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Biodegradation of Benzene, Toluene, Ethylbenzene, and *o*-, *m*-, and *p*-Xylenes by the Newly Isolated Bacterium *Comamonas* sp. JB

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Abstract A bacterium designated strain JB, able to degrade six benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylene (BTEX) compounds, was isolated from petroleum-contaminated soil. Taxonomic analyses showed that the isolate belonged to *Comamonas*, and until now, the genus *Comamonas* has not included any known BTEX degraders. The BTEX biodegradation rate was slightly low on the mineral salt medium (MSM), but adding a small amount of yeast extract greatly enhanced the biodegradation. The relationship between specific degradation rate and individual BTEX was described well by Michaelis-Menten kinetics. The treatment of petrochemical wastewater containing BTEX mixture and phenol was shown to be highly efficient by BTEX-grown JB. In addition, toxicity assessment indicated the treatment of the petrochemical wastewater by BTEX-grown JB led to less toxicity than untreated wastewater.

Keywords BTEX compounds · *Comamonas* sp. JB · Petrochemical wastewater

Introduction

Benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylenes, collectively known as BTEX compounds, are an important family of aromatic hydrocarbons that are components of petroleum, and its products such as gasoline and diesel fuel are widely used in industrial syntheses [1–4]. BTEX compounds are frequently found as the major organic pollutants in petrochemical, coking, and other indusial wastewater due to the application of petroleum and its products in industrial processes [5]. BTEX compounds are known to possess toxic to

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humans and are confirmed or suspected carcinogens. Thus, the Environmental Protection Agency classifies them as priority pollutants, making their removal from polluted environments critical [6]. Fundamental research examining the biodegradation of these BTEX compounds and the application of bacteria in bioremediation for reducing their concentrations in the environment has been conducted [7].

As is well known, bacteria that degrade BTEX compounds under aerobic conditions are widely distributed, and researchers have isolated many of these strains by enrichment broth culturing with BTEX compounds as the sole carbon and energy sources [8]. However, most of the isolation of BTEX degraders in soil environments belonged to the genera *Pseudomonas*, *Pseudoxanthomonas*, *Burkholderia*, *Sphingomonas*, *Thauera*, *Dechloromonas*, *Rhodococcus*, *Janibacter*, and *Acinetobacter* [7, 9–14]. Little is known about the degradation of all the BTEX compounds by genera *Comamonas*.

In this study, a novel BTEX compound-degrading bacteria *Comamonas* sp. JB was isolated from petroleum-contaminated soil and its feasibility of BTEX compound degradation was investigated. The relationship between specific degradation rate and BTEX concentration was described by Michaelis-Menten kinetics. The bioremediation of petrochemical wastewater containing BTEX compounds was also studied by strain JB, and ecotoxicological assessment of the treated effluent was carried out.

Materials and Methods

Chemicals

Benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylenes were purchased from J&K Scientific Ltd. (China). All other commercially available chemicals were of analytical grade.

Isolation and Identification of BTEX-Utilizing Strain JB

The petroleum-contaminated soil sample used to isolate BTEX-degrading bacteria was obtained in Liaoning province of China. The isolation process was performed in a mineral salt medium (MSM) which contained (g L^{-1}) KH_2PO_4 3.7, $K_2HPO_4\,3H_2O$ 5.2, NH_4Cl 2.0, Na_2SO_4 1.0, $MgSO_4$ 0.1, and 1 mL L^{-1} of trace metal solution. The petroleum-contaminated soil sample and the BTEX mixtures (100 mg L^{-1} for each benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylenes) were added to 50-mL MSM in 250-mL shake flasks at 30 °C on a reciprocal shaker at 180 rpm. After 10 times of sequential transfers, one isolate, designated JB, was isolated from the culture. 16S ribosomal rRNA was sequenced by Shanghai Sangon Biotechnology Co., Ltd., China. The 16S rRNA gene sequence and related ones retrieved from GenBank were aligned by Clustal W and constructed a phylogenic tree using NJ method of MEGA (version 4.1) with 500 bootstraps.

