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Degradation of o-xylene by a novel strain, *Zoogloearesiniphila* HJ1: Identification, kinetics and metabolic pathway

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Abstract

Biodegradation has become a popular alternative remediation technology due to its economical and ecological advantages. A strain able to degrade *o*-xylene effectively was selected and identified as *Zoogloearesiniphila* HJ1. The organism, strain HJ1, could degrade *o*-xylene with concentration as high as 256 mg L^{-1} at pH 6.8 and 26.3 °C. The Haldane model adequately described the relationship between specific growth rate and substrate concentration. The maximum specific growth rate and yield coefficient were 0.118 h^{-1} and 0.2572, respectively. A high mineralization rate of 67.9% was observed. Theenzyme activity and metabolic intermediates analysis showed that the ring cleavage of *o*-xylene was catalyzed by catechol 1,2-dioxygenase, and 3,4-dimethylcatechol was the main metabolic product. The present study demonstrated that the isolated strain, *Zoogloearesiniphila* HJ1, had a great application prospect for remediation of *o*-xylene-contaminated environment.

Keywords: *O*-xylene; *Zoogloearesiniphila*; Biodegradation; Kinetics; Metabolic pathway

1. Introduction

O-xyleneis a polluting agent derived from petroleum-like refining fuel, such as coating, colorant and solvent, and commonly found in polluted environmental media around the petrochemical industries and synthetic chemicals (Morlett-Chávez *et al.*, 2010). Much attention has been drawn to how to remove *o*-xyleneas which is toxic and more soluble in water than the rest of hydrocarbons. *O*-xylene is harmful to human health and can cause fatigue, confusion, headache, dizziness, and even death (Mazzeo and Marin-Morales, 2011). The U.S. Environmental Protection Agency has defined *o*-xylene as a priority pollutant and established the maximum permissible concentration level to be 10 ppm (Guo *et al.*, 2013). Therefore, its elimination from the environment becomes an important issue.

Biodegradation appears to be an effective and eco-friendly technology for remediation of contaminated sites under aerobic, microaerobic and anaerobic conditions (Martino *et al.*, 2012; W. Sun *et al.*, 2009; Zhang *et al.*, 2012). In recent years, biodegradation of typical organic compounds (such as benzene, toluene) to CO_2, H_2O and biomass by specialized bacteria has received great attention (Chakraborty *et al.*, 2006; Hildebrand and Mackenzie, 2008; Lu *et al.*, 2015; Ryu *et al.*, 2008). Since *o*-xylene, one type of the three isomers of xylene, has the lowest biodegradability, its biodegradation and metabolizing pathways have been rarely reported. Recent studies showed that some effective degraders were isolated and biodegradation of *o*-xylene by aerobic and anaerobic bacteria could be achieved, including *Pandoraea sp strain WL1*, *Cladophialopharo sp.*, *Rhodococcus sp. strain DK1*, *Pseudomonas putida F1* etc (D Kim *et al.*, 2004; Dockyu Kim *et al.*, 2010; Prenafetaboldú *et al.*, 2002; Wang *et al.*, 2015). Although these microorganisms can feasibly degrade o-xylene, their slow growth rates and

biodegradation rates under natural conditions have limited their use in bioremediation. Several methods (such as optimization of growth conditions, seeking of co-metabolic compounds, detection of key metabolites) have been tried to enhance biodegradation process, and make degraders show higher biodegradation activity compared to their original state (Shadi *et al.*, 2013; Yang *et al.*, 2008; Yao *et al.*, 2009). In addition, microorganism was cultured in optimal conditions as their introduction into bioreactors could accelerate biofilm formation and improve operational stability (Baptista *et al.*, 2008; Emanuelsson *et al.*, 2005). Accurate determination of biodegradation kinetics is required to design cost effective and reliable bioreactors for treatment of contaminated groundwater and industrial effluents (Feisther *et al.*, 2015). Therefore, isolation and characterization of target bacterial strains will be most important to bioremediation of typical recalcitrant compounds.

In the present study, a new identified bacterium that could degrade *o*-xylene was first isolated from the active sludge of a pharmaceutical factory. The isolator was identified as *Zoogloearesiniphila* and its degradation ability for *o*-xylene was not reported in previous studies. Investigation of growth conditions and kinetics was carried out for determination of degradation characteristics. Kinetic parameters of p-xylene degradation were determined and fitted to the Haldane equation (Feisther *et al.*, 2015). Then, based on measurement of special enzyme activity and detection of intermediates during degradation, a novel metabolic pathway for *o*-xylene was proposed. Such results provided fundamental data and theoretical guidance for further application of the isolator in the *o*-xylene bioremediation system.

2. Materials and methods

2.1. Experiment Materials

1) Active Sludge

Active sludge was collected from a SBR aeration tank of a pharmaceutical Factory in Xinchang, Zhejiang Province.

