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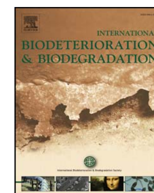

BTEX degradation by a newly isolated bacterium: Performance, kinetics, and mechanism

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ABSTRACT

A new isolate identified as *Rhodococcus* sp. ZJUT312 via taxonomic and 16S rRNA analysis was used for the efficient degradation of benzene, toluene, ethylbenzene, and xylene (BTEX). The strain ZJUT312 was able to degrade all the BTEX. Their average biodegradation rates followed the order *o*-xylene > ethylbenzene > toluene > benzene > *m*-xylene > *p*-xylene. The degradation rate of *o*-xylene reached as high as 0.117 mmol L^{-1} h^{-1}, which was one to two orders of magnitude higher than that of all other reported *o*-xylene degrading bacteria. GC-MS analysis revealed that *o*-xylene degradation pathway mainly proceeded with 2-methylbenzylalcohol as an intermediate and CO_2 as a final product. Mass balance analysis of the carbon element indicated that approximately 59% the *o*-xylene carbons were transferred to cell biomass and about 20% were mineralized into CO_2. Furthermore, the *o*-xylene degradation kinetics fitted well with Haldane's model. The maximum specific degradation rate (0.953 h^{-1}) was approximately 2.25 times higher than the reported data in the literature.

1. Introduction

BTEX (benzene, toluene, ethylbenzene, and xylene) are typical volatile monoaromatic hydrocarbons (Rahul et al., 2013; Yadav and Reddy, 1993). These compounds impose serious environmental issues, such as haze, photochemical smog, ozone depletion, and global warming (Jia et al., 2008; Zhang et al., 2018). They are also listed as priority pollutants by the United States Environmental Protection Agency (Dean, 1985). Therefore, BTEX removal processes have gained increasing attention.

Currently, various technologies, including absorption, adsorption, combustion, membrane separation, photocatalytic oxidation, and plasma, have been proposed for BTEX removal (Almasian et al., 2010; Bui and Hauser, 2016; Konggidinata et al., 2017; Korologos et al., 2012; Tomatis et al., 2016; Trusek-Holownia and Noworyta, 2015). Among them, the biotechnology-based processes are highly efficient, environmentally friendly, and cost-saving method for BTEX removal (Elmrini et al., 2004; Jorio et al., 2015; Zhu et al., 1998). Their successful application relies not only on efficient strains, which can rapidly consume BTEX as the carbon and energy sources, but also on the bioreactor design.

Several dedicated bacteria have been isolated to remove the BTEX (Chakraborty et al., 2005; Jeong et al., 2008; Jiang et al., 2015; Kim and Jeon, 2009; Prenafeta-Boldu et al., 2002; Zhang et al., 2013). However, most of the reported isolated bacteria can only degrade selected species of BTEX rather than all of them. For example, *Cladophialophora* sp. T1 can only degrade toluene and ethylbenzene (Prenafeta-Boldu et al., 2002). *Magnetospirillum* sp. 15-1 (Meyer-Cifuentes et al., 2017) and *Acinetobacter baumannii* DD1 (Zhou et al., 2016) cannot degrade (*m, p*)-xylene. *Pseudomonas* sp. BTEX-30 do not have the ability to degrade (*m, o*)-xylene.

Among BTEX, *o*-xylene is the most recalcitrant compound for microbial degradation (Jeong et al., 2008). Although several bacteria, such as *Comamonas* sp. JB, *Cladophialophora* sp. T1, *Janibacter* sp. SB2, *Pseudomonas putida* BCNU 106, *Pseudomonas Putida* F1, *Pseudoxanthomonas spadix* BD-a59, *Pseudomonas putida* OX1, *Pseudomonas* sp. FMB08, and *Rhodococcus* sp. BTO62, have been isolated for their ability to degrade *o*-xylene (Choi et al., 2013; Jeong et al., 2008; Jiang et al., 2015; Jin et al., 2013; Kim et al., 2008; Prenafeta-Boldu et al., 2002; Robledo-Ortíz et al., 2011), their degradation rates are slow (0.004–5 mg L^{-1} h^{-1}), hindering their large scale application. Based on the reported literature, the degradation pathway of *o*-xylene depends upon the inoculated microbes. *Cladophialophora* sp. T1 converted *o*-xylene to phthalates as end metabolites. (Prenafeta-Boldu et al., 2002).