Degradation of BTEX by Strain JB

Strain JB was cultured with BTEX mixture containing 30 mg L^{-1} of each component in MSM at 30 °C on a reciprocal shaker at 180 rpm. Cell suspensions of JB were prepared separately by centrifugating the cultures in late exponential phase at 8000×*g* for 10 min, washing cell pallets twice with MSM, and resuspending cells in MSM to get different turbidities.

The BTEX degradation abilities of strain JB were evaluated using 50 mg L^{-1} of each individual substrate or a BTEX mixture containing 30 mg L^{-1} of each component in MSM using the strain JB cell suspension with a turbidity at 660 nm of 0.05. The effect of yeast extract concentrations (10 to 40 mg L^{-1}) on the degradation of each individual substrate or a BTEX mixture was also tested. Studies on benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylene (20 to 150 mg L^{-1}) degradation at different times by strain JB cell suspension with a turbidity at 660 nm of 2.5 (BTEX-grown JB) were also carried out. Samples were taken at intervals to monitor the concentrations of BTEX as described below.

Petrochemical Wastewater Treatment by BTEX-Grown JB

The petrochemical wastewater used in this study was from the petrochemical wastewater treatment plant (WWTP) located in northeast China. The wastewater quality was as follows (mg L^{-1}): COD 1000, phenol 100, benzene 8.5, toluene 10.5, ethylbenzene 7.2, *o*-xylene 3.5, *m*-xylene 5.5, and *p*-xylene 8.7. The raw wastewater was diluted 1:1 (*v*/*v*) in deionized water, phenol and BTEX compounds were added to give prominence to degradation, and final concentrations of COD, phenol, benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylenes were 1700, 100, 30, 30, 30, 30, 30, and 30 mg L^{-1}, respectively. Study on the degradation of this petrochemical wastewater by BTEX-grown JB was carried out. Samples were taken at intervals to monitor the concentrations of BTEX, and the acute toxicity of effluent and influent samples were also tested by Microtox bioassays as described below.

Analytical Methods

After each batch of biodegradation, the samples were extracted with two volume of methylene chloride for at least 1 h by inversion. The concentrations of BTEX were analyzed by gas chromatography with an HP-5 capillary column (Agilent Technologies, 6890N) as previously described [7]. The gas chromatography oven was programmed to increase from 60 °C (held for 1 min) to 220 °C at 10 °C min^{-1}; after which, 220 °C was held for 3 min. The gas flow to the detector contained H_2 (40 mL min^{-1}) and synthetic air (450 mL min^{-1}), the detector temperature was 300 °C, the injection port temperature was 250 °C, and the 1-μL samples were loaded with an auto sampler with a split mode (5:1). The profiles of *m*-xylene and *p*-xylene mirrored each other, because they had the same retention time on the gas chromatography analysis chromatogram (calculated by dividing by 2). Phenol concentration was analyzed using high-performance liquid chromatography (HPLC) system (Shimadzu LC20A; Thermo Hypersil ODS-2 column, 5 μm, 250×4.6 mm) as previously described [15]. The acute toxicity of effluent and influent samples was assessed by Microtox bioassays using the luminescent bacteria *Vibrio fischeri* (NRRL B-11177) as previously described [15].

Results and Discussion

Isolation and Identification of the JB BTEX Degrader

A bacterial strain designated as JB was isolated from a petroleum-contaminated soil sample containing about 1500 mg kg^{-1} total petroleum hydrocarbons. Strain JB was a transparent-pigmented, gram-negative, aerobic, non-motile, and non-spore-forming bacterium. Its growth

was observed in the range of 15 to 35 °C (optimum, 25 to 30 °C) and between pH 6.0 and 9.0 (optimum, pH 7.5 to 8.0). The 1484-bp fragment of the 16S rRNA PCR product was sequenced, and the sequence was submitted to GenBank database (accession number: KM067129). It is indicated that strain JB had the highest similarity (ca. 99 %) with *Comamonas* sp. KZ-OAlF1 (FJ688377) (Fig. 1). Therefore, strain JB was identified as *Comamonas* sp. JB. Many other studies have reported BTEX-degrading bacteria belonging to the genera *Pseudomonas* and *Acinetobacter*, but to our knowledge, no one has reported a BTEX-degrading *Comamonas* species [7, 14].

