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Growth kinetics of *Pseudomonas putida* in the biodegradation of single and mixed phenol and sodium salicylate

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Abstract

The kinetics of biodegradation of single phenol and sodium salicylate (SA) and their binary mixtures in water by suspended *Pseudomonas putida* CCRC 14365 was studied at 30 °C and pH 7.0. Experiments were performed at different total substrate concentrations (0–4.25 mM) and/or mole fractions of phenol. The initial cell concentration was fixed at 0.025 g/L. Based on the parameters of the Haldane model for specific growth rate of the cells on single phenol and SA (correlation coefficient $R^2 > 0.9737$), phenol had larger degradation rate than SA, whereas the inhibition of *P. putida* by phenol was less significant than by SA. That is, the cells were more favored to degrade phenol than SA under comparable conditions. On the other hand, the specific growth rate of the cells on binary substrates could be described by an extended Haldane equation ($R^2 = 0.9256$). The substrate interactions were thus discussed according to the modeled parameters. The dynamics in the biodegradation of single and binary substrate systems was finally analyzed.

Keywords: Growth kinetics; *Pseudomonas putida*; Biodegradation; Phenol; Sodium salicylate; Binary substrates

1. Introduction

Phenol and its derivatives are the most common representatives of toxic organic pollutants, which have resulted in cumulative hazardous effects on the environments. They are encountered in the waste effluents of several processes and operations such as coal gasification, polymeric resin production, petroleum refining, coking plants, pharmaceuticals, explosive manufacture, plastic and varnish industries, and textile industries [1,2]. In addition to being potential carcinogens, phenols are toxic (reduces enzyme activity) or lethal to fish at relatively low levels of 5–25 mg/L [3]. As the phenol-bearing water is chlorinated, toxic polychlorinated phenols can be formed. They also impart objectionable tastes and odors to drinking water even at a very low level of 2.0 μg/L. Thus, these effluents require proper treatment before being discharged to the water bodies. The treatment alternatives such as activated carbon adsorption, ion exchange, solvent extraction, and chemical oxidation often suffer from serious drawbacks including high cost and the formation of hazardous by-products (secondary pollution) [4]. Of these methods, biodegradation is environmental friendly and cost effective [5]. A large number of studies on the degradation of phenols by *Pseudomonas putida* (*P. puitda*) have been made because of its high removal efficiency [6–9].

Organic chemical mixtures are prevalent in contaminated groundwater as well as in the waste effluents from industrial and municipal sources. The occurrence of contaminants in the mixture is an important problem, because the biodegradation of one component could be inhibited by other compounds in the mixture and because different conditions may be required to treat different compounds within the mixture. Researchers have noted that microbial degradation of a compound can be strongly impacted by other components in a mixture [10–12]. The terms homologous and heterologous have been used for compounds that serve the same and different roles, respectively [12]. The effect of other compounds in a mixture of homologous carbon and energy substrates on the degradation of a chemical can be positive, as in the case of increased growth at low substrate concentrations [13,14] or induction of required degradative enzymes [15]. More commonly, negative interactions are reported. Reasons for decreased degradation rate include competitive inhibition [16–18], toxicity [19], and the formation of toxic intermediates by non-specific

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Nomenclature

K	kinetic constant (mM)
K_I	substrate-inhibition constant (mM)
K_S	substrate-affinity constant (mM)
$K_{2,i}$	kinetic constant expressing competitive inhibition of substrate i degradation from another substrate
$K_{3,i}$	kinetic constant expressing uncompetitive inhibition of substrate i degradation from another substrate (mM^{-1})
R^2	correlation coefficient
SA	sodium salicylate
S_A, S_P	concentrations of sodium salicylate and phenol, respectively (mM)
S_T	total substrate concentration (mM)
t	time (h)
x_A, x_P	mole fractions of sodium salicylate and phenol, respectively
X	cell concentration (g/L)
Y_A, Y_P	cell yields on single sodium salicylate and phenol, respectively (g cell/mmol substrate)

Greek letter

μ	specific growth rate of cell (h^{-1})

enzymes [20]. To understand mixture effects, one must consider the metabolic role of each compound playing for the microorganisms, likely *via* the kinetic analysis of cell growth kinetics.