2) Culture Medium

The mineral medium for acclimation and isolation contained the following mineral salt (mg L^{-1}): $KH_2PO_4$1000, $Na_2HPO_4$4500, $(NH_4)_2SO_4$500, $MgSO_4$100, $CaCl_2$20, $MnSO_4{\cdot}4H_2O$ 100, $ZnSO_4{\cdot}7H_2O$100, $CuSO_4{\cdot}5H_2O$20, $CoCl_2{\cdot}6H_2O$20, $Na_2MoO_4{\cdot}2H_2O$20, $FeSO_4{\cdot}7H_2O$1, $H_3BO_3$0.02, pH7.3~7.55. The solution was autoclaved at 110°C for 40 min, under which the medium could not turn turbid. *O*-xylene was added as carbon and energy source.

2.2. Strain purification and identification

Preliminary characterizations were based on morphological and physiological land biochemical tests. Further identification was completed by 16S rRNA gene sequencing according to the method described (Guo *et al.*, 2013). Phylogenetic trees were generated from alignments by the neighbor-joining method. The reliability of inferred trees was tested via the bootstrap test with the MEGA4 program. The strain HJ1 (Genbank No. JQ751310, CCTCC NO. M2012235) was currently deposited in China Center for Type Cultural Collection (Wuhan).

2.3. Degradation and mineralization of o-xylene by the isolator

Experiments were carried out at 35°C on an orbital shaker at 160 rpm in the dark. Cells grown on MSM with *o*-xylene were harvested by centrifugation (16000g, 15 min), and the cell pellet was re-suspended in a fresh MSM. Aliquots of this suspension were placed into the serum bottles, and *o*-xylene was added as the carbon source. Incubation was performed in the dark on a rotary shaker (150 rpm). Growth was monitored by gas phase measurement of *o*-xylene and biomass density compared with *o*-xylene consumption and cell production in non-inoculated controls.

The final mineralization product CO_2 was measured by a gas chromatography. When CO_2 concentration in the sealed serum bottle reached its maximum value, and was not varied with the time, 1mL 30% HCl was added into the bottle to transform liquid phase HCO_3^- completely into CO_2, which was released into gas phase. CO_2 concentration was determined again after 0.5 h.

For research on the dynamics of single substrate degradation and cell yield, Haldane equation was employed to describe the process of degrading substrate at different initial concentrations, with equations represented as follows:

$$\mu_i = \frac{1}{X}\frac{dX}{dt} = \frac{\mu_{\max_i} S_i}{K_{S_i} + S_i + S_i^2/K_I} \quad v_i = \frac{1}{X}\frac{dS_i}{dt} = \frac{v_{\max_i} S_i}{K_{S_i} + S_i + S_i^2/K_I}$$

In the equations, X: concentration of stains, mg/L; t: time, h; S: concentration of the substrate, mg/L; μ_{max}: maximum specific growth rate, h^{-1}, K_S: half saturation coefficient,

mg/L; K_I: inhibition coefficient, mg/L; v_{max}: maximum specific degradation rate

2.4. Analytical techniques

2.4.1 Morphological observation

The observation was processed on Nikon general photon microscope and JEOL JEM-1230-TEM.

1) 16S rRNA identification

DNA was extracted using a TaKaRa MiniBEST Universal Genomic DNA Extraction Kit Ver. 4.0 (Takara Code: D824A). The bacterial universal primers BSF8/20(5'-AGAGT TTGAT CCTGG CTCAG-3) and BSR1541/20 (5'-AAGGA GGTGA TCCAG CCGCA-3') (TaKaRa Biotechnology, Dalian, China) were used for amplification, which was performed in a thermalcycle (PTC 200, Bio-Rad, USA) according to the method described by Zhang et al(Li *et al.*, 2011). Sequencing was measured by TaKaRa Biotechnology Co.,Ltd (Dalian, China) and all the 16S rRNA gene sequences were checked manually for phylogenetic analysis.

2) CO_2 analysis

Concentrations of *o*-xylene and CO_2 were measured by GC analysis of headspace. Headspace concentrations were converted to aqueous-phase concentrations using Henry's law constants. All samples, irrespective of incubation temperature of the cultures, were equilibrated at 25°C before GC analysis. An 800-µL headspace sample was injected into GC for *o*-xylene and CO_2analysis.

3) *O*-xyleneanalysis

O-xylene concentration was detected by a 6890N GC (Agilent Technologies, America), with a HP-Innowax capillary column (30 m×0.32 mm×0.5 µm) and a FID detector. The injector and detector temperatures were set at 180 °C and 200 °C, respectively. The oven temperature was programmed as follows: initial 90°C maintained for 4 min, increased to150°C at 30°C min^{-1} and maintained for 0.5 min. For CO_2 measurement, a HP-Plot-Q column (30 m×0.32 mm×20µm) and a TCD detector were used. The injector, oven and detector temperature were 90°C, 40°C and 100 °C, respectively.