BTEX Degradation by Growing Cells of Strain JB

The BTEX degradation abilities of strain JB were evaluated using 50 mg L^{-1} of each individual substrate or a BTEX mixture containing 30 mg L^{-1} of each component in MSM. BTEX loss during culture was negligible in the control without inoculation (data not shown). Figure 2 shows the individual BTEX concentration profiles with respect to incubation time. As shown in Fig. 2a, *m*- and *p*-xylenes (50 mg L^{-1}) are completely degraded within 3 days, benzene and toluene are exhausted within 5 days, and ethylbenzene and *o*-xylene are completely degraded within 8 days. Compared with bacteria *Pseudoxanthomonas spadix* BD-a59 and fungus *Cladophialophora* sp. T1 on the degradation of BTEX, strain JB exhibited higher degradation capability on all the BTEX compounds [7, 16]. In order to further improve the BTEX degradation abilities of strain JB, yeast extract was used, which could serve as a carbon source for microorganisms and might contain inducers that were necessary for efficient expression of the BTEX degradation genes, as previously suggested by [17] in their study of methyl *tert*-butyl ether degradation. When yeast extract was added in the MSM, the BTEX degradation was increased. Furthermore, with the amount of yeast extract increased, the BTEX degradation improved markedly, and the BTEX degradation was completely consumed within 32 h in cultures containing 40 mg L^{-1}. A similar finding was reported in a previous study, which showed that the BTEX degradation with yeast extract could accelerate the degradation rate, and the BTEX was completely consumed within 3 days in cultures containing 50 mg L^{-1}

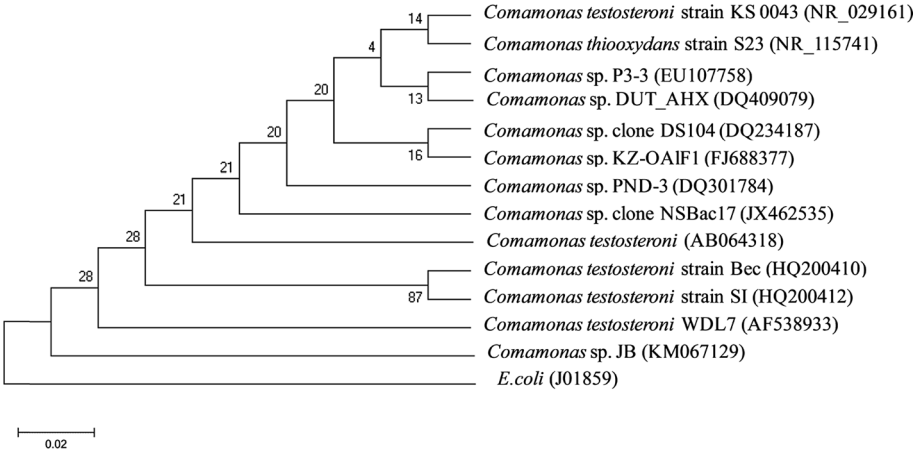

Fig. 1 The tree was constructed and bootstrapped (500 samples) from a sequence alignment of 16S rRNA genes using the neighbor-joining (NJ) method

