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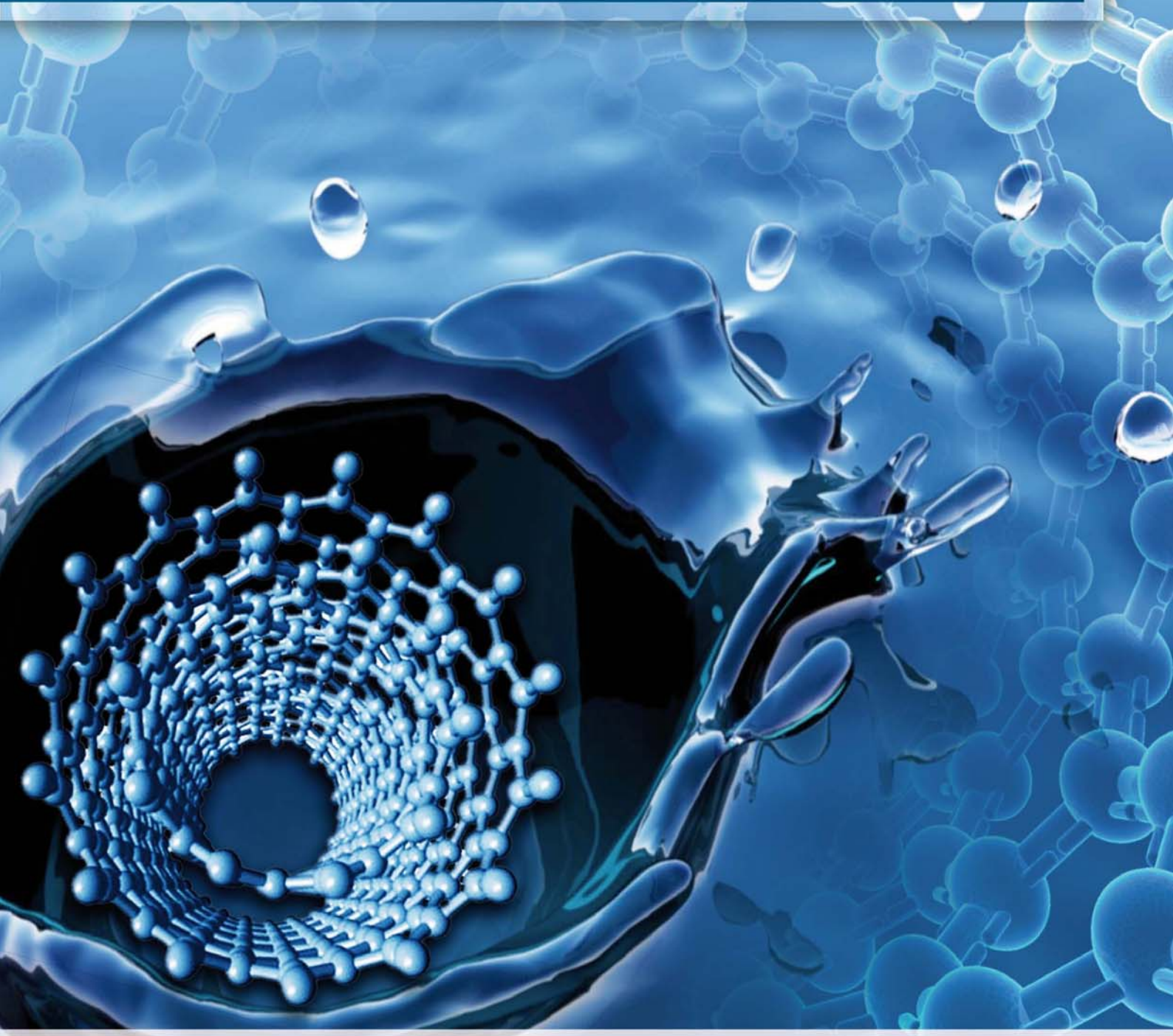

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CONTENTS

Aquatic environment

Application potential of carbon nanotubes in water treatment: A review
Xitong Liu, Mengshu Wang, Shujuan Zhang, Bingcai Pan ····· 1263

Characterization, treatment and releases of PBDEs and PAHs in a typical municipal sewage treatment plant situated beside an urban river, East China
Xiaowei Wang, Beidou Xi, Shouliang Huo, Wenjun Sun, Hongwei Pan, Jingtian Zhang, Yuqing Ren, Hongliang Liu ····· 1281

Factors influencing antibiotics adsorption onto engineered adsorbents
Mingfang Xia, Aimin Li, Zhaolian Zhu, Qin Zhou, Weiben Yang ····· 1291

Assessment of heavy metal enrichment and its human impact in lacustrine sediments from four lakes in the mid-low reaches of the Yangtze River, China
Haijian Bing, Yanhong Wu, Enfeng Liu, Xiangdong Yang ····· 1300

Biodegradation of 2-methylquinoline by *Enterobacter aerogenes* TJ-D isolated from activated sludge
Lin Wang, Yongmei Li, Jingyuan Duan ····· 1310

Inactivation, reactivation and regrowth of indigenous bacteria in reclaimed water after chlorine disinfection of a municipal wastewater treatment plant
Dan Li, Siyu Zeng, April Z. Gu, Miao He, Hanchang Shi ····· 1319

Photochemical degradation of nonylphenol in aqueous solution: The impact of pH and hydroxyl radical promoters
Aleksandr Dulov, Niina Dulova, Marina Trapido ····· 1326