4) Metabolites analysis

Metabolites at different sampling intervals were extracted from the culture by equal volume of ethyl acetate, dried over by anhydrous sodium sulphate and evaporated to dryness under high-purity N_2 stream. GC/MS (Agilent 7890N/MS 5975) equipped with a HP-5MS capillary column (30 m × 0.25 mm × 0.25µm) was used for analyzing these metabolites. Conditions for GC/MS were as follows: oven temperature 100 °C for 1 min, ramped to 280°C at 5°C min^{-1} and held for 10 min. Injector temperature was 180 °C. The MS interface temperature was set at 280°C and the ionization source at 250°C. The mass spectra were obtained at 70 eV. All metabolites were identified by GC/MS through matching retention times and ion spectra with authentic standard and NIST library data.

5) Determination of enzyme activity

The activity of catechol 1,2-dioxygenase and catechol 2,3-dioxygenase was determined by the formations of cis, cis-muconate, 2-hydroxymuconic semialdehyde, and the characteristic absorbance was respectively 260 and 375 nm measured by a spectrophotometer (UV2910, Shimadzu,Japan) (Cao *et al.*, 2008). Enzyme reaction was conducted in 4 mL deionized water, which contained 33 µmol Tris-HCl, 1.3 µmol Na_2EDTA, 0.5 µmol catechol and 100 µL enzyme. Enzyme activity was calculated as follows:

$$U/mg=\Delta A\times V/(\varepsilon\times M)$$

Where, ΔA is the change of absorbance per minute at the wavelength of 260 nm and 375 nm; V is the total reaction volume detected by enzyme activity, L; ε is the molar extinction coefficient of catechol at 260 nm and 365 nm, $(mmol\cdot cm)^{-1}$; M is protein content, mg.

3. Results and discussion

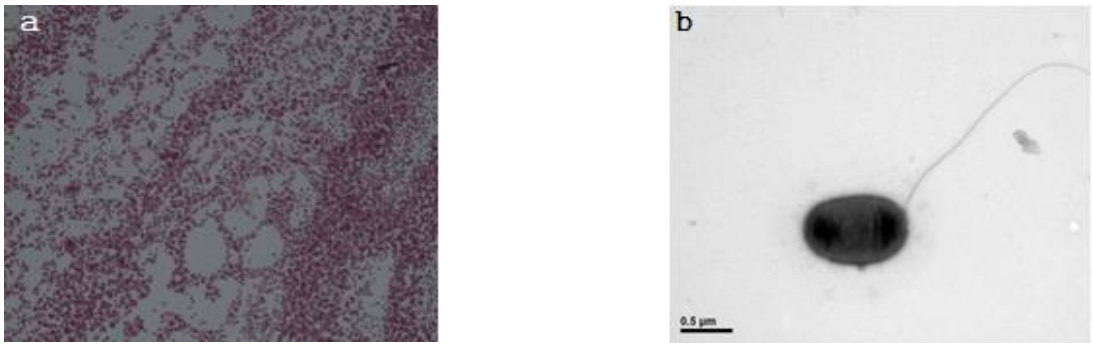
3.1. Identification of the isolated strain

1) Morphological observation

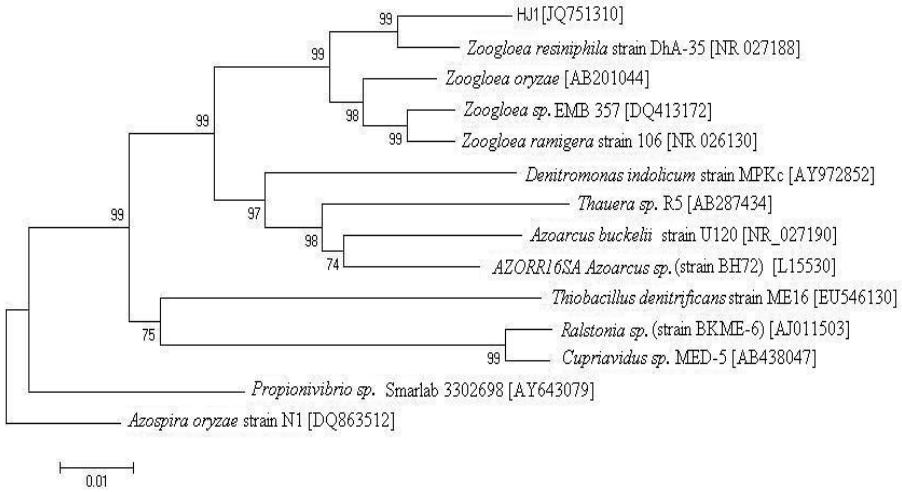
By strain screening and rescreening, an isolator (coded as HJ1) with high *o*-xylene degradation ability was selected. Results of Gram staining showed that the strain was gram-negative with a flagellum (Fig. 1a) and its oxidase was positive. The colonial morphology of strain HJ1 was circular, the edge of which is neat, moist and smooth. The shape of strain HJ1 was short rod, with the mean size of (0.8~1.0) µm×(1.2~1.5) µm (Fig. 1b).

