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Phenol biodegradation by the yeast *Candida tropicalis* in the presence of *m*-cresol

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Abstract

Biodegradation of phenol and *m*-cresol using a pure culture of *Candida tropicalis* was studied. The results showed that *C. tropicalis* could degrade 2000 mg l^{-1} phenol alone and 280 mg l^{-1} *m*-cresol alone within 66 and 52 h, respectively. The capacity of the strain to degrade phenol was obviously higher than that to degrade *m*-cresol. The presence of *m*-cresol intensely inhibited phenol biodegradation. Only 1000 mg l^{-1} phenol can be completely degraded in the presence of 280 mg l^{-1} *m*-cresol. On the contrary, the phenol of low concentration from 100 to 500 mg l^{-1} supplied a sole carbon and energy source for *C. tropicalis* in the initial phase of biodegradation and accelerated the assimilation of *m*-cresol, resulting in the fact that *m*-cresol biodegradation velocity was higher than that without phenol. Besides, the capacity of *C. tropicalis* for *m*-cresol biodegradation was increased up to 320 mg l^{-1} with the presence of 60–100 mg l^{-1} phenol. In addition, the intrinsic kinetics of cell growth and substrate degradation were investigated with phenol and *m*-cresol as single and mixed substrates in batch cultures. The results illustrated that the models proposed adequately described the dynamic behaviors of biodegradation by *C. tropicalis*.

Keywords: Biodegradation; *Candida tropicalis*; *m*-Cresol; Kinetics; Phenol

1. Introduction

Phenol, a compound regarded as a priority contaminant by the US Environmental protection agency [1], is a characteristic pollutant in wastewaters and effluents from crude oil, ceramic plants, steel plants, coal conversion processes and phenolic resin industries and has been detected recently in river water, and in effluents from wastewater treatment plants [2–6]. Its methylated derivative cresol has been detected not only in leachate from creosote sites, and as such, giving rise to groundwater pollution [7], but also has been found in a huge range of industrial effluents [8]. Once wastewater containing phenolic compounds is discharged into the receiving body of water, it endangers fish life, even at a relatively low concentration, e.g. 5–25 mg l^{-1} [9–11]. For drinking water, a guideline concentration of 1 μg l^{-1} has been prescribed [12]. Due to the toxic properties of both phenol and cresol [13,14], the efficient removal of these compounds from industrial aqueous effluents is of great practical significance for environmental protection.

Traditionally, phenol and its derivatives have been removed from industrial effluents by physico-chemical methods, but recently developed biodegradation techniques have the potential to mineralize these toxic compounds completely at relatively low processing costs [15,16] and rare possibility of secondary pollution [17]. In spite of phenolic toxic properties, a lot of bacteria have been demonstrated to mineralize 100–1200 mg l^{-1} phenol or/and *m*-cresol under aerobic conditions as the sources of carbon and energy [18–20]. Recently, its noteworthy that Jiang and Wen isolated a strain of yeast *Candida tropicalis* able to degrade phenol up to 2000 mg l^{-1} from acclimated activated sludge [21]. However, none has been known about the biodegradation of phenol and *m*-cresol dual-substrate system by *C. tropicalis*.

Objectives of the present study are to investigate biodegradation of phenol and *m*-cresol as single substrate by *C. tropicalis*, to investigate the interaction of phenol and *m*-cresol in dual-substrate system, and to research the cell growth and substrate degradation intrinsic kinetics of *C. tropiccalis* in single and dual substrate biodegradation system, respectively.

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Nomenclature

A	growth associated constant for substrate consumption
B	non-growth associated constant for substrate consumption (h^{-1})
f	substrate interaction coefficient
k_j	($j=+1, -1, +2, +3, -3, +4, -4, +5, -5, +6, -6$) reaction rate constants shown in equations (7)–(11) (units: k_{+1}, k_{+3}, k_{+4}, k_{+5}, k_{+6}, $((mg\,l^{-1})^{-1}\,h^{-1})$; k_{-1}, k_{+2}, k_{-3}, k_{-4}, k_{-5}, k_{-6} (h^{-1}))
k'_j	($j=+1, -1, +2, +3, -3, +4, -4, +5, -5, +6, -6, +7, -7, +8, -8$) reaction rate constants shown in equations (12)–(18) (units: $k'_{+1}, k'_{+3}, k'_{+4}, k'_{+5}, k'_{+6}, k'_{+7}, k'_{+8}$, $((mg\,l^{-1})^{-1}\,h^{-1})$; $k'_{-1}, k'_{+2}, k'_{-3}, k'_{-4}, k'_{-5}, k'_{-5}, k'_{-6}, k'_{-7}, k'_{-8}$ (h^{-1}))
K, M, Q	substrate interaction coefficient $((mg\,l^{-1})^{-1})$
K_{i1}	self-inhibition constant of phenol ($mg\,l^{-1}$)
K_{i2}	self-inhibition constant of *m*-cresol ($mg\,l^{-1}$)
K_{S1}	saturation constant for cell growth on phenol ($mg\,l^{-1}$)
K_{S2}	saturation constant for cell growth on *m*-cresol ($mg\,l^{-1}$)
K'_{i2}	self-inhibition constant of *m*-cresol ($(mg\,l^{-1})^2$)
S	initial substrate concentration ($mg\,l^{-1}$)
t	time (h)
X	biomass concentration ($mg\,l^{-1}$)
$Y_{P/S}$	product yield coefficient on substrate
$Y_{X/S}$	cell yield coefficient on substrate