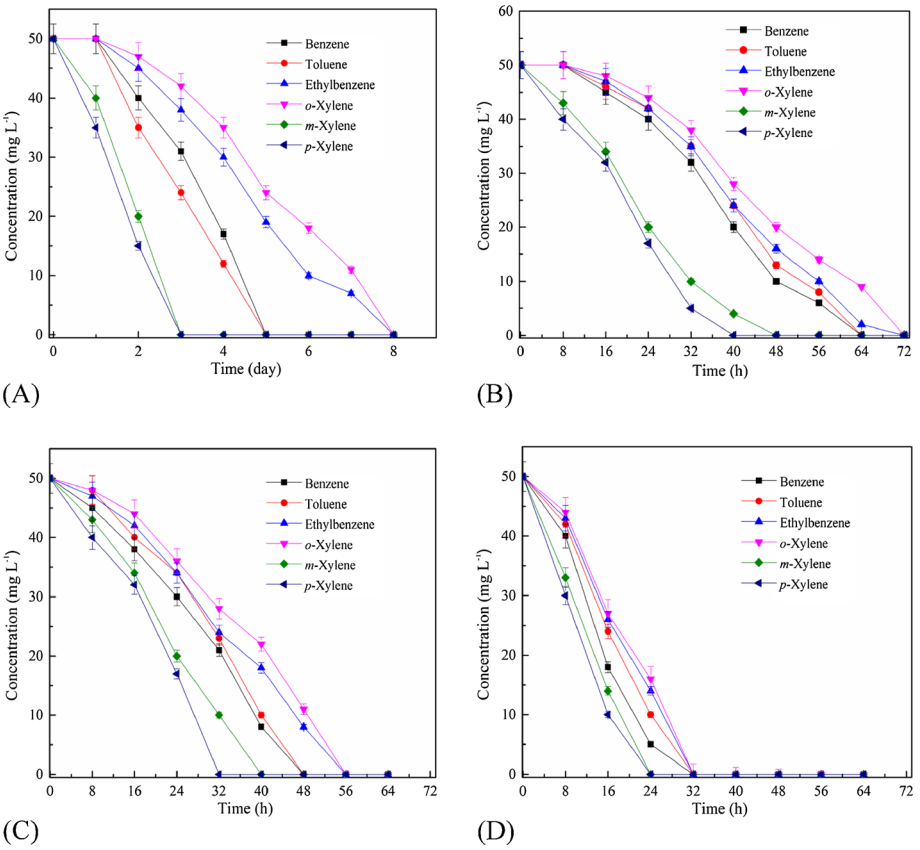

Fig. 2 The effects of yeast extract on degradation of individual BTEX compounds by strain JB in MSM without yeast extract (**a**), with 10 mg L^{-1} yeast extract (**b**), with 20 mg L^{-1} yeast extract (**c**), and with 40 mg L^{-1} yeast extract (**d**)

yeast extract by strain BD-a59 [7]. To further define the degradation relationships among the six BTEX compounds, we determine degradation profiles for each compound in the mixture (1:1:1:1:1:1) in Fig. 3. The BTEX degradation in the mixture was improved as the amount of yeast extract increased, which was the same as those of individual BTEX compounds. The degradation rate of the BTEX mixture also increased with increasing amounts of yeast extract; the BTEX mixture was completely degraded in 40 h at 100 mg L^{-1} yeast extract. These results indicated that strain JB as a pure strain of a bacterium not only showed high degradation activities on each individual BTEX compounds and BTEX mixture, but also could accelerate the degradation rate with additional yeast extract.

BTEX Degradation by BTEX-Grown JB

To further study the BTEX degradation, the effect of individual BTEX concentration on the degradation at a function time was carried out by BTEX-grown JB. BTEX-grown JB cell suspensions can degrade BTEX quickly with the concentration of each substrate increasing from 25 to 150 mg L^{-1}, especially for *m*- and *p*-xylenes. The relationships between specific degradation