2) 16S rRNA sequence analysis

The 16S rRNA of strain HJ1 (Genbank No. JQ751310 CCTCC NO. M2012235) was sequenced to determine its phylogenetic position as depicted in Fig. 1c. Since 16S rRNA sequence of strain HJ1 shows 99% similarity to that of *Zoogloearesiniphila* strain DhA-35 (NR027188).



(a) (b)



(c)

Figure 1. Morphological observation and 16S rRNA sequence analysis: (a) Gram stain; (b) TEM image; (c) Phylogenetic tree of the strain with other closely related strain

3.2. Degradation characteristics of o-xylene by strain HJ1

1) Effect of pH and temperature

Initial pH and growth temperature were two important factors that affected bacteria growth and degradation activity for special compounds (Sharma *et al.*, 2009). Fig.2 shows the growth and degradation characteristics with initial *o*-xylene of 128 mg L^{-1} under different pH values and temperatures. Incubated for 24h, removal efficiency of *o*-xylene arrived at its maximum value 99.9% (biomass being 43.7 mg L^{-1}) under pH of 7. As for growth temperature, strain HJ1 could grow very well at 35°C, and removal efficiency was 99.8% (biomass being 44.2 mg L^{-1}). Song indicated that pH and temperature of o-xylene degradation was 7.5 and 27 °C in soil, respectively (Song, 2003), with temperature slightly lower than the strain HJ1. Biodegradation rate for *o*-xylene was nearly 20% faster than that obtained under non-optimized cultural conditions (pH 7 and temperature 30 °C).

2) Effect of initial *o*-xylene concentration

The ability of strain HJ1 to degrade *o*-xylene was evaluated by monitoring degradation efficiency of *o*-xylene at different initial concentrations ranging from 30-300 mg L^{-1} under optimal conditions. Fig. 3 shows *o*-xylene concentration and cell concentration profiles with respect to incubation time. As shown in Fig. 3, a complete reduction was achieved at about 10 h with an initial concentration of 32 mg L^{-1} or at about 105 h with an initial concentration of 256 mg L^{-1}. When *o*-xylene concentration was increased to 288 mg L^{-1}, strain HJ1 failed to completely degrade *o*-xylene and 40.5% remained in the cultural medium, suggesting that *o*-xylene concentrations in excess of 288mg L^{-1} would inhibit the growth of strain HJ1 strongly. The growth yield of strain HJ1 was linear up to an

approximately *o*-xylene concentration of 160 mg L^{-1} and no direct relationship was found between these parameters when concentration reached 192 mg L^{-1} and above. Such a phenomenon was caused by the toxicity of higher-concentration *o*-xylene and the corresponding energy-dependent adaptive response of the strain.

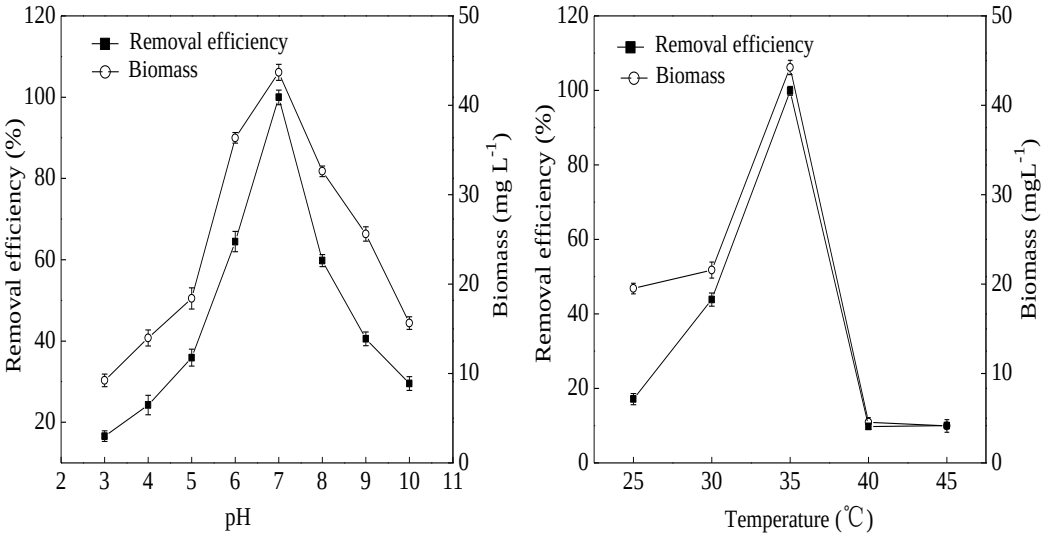


Figure 2. Effect of pH and temperature on *o*-xylene degradation by strain HJ1.

