression and/or allosteric feedback inhibition, as used in the cybernetic model formulation, the optimal allocation of energy content of a growth substrate during microbial growth is implicit in terms of Y_m and b, and the parameters n, W and f_l capture essential

kinetic features of microbial cultures that exhibit broad specificities.

The determination of n value for a given microbial system, therefore, should be the best available strategy for selecting microbial culture able to cometabolically degrade a particular nongrowth substrate. If $n \leq 1$, an adequate amount of a growth substrate could be provided to ensure a complete degradation of nongrowth substrates. On the other hand, if $n > 1$, the selection of an alternative microbial system may be required, since the cometabolic transformation neither by pre-cultured cells nor in the presence of growth substrate would result in the complete degradation of nongrowth substrates. Nevertheless, even for the case where $n > 1$, the use of cometabolic capabilities of microbial cells in the presence of a growth substrate is expected to be more expedient, since it can provide possible recovery from the effect of product toxicity and, more importantly, the utilization of the available reducing power for transformation of a nongrowth substrate by cell cultures can be better predicted and explained.

4. Conclusions

A kinetic model of competitive cometabolism for predicting different degradation patterns of nongrowth substrate in the presence of growth substrate is presented. The critical evaluation of the model framework indicates that cometabolic transformation depends on two factors: (1) specificity constant ratio of growth to nongrowth substrates and (2) ratio of the initial growth to nongrowth substrate concentration. The specificity value of a particular microbial culture characterizes the inherent competition involved in cometabolic transformation in the presence of growth substrate, and the initial concentrations of substrates determine whether a sufficient reducing power could be diverted to degradation of the nongrowth substrate. The newly introduced model parameters in this study, particularly n, facilitate the evaluation of cometabolic degradation for a variety of environmentally toxic pollutants with a minimum experimental effort. The growth substrate transformation capacity W and f_i, as defined in this paper, are constant parameters for a given microbial system that, in conjunction with the specificity ratio (n) and substrate concentrations, further explain the reducing power condition and quantify different fractions of reductant supply that can be channeled into cometabolic transformation. The effect of possible product toxicity was not explicitly accounted for in the model. However, the indication that growing cells can recover from the effect of product toxicities in the presence of a growth substrate may be interpreted such that an increased ratio of initial growth to nongrowth substrate concentration could counter the effect of possible product toxicities. Finally, although the model predicts experimental data from literature and from this study, the general utility of the proposed model in other microbial systems, especially in growth substrate-acclimated activated sludge systems, awaits further verification to enhance the current understanding on the kinetics of competitive cometabolism.

CRediT authorship contribution statement

Michael H. Kim: Conceptualization, Methodology, Writing - original draft. **Chihhao Fan:** Data curation, Writing - original draft. **Shu-Yuan Pan:** Visualization. **Ingyu Lee:** Data curation. **YuPo Lin:** Data curation. **Hyunook Kim:** Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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