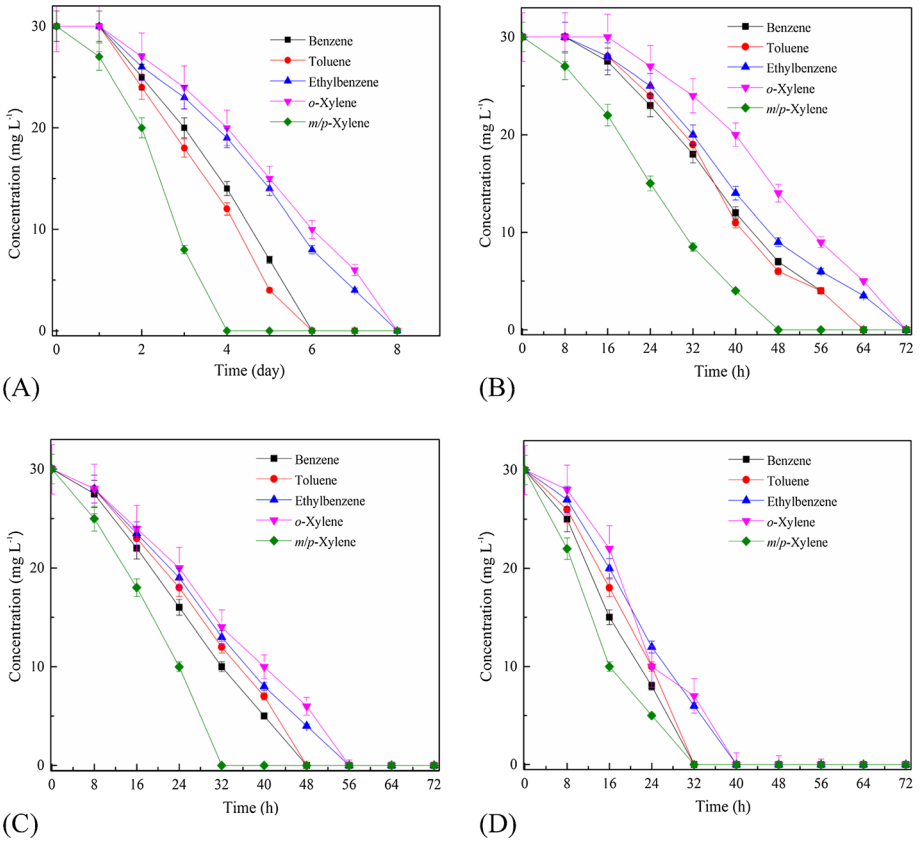

Fig. 3 The effects of yeast extract on degradation of a BTEX mixture by strain JB in MSM without yeast extract (**a**), with 20 mg L^{-1} yeast extract (**b**), with 40 mg L^{-1} yeast extract (**c**), and with 80 mg L^{-1} yeast extract (**d**)

rate and initial BTEX concentration are also described well with Michaelis-Menten kinetics by GraphPad Prism 5 software. The kinetic constants estimated from the experiment data are shown in Table 1. BTEX-grown JB cell suspensions exhibited the highest degradation rate (V_{max} 9.452 mg g $cell^{-1}$ h^{-1}) for *p*-xylene, which was degraded at a rate of 1.529, 1.60, 1.463, 1.83, and 1.008-fold higher than the degradation of benzene, toluene, ethylbenzene, *m*-xylene, and *o*-xylene, respectively (Table 1). Likewise, the specific affinity (V_{max}/K_m) obtained for every individual BTEX component suggested that BTEX-grown W1 cell suspensions possessed the highest efficiency for *p*-xylene, followed by *m*-xylene, benzene, ethylbenzene, toluene, and finally *o*-xylene, which was slightly, hardly degraded. This phenomenon was significantly different from the previous report on the BTEX degradation by fungus *Cladophialophora* sp. strain T1 [16]. In that study, toluene and ethylbenzene were consumed preferentially, followed by *o*-, *m*-, and *p*-xylenes and finally benzene, which could not be degraded.

Petrochemical Wastewater Treatment by BTEX-Grown JB

In this study, the petrochemical wastewater from northeast China with diluting 1:1 (*v*/*v*) was used, and benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylenes were added to give