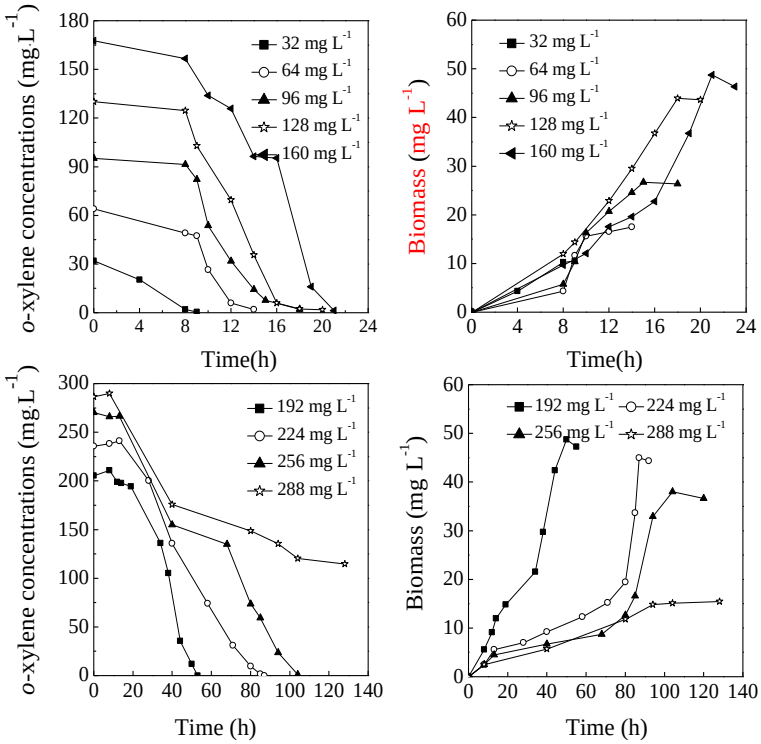


Figure 3. Different amounts of *o*-xylene as an increasing substrate by strain HJ1 under optimal conditions

In previous studies, a few species were reported to have the ability for biodegradation of *o*-xylene. Even some researchers indicated that *o*-xylene could only be completely degraded in the presence of other aromatic compounds (such as benzene, toluene), and *o*-xylene was not utilized as the sole carbon or energy source (Robledo-Ortíz *et al.*, 2011; Zhang *et al.*, 2012). Compared to strains described elsewhere, this isolate HJ1 was able to degrade *o*-xylene individually and could degrade rather higher concentrations of *o*-xylene than the reported ones. Strain

HJ1 tolerated *o*-xylene in 256 mg L^{-1} and completely degraded it in 105 h, while strain *P. Putida* F1 was previously reported to degrade 30 mg L^{-1} *o*-xylene with removal efficiency of 80% (Robledo-Ortíz *et al.*, 2011). From a practical perspective, this isolate is important for further application in bioremediation as it is relatively resistant to elevated *o*-xylene concentrations and refuses to utilize *o*-xylene as the sole carbon source without any other sources.

3.3. Kinetics and biotic losses

Batch experiments were conducted to evaluate the kinetics of *o*-xylene utilization and calculate the growth yield. Growth kinetics was determined using the Haldane model (Datta *et al.*, 2014; Feisther *et al.*, 2015).The mathematical programme of Origin 8.0 was used for analyzing the Haldane equations. The plot between specific growth rate and *o*-xylene concentration was shown in Fig.4a. The specific growth increased with *o*-xylene concentration and reached its maximum at approximately 96 mg L^{-1}. Concentrations above this level affected the specific growth rate negatively as substrate inhibition became stronger. Then, the correlation coefficient was tested at the initial concentration of 96 mg L^{-1}. Kinetic parameters of o-xylene degradation were fitted to the Haldane equations (Feisther *et al.*, 2015) and correlation coefficient (R^2) was 0.9978. Kinetic parameters that fitted to the model were: 0.118 h^{-1} for μ_{max}, 18.46 mg L^{-1} for K_s, and 169.75 mg L^{-1} for K_I. A microbial consortium was isolated for biodegradation of *o*-xylene and its specific growth rate of μ_{max}=0.14 h^{-1} was obtained from the Haldane model (Goudar and Strevett, 1998), slightly higher than that of strain HJ1. Fig.4b shows that the plot was used to determine the biomass yield coefficient of strain HJ1 on *o*-xylene. The value of yield coefficient was 0.2572. As illustrated before, initial concentrations higher than 192 mg L^{-1} would inhibit the growth of strain HJ1, so biomass amount could not fit the line very well. With the increase of initial concentration, the deviation phenomenon was more evident.

