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Kinetic modeling of growth and biodegradation of phenol and *m*-cresol using *Alcaligenes faecalis*

Jing Bai [a,b,*], Jian-Ping Wen [a], Hong-Mei Li [a,d], Yan Jiang [a,c]

[a] *Department of Biochemical Engineering, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, PR China*
[b] *Institute of Modern Physics, Chinese Academy of Science, Lanzhou 730000, PR China*
[c] *School of Life Sciences and chemistry, Harbin college, Harbin 150016, PR China*
[d] *Yancheng Textile Vocational and Technical College, Yancheng 224005, PR China*

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Abstract

A phenol-degrading microorganism, *Alcaligenes faecalis*, was used to study the substrate interactions during cell growth on phenol and *m*-cresol dual substrates. Both phenol and *m*-cresol could be utilized by the bacteria as the sole carbon and energy sources. When cells grew on the mixture of phenol and *m*-cresol, strong substrate interactions were observed. *m*-Cresol inhibited the degradation of phenol, on the other hand, phenol also inhibited the utilization of *m*-cresol, the overall cell growth rate was the co-action of phenol and *m*-cresol. In addition, the cell growth and substrate degradation kinetics of phenol, *m*-cresol as single and mixed substrates for *A. faecalis* in batch cultures were also investigated over a wide range of initial phenol concentrations (10–1400 mg L^{-1}) and initial *m*-cresol concentrations (5–200 mg L^{-1}). The single-substrate kinetics was described well using the Haldane-type kinetic models, with model constants of $\mu_{m1} = 0.15\ h^{-1}$, $K_{S1} = 2.22$ mg L^{-1} and $K_{i1} = 245.37$ mg L^{-1} for cell growth on phenol and $\mu_{m2} = 0.0782\ h^{-1}$, $K_{S2} = 1.30$ mg L^{-1} and $K_{i2} = 71.77$ mgL^{-1}, $K'_{i2} = 5480\ (\text{mg}\,L^{-1})^2$ for cell growth on *m*-cresol. Proposed cell growth kinetic model was used to characterize the substrates interactions in the dual substrates system, the obtained parameters representing interactions between phenol and *m*-cresol were, $K = 1.8 \times 10^{-6}$, $M = 5.5 \times 10^{-5}$, $Q = 6.7 \times 10^{-4}$. The results received in the experiments demonstrated that these models adequately described the dynamic behaviors of phenol and *m*-cresol as single and mixed substrates by the strain of *A. faecalis*.

Keywords: *Alcaligenes faecalis*; Biodegradation; Phenol; *m*-Cresol; Inhibition; Kinetics

1. Introduction

Phenol, a compound regarded as a priority contaminant by the Chinese Environmental Protection Agency, is a characteristic pollutant in wastewaters and effluents from crude oil and coal conversion processes and has been detected recently in river water and in effluents from wastewater treatment plants [1–3]. Its methylated derivative *o*-, *m*- and *p*-cresol has been detected not only in leachate from creosote sites, and as such, has given rise to groundwater pollution, but has also been found in a huge range of industrial effluents. Due to the toxic properties of both phenol and cresol, the efficient removal of these compounds by microorganisms is of great importance.

To treat phenolic compounds, biological methods are preferable because this is economical, and there is a low possibility of the production of byproducts. The biodegradation of phenol have been widely examined in the past three decades and different microorganisms were used by different researchers in this kind of study [4–7]. Several other studies have shown that cresols can be degraded by a wide variety of microorganisms [8–10]. The microorganisms used are usually aerobes because the aerobes are efficient at degrading toxic compounds and usually transform the organic compounds into inorganic compounds (CO_2, H_2O). As phenol and cresol are most important components in phenolic wastewater; recently, the inhibitions of cresol on aerobic biodegradation of phenol have been investigated. Kar et al. [11] observed that phenol and *p*-cresol mutually inhibited each other; the inhibition of *p*-cresol to phenol degradation was stronger than reverse, but *o*-cresol enhanced phenol degradation marginally. Paraskevi et al. [12] demonstrated that the addition of *o*-cresol strongly inhibited phenol

* Corresponding author at: Department of Biochemical Engineering, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, PR China. Tel.: +86 22 27890492; fax: +86 22 27890492.
E-mail address: baijing_tju@yahoo.com.cn (J. Bai).

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transformation in respect to the strong competitive inhibition between the substrates. Perron and Welander [13] paid attention on the interaction of phenol and cresol when they were degraded at low temperatures. However, their researches focused on the substrate interactions during phenol biodegradation in the cresol–phenol mixtures only qualitatively and hitherto, no other reports were found on the substrate interaction kinetics during phenol degradation.

Knowledge of the kinetics of biodegradation is important for the evaluation of the persistence of organic pollutant and the design of biodegradation facilities [14]. Therefore, further detailed research is needed to quantify these substrate interactions in the degradation of phenol and *m*-cresol mixtures. The aim of our present work is to investigate and quantify the kinetics of cell growth and biodegradation of phenol and *m*-cresol as the single and mixed substrates using *Alcaligenes faecalis*.