Table 1 The kinetic parameters of BTEX degradation by Michaelis-Menten kinetics

Substrate	V_{max} (mg g cell^{-1} h^{-1})	K_m (mg L^{-1})	V_{max}/K_m (L g cell^{-1} h^{-1})	R^2
Benzene	6.813	50.63	0.1346	0.9068
Toluene	5.908	48.64	0.1215	0.9106
Ethylbenzene	6.462	49.35	0.1309	0.9035
o-Xylene	5.165	48.26	0.1070	0.9189
m-Xylene	9.376	59.98	0.1564	0.9200
p-Xylene	9.452	57.59	0.1641	0.8989

prominence to bioremediation. Nevertheless, phenol was always the major organic compounds in the petrochemical wastewater, and 100 mg L^{-1} phenol was detected in this petrochemical wastewater. Strain JB could use phenol as a growth substrate (data not shown). The biodegradation of the BTEX mixture and phenol in petrochemical wastewater was conducted by BTEX-grown JB. Figure 4 shows that 100 mg L^{-1} phenol could be degraded completely in 8 h, *m*-/*p*-xylenes were completely consumed in 12 h, and benzene, toluene, ethylbenzene, and *o*-xylene were degraded completely in 32 h by BTEX-grown JB. These results indicated that strain JB exhibited high degradation capability on the petrochemical wastewater containing BTEX mixture and phenol.

Toxicity Assessment (Microtox Test)

In this study, eco-toxicity estimation was conducted by using the Microtox test (bacterium *V. fischeri*) to determine the change in effluent toxicity during the bioremediation of petrochemical wastewater by strain JB as previously described [15]. The influent contained high levels of BTEX, and phenol had high toxicity against strain *V. fischeri* as indicated by IR value that exceeded 95 %. When petrochemical wastewater was incubated with strain JB, the IR

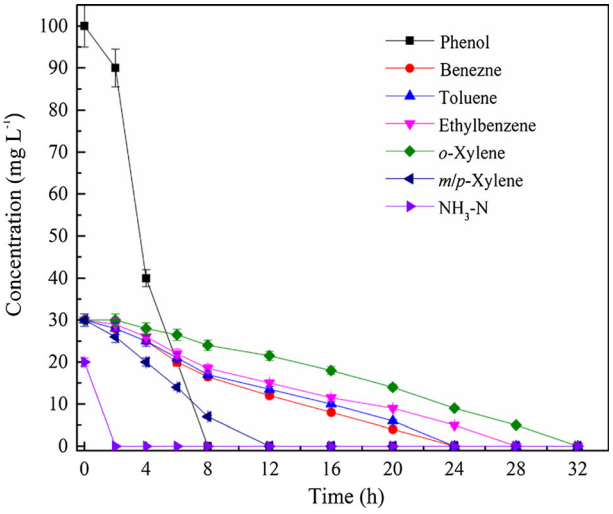

Fig. 4 The degradation of petrochemical wastewater containing phenol, benzene, toluene, ethylbenzene, *o*-xylene, and *m*/*p*-xylene by BTEX-grown JB

value of effluent was sharply decreased from 95 to (high toxicity) 35 % (moderate toxicity) within 16 h. This may be due to degradation of both the tested pollutants (BTEX mixture and phenol) and other pollutants in the petrochemical wastewater. It indicated that the bioremediation of the petrochemical wastewater containing BTEX mixture and phenol by strain JB led to less toxicity than the untreated petrochemical wastewater. These results confirmed that *Comamonas* sp. JB could be used as a promising biocatalyst for the bioremediation of the petrochemical coking wastewater.

Conclusions

In this study, we isolated a novel bacterium JB belonging to the genus *Comamonas* that had the high ability to degrade all the six BTEX components. A small amount of yeast extract significantly enhanced BTEX degradation by strain JB. The degradation kinetics could be described by Michaelis-Menten equation. Strain JB exhibited high degradation capability on the petrochemical wastewater containing BTEX mixture and phenol. Thus, this bacterium may have been a prominent BTEX degrader and shows the potential applications for the bioremediation of petrochemical wastewater.