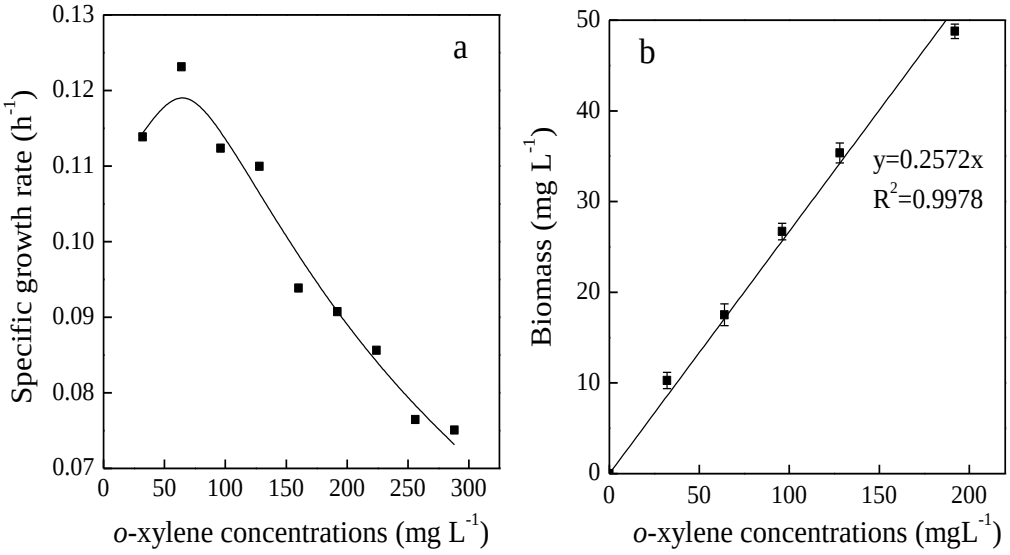


Figure 4. Relationship between *o*-xylene concentration and microbial growth rate: (a) Haldane's growth model fitted to the results of batch growth experimental data; (b) plot to calculate the yield coefficient for strain HJ1 growth on *o*-xylene

To investigate baiotic losses and sorption in strain HJ1 growth on *o*-xylene, initial concentration was set at 96 mg L^{-1}. The control group was compared to the experiment group lacking strain HJ1 (Tab.1). After 18 h, *o*-xylene concentration of the control group and the experiment group was 95.42 and 6.58 mg/ L, respectively. It was shown that baiotic losses and sorption had little effect on *o*-xylene concentration. In addition, biomass of the experiment group increased to 26.36 mg/L. Strain HJ1 growth was determined as the main factor for reduction of *o*-xylene concentration.

3.4. Mineralization of o-xylene by strain HJ1

In the biodegradation process, organic pollutants were used as essential carbon source for microbial growth and aerobically degraded to H_2O and CO_2 (Ralebitsosenior *et al.*, 2012). Therefore, CO_2 production was an important parameter to evaluate the degradability of organic

compounds, which provides further valuable information on the complete removal. CO_2 production as a function of *o*-xylene consumed at different concentrations was presented in Fig. 5, indicating a linear relationship.

Table 1. Biotic losses and sorption of *o*-xylene

	Control group （mg/L）		Experiment group（mg/L）	
	Concentration	Biomass	Concentration	Biomass
0 h	96	0	96	0
18 h	95.42	0	6.58	26.36

The mean experimental data below 192 mg·L^{-1} were found to lie reasonably around y=1.821x, showing that 1 mg *o*-xylene could be completely mineralized to produce 1.821 mg CO_2. In theory, the stoichiometric ratio should be 3.32 in the case of complete oxidation of *o*-xylene to H_2O and CO_2. Therefore, carbon mineralization of *o*-xylene by strain HJ1 was around 54.8%.