2. Kinetics models

In our work, as the flasks were covered with six layer gauzes, it was presumed that the aeration provided by shaking the flasks was sufficient to keep the oxygen concentration constant and not limited, the influence of oxygen was not considered. Thus, the *A. faecalis* growth rate and substrate degradation rate were only limited by substrate concentration at fixed initial pH, temperature and shaking rate.

To develop the cell growth kinetics model in binary substrates system, our strategy is first to specify the cell growth model for cells acting on phenol and *m*-cresol alone, and then to quantify the substrates interactions.

Because of the inhibition of high phenol or *m*-cresol concentration on the cell growth, the Haldane type kinetic models [15] were selected for assessing the dynamic behavior of *A. faecalis* growth on phenol or *m*-cresol alone.

2.1. Cell growth on phenol and m-cresol

On the basis of the experimental results (that both substrates exerted substrate-inhibition on the cells and cross-inhibition occurred between phenol and *m*-cresol) and considering that the inhibitory effects of *m*-cresol on the cell growth behaviors are larger than those of phenol, the following sequences of reactions based on enzymatic reactions are proposed:

$$X + S_1 \underset{k_{-1}}{\overset{k_{+1}}{\Leftrightarrow}} X' \xrightarrow{k_{+2}} 2X \tag{1}$$

$$X' + S_1 \underset{k_{-3}}{\overset{k_{+3}}{\Leftrightarrow}} X'S_1 \tag{2}$$

$$X' + S_2 \underset{k_{-4}}{\overset{k_{+4}}{\Leftrightarrow}} X'S_2 \tag{3}$$

$$X'S_1 + S_2 \underset{k_{-5}}{\overset{k_{+5}}{\Leftrightarrow}} X'S_1S_2 \tag{4}$$

$$X'S_2 + S_2 \underset{k_{-6}}{\overset{k_{+6}}{\Leftrightarrow}} X'S_2^2 \tag{5}$$

$$X + S_2 \underset{k'_{-1}}{\overset{k'_{+1}}{\Leftrightarrow}} X'' \xrightarrow{k'_{+2}} 2X \tag{6}$$

$$X'' + S_2 \underset{k'_{-3}}{\overset{k'_{+3}}{\Leftrightarrow}} X''S_2 \tag{7}$$

$$X'' + S_1 \underset{k'_{-4}}{\overset{k'_{+4}}{\Leftrightarrow}} X''S_1 \tag{8}$$

$$X''S_2 + S_2 \underset{k'_{-5}}{\overset{k'_{+5}}{\Leftrightarrow}} X''S_2^2 \tag{9}$$

$$X''S_2 + S_1 \underset{k'_{-6}}{\overset{k'_{+6}}{\Leftrightarrow}} X''S_2S_1 \tag{10}$$

$$X''S_1 + S_2 \underset{k'_{-7}}{\overset{k'_{+7}}{\Leftrightarrow}} X''S_1S_2 \tag{11}$$

$$X''S_1 + S_1 \underset{k'_{-8}}{\overset{k'_{+8}}{\Leftrightarrow}} X''S_1^2 \tag{12}$$

Eqs. (1), (2), (6), (7) and (9) represent substrate-inhibition of phenol and *m*-cresol, respectively. The cross-inhibition between phenol and *m*-cresol is represented by Eqs. (3)–(5), (8) and (10)–(12). By considering the above mechanism and assuming pseudosteady state for formation of the cellular intermediates, the cell growth equation, for the mixed two growth substrates (phenol and *m*-cresol), can be obtained. Cellular intermediates in this mechanism are X', $X'S_1$, $X'S_2$, $X'S_1S_2$, $X'S_2^2$, X'', $X''S_2$, $X''S_1$, $X''S_2^2$ and $X''S_1^2$. From Eqs. (1) to (5),

$$\begin{aligned}\frac{dX'}{dt} &= k_{+1}XS_1 - k_{-1}X' - k_{+2}X' + k_{+3}X'S_1 - k_{-3}[X'S_1] \\ &\quad + k_{+4}X'S_2 - k_{-4}[X'S_2] + k_{+5}[X'S_1]S_2 - k_{-5}[X'S_1S_2] \\ &\quad + k_{+6}[X'S_2]S_2 - k_{-6}[X'S_2^2] = 0\end{aligned} \tag{13a}$$

Eqs. (2)–(5) will provide

$$\frac{d[X'S_1]}{dt} = k_{+3}X'S_1 - k_{-3}[X'S_1] = 0 \tag{13b}$$

$$\frac{d[X'S_2]}{dt} = k_{+4}X'S_2 - k_{-4}[X'S_2] = 0 \tag{13c}$$

$$\frac{d[X'S_1S_2]}{dt} = k_{+5}[X'S_1]S_2 - k_{-5}[X'S_1S_2] = 0 \tag{13d}$$

$$\frac{d[X'S_2^2]}{dt} = k_{+6}[X'S_2]S_2 - k_{-6}[X'S_2^2] = 0 \tag{13e}$$

By solving Eqs. (13a)–(13e) and (14a)–(14e) can be obtained:

$$X' = \frac{k_{+1}}{k_{-1} + k_{+2}}XS_1 \tag{14a}$$

$$[X'S_1] = \frac{k_{+3}}{k_{-3}}\frac{k_{+1}}{k_{-1} + k_{+2}}XS_1^2 \tag{14b}$$