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References

1. Assinder, S. J., & Williams, P. A. (1990). The TOL plasmids: determinants of the catabolism of toluene and the xylenes. *Advances in Microbial Physiology, 31*, 1–69.
2. Chang, J. H., Chang, Y. K., Ryu, H. W., & Chang, H. N. (2000). Desulfurization of light gas oil in immobilized-cell systems of *Gordona* sp. CYKS1 and *Nocardia* sp. CYKS2. *FEMS Microbiology Letters, 82*, 309–312.
3. Rahul, Mathur, A. K., & Balomajumder, C. (2013). Biological treatment and modeling aspect of BTEX abatement process in a biofilter. *Bioresource Technology, 142*, 9–17.
4. Jennifer, V. L., & Andrew, J. D. (2008). Kinetics and interactions of BTEX compounds during degradation by a bacterial consortium. *Process Biochemistry, 43*, 1068–1076.
5. Costa, A. S., Romão, L. P., Araújo, B. R., Lucas, S. C., Maciel, S. T., Wisniewski, A., Jr., & Alexandre, M. R. (2012). Environmental strategies to remove volatile aromatic fractions (BTEX) from petroleum industry wastewater using biomass. *Bioresource Technology, 105*, 31–39.
6. Dean, B. J. (1985). Recent findings on the genetic toxicology of benzene, toluene, xylenes and phenols. *Mutation Research, 154*, 153–181.
7. Kim, J. M., Le, N. T., Chung, B. S., Park, J. H., Bae, J. W., Madsen, E. L., & Jeon, C. O. (2008). Influence of soil components on the biodegradation of benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylenes by the newly isolated bacterium *Pseudoxanthomonas spadix* BD-a59. *Applied and Environmental Microbiology, 74*, 7313–7320.
8. Yadav, J. S., & Reddy, C. A. (1993). *Applied and Environmental Microbiology, 59*, 756–762.
9. Chakraborty, R., O'Connor, S. M., Chan, E., & Coates, J. D. (2005). Anaerobic degradation of benzene, toluene, ethylbenzene, and xylene compounds by *Dechloromonas* strain RCB. *Applied and Environmental Microbiology, 71*, 8649–8655.
10. Kim, D., Kim, Y. S., Kim, S. K., Kim, S. W., Zylstra, G. J., Kim, Y. M., & Kim, E. (2002). Monocyclic aromatic hydrocarbon degradation by *Rhodococcus* sp. strain DK17. *Applied and Environmental Microbiology, 68*, 3270–3278.

11. Shinoda, Y., Sakai, Y., Uenishi, H., Uchihashi, Y., Hiraishi, A., Yukawa, H., Yurimoto, H., & Kato, N. (2004). Aerobic and anaerobic toluene degradation by a newly isolated denitrifying bacterium, *Thauera* sp. strain DNT-1. *Applied and Environmental Microbiology, 70*, 1385–1392.
12. Zylstra, G. J., & Kim, E. (1997). Aromatic hydrocarbon degradation by *Sphingomonas yanoikuyae* B1. *Journal of Industrial Microbiology and Biotechnology, 19*, 408–414.
13. Jin, H. M., Choi, E. J., & Jeon, C. O. (2013). Isolation of a BTEX-degrading bacterium, *Janibacter* sp. SB2, from a sea-tidal flat and optimization of biodegradation conditions. *Bioresource Technology, 145*, 57–64.
14. Kim, J. M., & Jeon, C. O. (2009). Isolation and characterization of a new benzene, toluene, and ethylbenzene degrading bacterium, *Acinetobacter* sp. B113. *Current Microbiology, 58*, 70–75.
15. Shi, S. N., Qu, Y. Y., Ma, F., & Zhou, J. T. (2014). Bioremediation of coking wastewater containing carbazole, dibenzofuran, dibenzothiphene and naphthalene by a naphthalene-cultivated *Arthrobacter sp*. W1. *Bioresource Technology, 164*, 28–33.
16. Prenafeta-Boldú, F. X., Vervoort, J., Grotenhuis, J. T., & Van Groenestijn, J. W. (2002). Substrate interactions during the biodegradation of benzene, toluene, ethylbenzene, and xylene (BTEX) hydrocarbons by the fungus *Cladophialophora* sp. strain T1. *Applied and Environmental Microbiology, 8*, 2660–2665.
17. Hatzinger, P. B., McCalay, K., Vainberg, S., Tugusheva, M., Condee, C. W., & Steffan, R. J. (2001). Biodegradation of methyl tert-butyl ether by a pure bacterial culture. *Applied and Environmental Microbiology, 67*, 5601–5607.