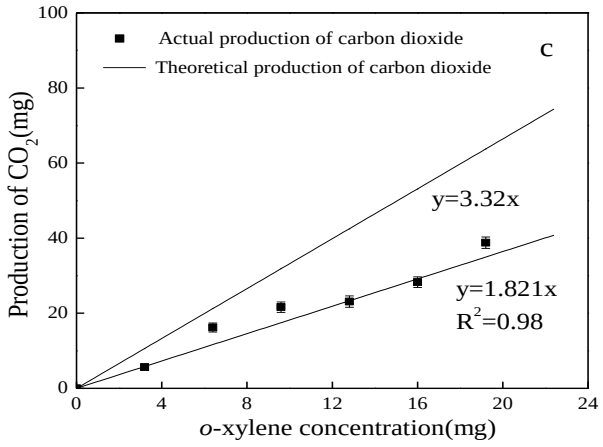


Figure 5. CO_2 productions of *o*-xylene with different initial concentrations

The strain *Mycobacterium cosmeticum*byf-4 utilized *o*-xylene as the sole carbon source and the mineralization rate was approximately 65.5% (Zhang *et al.*, 2012). Compared with other aromatic compounds, such low mineralization rates suggested that *o*-xylene was relatively difficult for complete mineralization into compounds by microorganisms.

The discrepancy between the theoretical ratio (3.32) and the experimental ratio (1.82) indicated that CO_2 was not the only final conversion of carbon by strain HJ1, and remaining between them might be other conversions accumulated during biodegradation, such as biomass and extracellular polymers (Delhoménie *et al.*, 2002).Through metabolic effects of strain HJ1, 0.9057 mg C contained by 1 mg *o*-xylene could be converted to 0.4966 mg C-CO_2 and 0.1187 mg C-biomass, from which a carbon recovery efficiency of 67.93% was calculated .

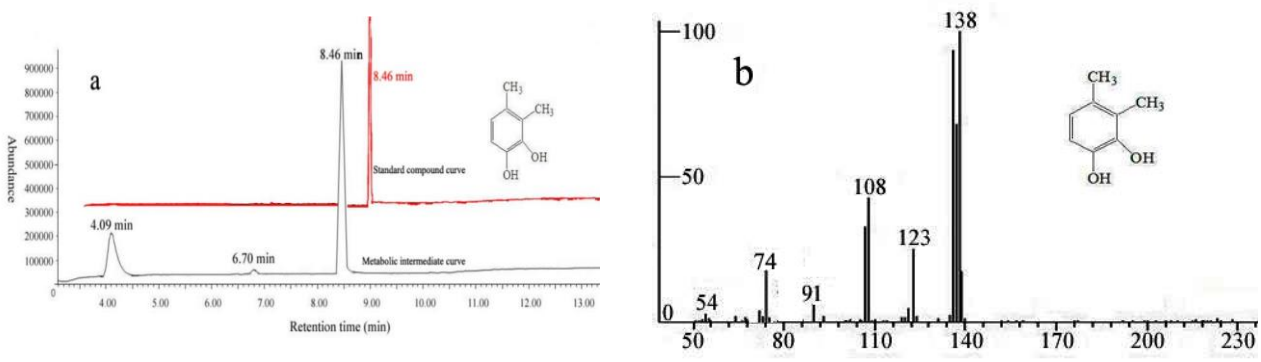
3.5. Possible metabolic intermediates of o-xylene and its pathway

Resting cells were inoculated in the sterilized phosphate buffer (pH 7.2) solution for determination of metabolic intermediates. The initial substrate concentrations were 50 and 200 mg L^{-1}, and each was continuously detected for 64 or 200 h. The identities of main metabolic intermediates were confirmed by GC-MS. Through identification by comparison with commercially available authentic standards and by matching against a mass spectra library, 3, 4-dimethylcatechol was detected as a main intermediate, (Fig.6 shows its gas chromatogram and mass spectrum). Kim *et al.* indicated that there were several different pathways of *o*-xylene by different microorganisms. It has been proposed that identified intermediates were 3,4-dimethylcatechol or 4,5-dimethylcatechol from the successive monooxidations of the aromatic ring to form 2,3-dimethylphenol or 3,4-dimethylphenol. Therefore, detection of 3, 4-dimethylcatechol showed that strain HJ might contain monooxidation enzyme that could oxidize the aromatic ring.

After formation of dihydroxylated compounds, there were two manners of ring cleavage, ortho (between the two hydroxylated carbons) or meta (between a hydroxylated and non-hydroxylated carbon) (Bozinovski *et al.*, 2014; Prabhu and Phale, 2003; J. Q. Sun *et al.*, 2009; Zeinali *et al.*, 2008).Since the ring-cleavage intermediates, which processed polar groups, were hardly detected by GC/MS, dioxygenase activity was applied to indirectly testify the manner of ring cleavage.