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Nocardia sp. and *Corynebacterium* sp. C125 degrade *o*-xylene through an initial aromatic dioxygenase to generate a *cis*-dihydrodiol (Gibson and Subramanian, 1984; Schraa et al., 1987). Two metabolic pathways of *o*-xylene by *Rhodococcus* sp. B3 were found to exist simultaneously, and both of them are initiated by monooxygenases (Bickerdike et al., 1997). One pathway was found to involve the oxidation of the methyl group to generate 2-methylbenzylalcohol under aerobic conditions (Bickerdike et al., 1997). The other one was proceeded via oxidation of the aromatic ring to form 2,3-dimethylphenol (Bickerdike et al., 1997). In the case of *Pseudomonas stutzeri* OX1, *o*-xylene is degraded via two simultaneous monooxidations of the aromatic ring to form 2,3-dimethylphenol and 3,4-dimethylphenol, which are then converted into 3,4-dimethylcatechol and 4,5-dimethylcatechol, respectively (Baggi et al., 1987; Kim et al., 2002).

This work aims to: 1) isolate a novel bacterium with a high degradative activity toward *o*-xylene and a broad substrate specificity toward BTEX, 2) determine the kinetics of the *o*-xylene degradation, and 3) determine the mass balance during *o*-xylene degradation process.

2. Material and methods

2.1. Chemicals

Benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene, *p*-xylene, and ethyl acetate with a purity over 99.9% were purchased from Sigma–Aldrich, China (Shanghai, China). 2-methylbenzylalcohol (purity ≥ 97.0%) was obtained from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China), KH_2PO_4 (purity ≥ 99.5%) and $Na_2HPO_4{\cdot}12H_2O$ (purity ≥ 99.0%) were from Guangdong Guanghua Reagent (Shantou, China). $(NH_4)_2SO_4$ (purity ≥ 99.0%), $MgSO_4$ (purity ≥ 99.5%), and $CaCl_2$ (purity ≥ 96.0%) were from Shanghai Shisihewei Reagent (Shanghai, China). Soluble-starch, grease, cellulose, protein, starch, tryptone, and yeast extract were purchased from Nuoyang Biotechnology Company (Hangzhou, China). All the other chemicals used in this study were analytical grade and purchased from Huipu Company (Hangzhou, China).

2.2. Medium

The mineral medium (MSM) used in this study contains (per liter): 1 g KH_2PO_4, 4.5 g $Na_2HPO_4{\cdot}12H_2O$, 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4$, 0.02 g $CaCl_2$, 0.1 mg $MnSO_4{\cdot}4H_2O$, 0.1 mg $ZnSO_4{\cdot}7H_2O$, 0.02 mg $CuSO_4{\cdot}5H_2O$, 0.02 mg $CoCl_2{\cdot}6H_2O$, 0.02 mg $Na_2MoO_4{\cdot}2H_2O$, 1 mg $FeSO_4{\cdot}7H_2O$, and 0.02 mg H_3BO_3. The pH value of the MSM was adjusted to 7.0 using 1.0 mol L^{-1} NaOH and then it was autoclaved at 110° C for 40 min. The Luria-Bertani (LB) broth includes (per liter): 10 g tryptone, 5 g yeast extract, and 10 g NaCl, and was autoclaved at 121° C for 20 min. All solid LB contained 2.0 wt% agar.

2.3. Isolation and identification of o-xylene degradation bacterium

The bacterium was isolated from an activated sludge obtained from the wastewater treatment plant of Chengshilvneng Limited Company (Hangzhou, China). To enrich for the *o*-xylene-degrading bacteria, the collected activated sludge was cultured in 50 mL of MSM with 1 mmol L^{-1} of *o*-xylene at 30 °C and 160 rpm in batch mode for 3 months. Thereafter, 5 mL of the enriched culture was inoculated to a fresh medium. After repeating the same procedure for 3 times, the culture was inoculated to a solid LB medium. After incubation at 30 °C for 1–3 days, a single colony was selected and inoculated to the fresh MSM with *o*-xylene as the sole carbon and energy source to test its *o*-xylene-degrading ability. The bacterium was confirmed to be purified by optical and scanning electron microscope analyses and it was designated as strain ZJUT312.