Moreover, successful design of large-scale bioreactors for pollutant degradation requires the ability to accurately predict the cell growth under both steady and dynamic conditions. At the same time, the mathematical models should not be too complicated in order to reduce experimental work required to determine kinetic parameters. Although the mathematical models of mixed homologous consumption and microbial growth have been proposed [10,16,21,22], this body of literature is much smaller than that for the modeling of growth kinetics on single substrate [14]. In addition, most models have been tested within only limited substrate concentrations.

The aim of this work was to examine the growth kinetics of *P. putida* during the degradation of single and binary phenol and sodium salicylate (SA) in a batch reactor as well as to determine the substrate interactions. It has been reported that SA and sodium glutamate not only support the growth of *P. putida* but also is responsible for inducing some specific enzymes for the degradation of non-growth substrates such as 4-chlorophneol and carbazole [23–25]. This class of biological transformation is called co-metabolism, referring to the transformation of a non-growth substrate by cells growing on a growth substrate or by resting cells [26]. Few studies have been made to explore the mixture effect when one sodium salt of such carboxylic acids and one growth substrate such as phenol are present [23–25]. The initial cell concentration was fixed at 0.025 g/L, and the temperature and pH were fixed at 30 °C and 7.0, respectively. Experiments were performed in the substrate level range of 0.53–3.18 mM. In binary substrate solutions, one substrate level was fixed at 1.06 mM and the other substrate level was changed from 0.27 to 4.25 mM.

2. Model description

2.1. Inhibition in single substrate systems

The specific growth rate of cell in a batch system, μ (h^{-1}), is defined as [27]

$$\mu = \frac{1}{X}\frac{dX}{dt} = \frac{d\ln X}{dt} \quad (1)$$

where X is the cell concentration in either g/L (dry basis) or in absorbance unit at 600 nm (OD). The value of μ is determined at the exponential phase of the growth curve.

Expression of μ in Eq. (1) represents the specific growth rate of cell on single substrate, which is a function of the concentration of the resource. The Haldane model (Eq. (2)) was tried here due to its wide applicability for representing the growth kinetics of inhibitory substrates [27]:

$$\mu = \frac{\mu_{max} S}{K_S + S + (S^2/K_I)} \quad (2)$$

where S is the substrate concentration (mM) and μ_{max} is the maximum growth rate (h^{-1}). Here, K_S is the substrate-affinity constant (mM) and K_I is the substrate-inhibition constant (mM). A larger K_I value indicates that the culture is less sensitive to substrate inhibition [28].

Four alternatives shown in Table 1 were also tested in this work [29–32]. These results enable us to confirm whether the obtained kinetic parameters (μ_{max}, K_S, and K_I) are reasonable or not.

2.2. Inhibition in binary substrate systems

Of the aforementioned models on single substrate, the Haldane model is selected for further analysis due to its acceptable fitness and mathematical simplicity, as we will see in Section 4.2. Although there may be many alternate expressions [14,24,25,33,34], one possible expression for the specific growth

Table 1
Other growth kinetic models of biomass with substrate inhibition

Model	Equation		Reference
Yano et al.	$\mu = \frac{\mu_{max} S}{S + K_S + (S^2/K_I)[1 + (S/K)]}$	(3)	[29]
Aiba et al.	$\mu = \frac{\mu_{max} S}{(S + K_S)} \exp\left(\frac{-S}{K_I}\right)$	(4)	[30]
Edwards	$\mu = \mu_{max}\left[\exp\left(\frac{-S}{K_I}\right) - \exp\left(\frac{-S}{K_S}\right)\right]$	(5)	[31]
Webb	$\mu = \frac{\mu_{max} S[1 + (S/K)]}{S + K_S + (S^2/K_I)}$	(6)	[32]