Catechol 1,2-dioxygenase and catechol 2,3-dioxygense activity assays were performed by measurement of absorbance at 260 or 375 nm, since these wavelengths were special forcis, cis-muconic acid or 2-hydroxymuconic semialdehyde(2-HMSA), respectively(Cao *et al.*, 2008). Catechol-1,2-dioxygenase activity to hydroquinone and catechol was conducted on cells grown on different carbon sources, including *o*-xylene. The highest activity was observed with YE+*o*-xylene or only *o*-xylene as assay substrates; the activity was induced with LB, glucose and YE

as substrates which were reduced gradually, but at significant levels. However, catechol-2,3-dioxygense activity was not detected in any cell extracts. It suggested that hydroquinone and catechol were further oxidized to the ring cleavage products by the action of relative nonspecific 1,2-dioxygenase enzymes (Tab. 2), i.e., further breakdown of these compounds by HJ1 proceeded via the ortho-cleavage. It was also indicated that the relative enzyme was an induced enzyme, which had the activity in the presence of *o*-xylene.



(a) (b)

Figure 6. (a) GC/MS chromatogram of *o*-xylene biodegradation intermediates by strain HJ1; (b) the mass spectrums of the mainintermediates

Table 2. Activity and inducibility of enzymes involved in *o*-xylene degradation pathway in strain HJ1.

Substrate induced	Enzyme activity（U/mg protein）			
	Hydroquinone		Catechol	
	$C_{12}O$	$C_{23}O$	$C_{12}O$	$C_{23}O$
LB	1.1	0	12.3	0
Glucose	0.8	0	2.7	0
YE	0.9	0	2.4	0
O-xylene	7.3	0.6	26.9	1.0
YE+o-xylene	10.6	0	55.1	1.5

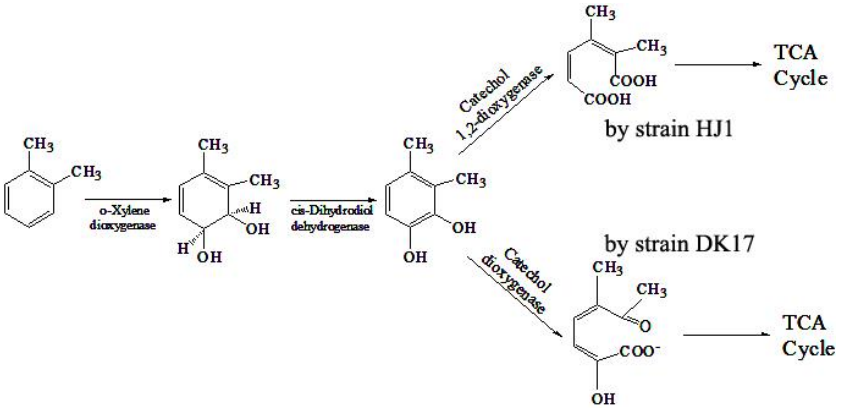


Figure 7. Possible metabolic pathways of *o*-xylene by *Zoogloearesiniphila* stain HJ1 and *Rhodococcus* sp. strain DK17

Based on these results, a different possible pathway was proposed as shown in Fig.7 for initial attack on *o*-xylene. Before the ring cleavage, the pathway was similar to the one reported by Kim *et al.* (D Kim *et al.*, 2004; Dockyu Kim *et al.*, 2010), in which *Rhodococcus* sp. strain DK17 was utilized for degradation of *o*-xylene. *O*-xylene was firstly converted to 1,2-dihydroxy-3,4-dimethyl-cyclohexane-3,5-diene, which was then transformed into 3,4-

dimethylcatechol (detected in the study) under the function of dioxygenase and cis-dihydrodiol dehydrogenase, respectively. But for the ring cleavage, the manner processed by strain HJ was different from others. 3,4-dimethylcatechol was converted to ring-opening products in the manner of ortho, and then such ring-opening products entered the cycle of TCA to generate the completely mineralized products (CO_2, H_2O etc.) and biomasses.

4. Conclusions

The *Zoogloea* species HJ1 (Genbank No JQ751310) capable of effectively degrading *o*-xylene was isolated and identified in the present study. The optimum values of pH and temperature were 6.8 and 26.3°C. Under optimal conditions, 256mg L^{-1} *o*-xylene could be completely degraded. Specific growth rate complied with the Haldane kinetic equation; the maximum value was 0.118 h^{-1} when *o*-xylene concentration reached 20 mg L^{-1}. With *o*-xylene concentration higher than 288 mg L^{-1}, the strain growth and degradation was significantly inhibited. The cell yield coefficient of 0.2572 mg (mg*o*-xylene) $^{-1}$ by the isolated strain and CO_2 production rate of up to 54.8% proved that removal of substrate was mainly via mineralization and incorporation into cell material. New pathways for *o*-xylene degradation were proposed by identification of metabolites and assay of ring cleavage enzymes in cell extracts. Results showed that 3, 4-dimethylcatechol was an important intermediate and the manner of ring cleavage was ortho. The isolated strain HJ would have a potential application for bioremediation of *o*-xylene pollution, and the enzyme possessed by strain HJ would have engineering application value for purification of aromatic compounds.

Acknowledgments

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