Genomic DNA of the isolate was extracted using a genomic DNA purification kit (Cwibio Beijing, China, CW0552S) following the manufacturer's protocol. The universal forward (CAGAGTTTGATCCTGGCT) and reverse primers (AGGAGGTGATCCAGCCGCA) were used to amplify the 16S rRNA gene via Polymerase Chain Reaction (PCR). PCR was operated for 25 cycles under the following procedures: denaturation at 96° C for 10 s, annealing at 50° C for 5 s, and extension at 60° C for 4 min. The resulted PCR product was sequenced by the Shanghai Sangon Biotech Corporation (Shanghai, China). The amplified sequences were compared with those available in the GenBank database using a nucleotide-nucleotide BLAST algorithm and a phylogenic tree was developed using the NJ method of MEGA 7.0 with 1000 bootstraps.

2.4. BTEX degradation by strain ZJUT312

The strain ZJUT312 cultured in LB broth at 30° C was harvested by centrifugation (12000 rpm, 3 min) at exponential phase after approximately 12 h of cultivation. The harvested cells were washed twice with deionized water and used for the BTEX degradation. The biodegradation performance and broad substrate range of strain ZJUT312 were investigated with the individual BTEX compounds (1 mmol L^{-1}) in a 330 mL serum vial (sealed with PTFE screw cap) containing 50 mL of MSM with 30 mg (dry cell weight, DCW) L^{-1} of stain ZJUT312 (1.5 mg DCW) under aerobic condition for 100 h. It should be noted that the oxygen in the headspace was sufficient for the aerobic respiration. The effects of the initial substrate concentrations (0.5–3 mmol L^{-1}) and cell concentrations (10–50 mg DCW·L^{-1}) on the performance of TE(*o*)X degradation were also investigated. The abiotic serum vials were also tested under the same conditions as blank control.

The intermediates and carbon mass balance during *o*-xylene degradation were determined by inoculating 30 mg DCW·L^{-1} strain ZJUT312 into 660 mL serum bottles containing 100 mL MSM with 1 mmol L^{-1} *o*-xylene. Both gas and liquid phase samples were collected at the desirable time interval. The kinetics of *o*-xylene degradation was determined at various *o*-xylene concentrations (0.2–3 mmol L^{-1}) and 30 mg DCW·L^{-1} of strain ZJUT312 at 30 °C and 160 rpm within 118 h. The Haldane's inhibitory model was adopted to describe the *o*-xylene degradation and cell growth profile as follows:

$$\mu_o = \frac{1}{X}\frac{dX}{dt} = \frac{\mu_{max}S}{K_S + S + \frac{S^2}{K_I}} \cdot \nu_o = \frac{1}{X}\frac{ds}{dt} = \frac{\nu_{max}S}{K_S + S + \frac{S^2}{K_I}} \tag{1}$$

where μ_o represents the specific cell growth rate (h^{-1}), ν_o is the specific degradation rate of *o*-xylene (h^{-1}), X is the cell concentration (mg DCW·L^{-1}), S is the substrate concentration (mmol L^{-1}), μ_{max} is the maximum specific cell growth rate (h^{-1}), ν_{max} is the maximum specific degradation rate (h^{-1}), K_s is the half-saturation constant (mmol L^{-1}), and K_I is the inhibition constant (mmol L^{-1}).

2.5. Analytical method

The cell concentration of strain ZJUT312 was determined from a standard curve representing the relationship between the dry cell weight and optical density at 600 nm with a HITACHI U-2910 spectrophotometer (Hitachi High Technologies America). BTEX and CO_2 concentrations in the headspace were determined using a 6890 gas chromatograph (GC, Agilent Technologies). The corresponding concentrations of BTEX in aqueous-phase were estimated based on Henry's law according to Eq. (2) (Prenafeta-Boldu et al., 2002; Verce et al., 2001):

$$C_{aq} = \frac{M_{TOT}}{V_{aq} + H_c V_{hs}} \tag{2}$$

where C_{aq} is BTEX concentration in the aqueous phase (μmol L^{-1}); M_{TOT} is the total molecular amount of BTEX added to the vials (μmol); V_{aq} is the volume of liquid in the serum vials (L); V_{hs} is the volume of headspace in the vials (L); and H_c is Henry's constant (Jin et al., 2013;

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