Greek symbols

α	growth associated constant for product formation
β	non-growth associated constant for product formation (h^{-1})
γ_s	substrate degradation rate ($mg\,l^{-1}\,h^{-1}$)
μ_S	specific substrate degradation rate (h^{-1})
μ_{S1}	specific degradation rate of phenol in dual substrates (h^{-1})
μ_{S2}	specific degradation rate of *m*-cresol in dual substrates (h^{-1})
μ_X	overall specific growth rate in dual substrates (h^{-1})
μ_{X1}	specific growth rate on phenol in dual substrates (h^{-1})
μ_{X2}	specific growth rate on m-cresol in dual substrates (h^{-1})

Subscripts

1	growth substrate, phenol
2	growth substrate, *m*-cresol

Superscript

0	single growth substrate

2. Materials and methods

2.1. Microorganism and cultivation conditions

Wild-type *C. tropicalics* was isolated in the lab from acclimated activated sludge collected from a municipal gasworks in China, and was identified based on physiological and biochemical tests and Biolog Automated Microbial Identification System by the Chinese Academy of Agricultural Sciences.

C. tropicalis was grown and maintained in YEPD medium [22]. A mineral salt medium supplemented with phenol and *m*-cresol was used for biodegradation studies, with the ingredients ($g\,l^{-1}$) of $(NH_4)_2SO_4$ 0.4, K_2HPO_4 0.4, KH_2PO_4 0.2, NaCl 0.1, $MgSO_4$ 0.1, $MnSO_4{\cdot}H_2O$ 0.01, $Fe_2(SO_4)_3{\cdot}H_2O$ 0.01, $Na_2MoO_4{\cdot}2H_2O$ 0.01, varying phenol and/or *m*-cresol. All cultivation was conducted at 30 °C, and shaking flasks were incubated in a rotary shaker with a speed of 200 rpm.

2.2. Phenol and m-cresol biodegradation

The experiments began with inoculating 10 ml YEPD medium with *C. tropicalis*. After 18 h of incubation, 2 ml of this cell culture was added to 500 ml shaking flasks with 100 ml fresh YEPD medium. Cells ($OD_{600}=1.3$) were harvested as inoculum. High inoculum concentration lessened contaminants toxic property and increased phenol and *m*-cresol biodegradation velocity [21]. In all the experiments, 5% subculture was inoculated into 100 ml mineral salt medium with varying initial phenol and/or *m*-cresol concentrations. In the process of batch culture, all samples were periodically taken for biomass and substrate concentrations.

2.3. Analytical methods

Cell density was monitored spectrophotometrically by measuring the absorbance at wavelength 600 nm [23]. Then biomass concentrations on a dry weight basis were measured by filtering cell suspension with the filler and drying the filter paper and cells to a constant weight for 24 h at 105 °C. To measure concentration of residual substrate, immediately after measurements of optical density, samples of suspended culture were centrifuged at 7500 rpm for 10 min. The cell free supernatants were used to determine the substrate concentration by high performance liquid chromatography (HPLC) using a LabAlliance (model Series III) system, with a C18 column (250mm × 4.6 mm, LabAlliance, USA). Elution was performed with 400/300 (v/v) methanol/water at a flow rate of 1.0 ml min^{-1}, and detection was realized with a UV-detector (Model 500, LabAlliance, USA) at 280 nm. The retention time for phenol was 4.89 min and for *m*-cresol was 6.12 min.

2.4. Statistics

All experiments were repeated three times. The data shown in the corresponding figures in Section 3 were the mean values of the experiments.