A pilot-scale study of cryolite precipitation from high fluoride-containing wastewater in a reaction-separation integrated reactor
Ke Jiang, Kanggen Zhou, Youcai Yang, Hu Du ····· 1331

Atmospheric environment

Effect of phosphogypsum and dicyandiamide as additives on NH_3, N_2O and CH_4 emissions during composting
Yiming Luo, Guoxue Li, Wenhai Luo, Frank Schuchardt, Tao Jiang, Degang Xu ····· 1338

Evaluation of heavy metal contamination hazards in nuisance dust particles, in Kurdistan Province, western Iran
Reza Bashiri Khuzestani, Bubak Souri ····· 1346

Terrestrial environment

Utilizing surfactants to control the sorption, desorption, and biodegradation of phenanthrene in soil-water system
Haiwei Jin, Wenjun Zhou, Lizhong Zhu ····· 1355

Detoxifying PCDD/Fs and heavy metals in fly ash from medical waste incinerators with a DC double arc plasma torch
Xinchao Pan, Jianhua Yan, Zhengmiao Xie ····· 1362

Role of sorbent surface functionalities and microporosity in 2,2′,4,4′-tetrabromodiphenyl ether sorption onto biochars
Jia Xin, Ruilong Liu, Hubo Fan, Meilan Wang, Miao Li, Xiang Liu ····· 1368

Environmental biology

Systematic analysis of microfauna indicator values for treatment performance in a full-scale municipal wastewater treatment plant
Bo Hu, Rong Qi, Min Yang ····· 1379

Function of *arsATorf7orf8* of *Bacillus* sp. CDB3 in arsenic resistance
Wei Zheng, James Scifleet, Xuefei Yu, Tingbo Jiang, Ren Zhang ····· 1386

Enrichment, isolation and identification of sulfur-oxidizing bacteria from sulfide removing bioreactor
Jianfei Luo, Guoliang Tian, Weitie Lin ····· 1393

Environmental health and toxicology

In vitro immunotoxicity of untreated and treated urban wastewaters using various treatment processes to rainbow trout leucocytes
François Gagné, Marlène Fortier, Michel Fournier, Shirley-Anne Smyth ····· 1400
Using lysosomal membrane stability of haemocytes in *Ruditapes philippinarum* as a biomarker of cellular stress to assess contamination by caffeine, ibuprofen, carbamazepine and novobiocin
Gabriela V. Aguirre-Martínez, Sara Buratti, Elena Fabbri, Angel T. DelValls, M. Laura Martín-Díaz ····· 1408

Environmental catalysis and materials

Effect of transition metal doping under reducing calcination atmosphere on photocatalytic property of TiO_2 immobilized on SiO_2 beads
Rumi Chand, Eiko Obuchi, Katsumi Katoh, Hom Nath Luitel, Katsuyuki Nakano ····· 1419
A high activity of Ti/SnO_2-Sb electrode in the electrochemical degradation of 2,4-dichlorophenol in aqueous solution
Junfeng Niu, Dusmant Maharana, Jiale Xu, Zhen Chai, Yueping Bao ····· 1424
Effects of rhamnolipid biosurfactant JBR425 and synthetic surfactant Surfynol465 on the peroxidase-catalyzed oxidation of 2-naphthol
Ivanec-Goranina Rūta, Kulys Juozas ····· 1431

The 8th International Conference on Sustainable Water Environment

An novel identification method of the environmental risk sources for surface water pollution accidents in chemical industrial parks
Jianfeng Peng, Yonghui Song, Peng Yuan, Shuhu Xiao, Lu Han ····· 1441
Distribution and contamination status of chromium in surface sediments of northern Kaohsiung Harbor, Taiwan
Cheng-Di Dong, Chiu-Wen Chen, Chih-Feng Chen ····· 1450
Historical trends in the anthropogenic heavy metal levels in the tidal flat sediments of Lianyungang, China
Rui Zhang, Fan Zhang, Yingjun Ding, Jinrong Gao, Jing Chen, Li Zhou ····· 1458
Heterogeneous Fenton degradation of azo dyes catalyzed by modified polyacrylonitrile fiber Fe complexes: QSPR (quantitative structure peorperty relationship) study
Bing Li, Yongchun Dong, Zhizhong Ding ····· 1469
Rehabilitation and improvement of Guilin urban water environment: Function-oriented management
Yuansheng Pei, Hua Zuo, Zhaokun Luan, Sijia Gao ····· 1477
Adsorption of Mn^{2+} from aqueous solution using Fe and Mn oxide-coated sand
Chi-Chuan Kan, Mannie C Aganon, Cybelle Morales Futalan, Maria Lourdes P Dalida ····· 1483
Degradation kinetics and mechanism of trace nitrobenzene by granular activated carbon enhanced microwave/hydrogen peroxide system
Dina Tan, Honghu Zeng, Jie Liu, Xiaozhang Yu, Yanpeng Liang, Lanjing Lu ····· 1492

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Biodegradation of 2-methylquinoline by *Enterobacter aerogenes* TJ-D isolated from activated sludge

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Abstract

Bacterial strain *Enterobacter aerogenes* TJ-D capable of utilizing 2-methylquinoline as the sole carbon and energy source was isolated from acclimated activated sludge under denitrifying conditions. The ability to degrade 2-methylquinoline by *E. aerogenes* TJ-D was investigated under denitrifying conditions. Under optimal conditions of temperature (35°C) and initial pH 7, 2-methylquinoline of 100 mg/L was degraded within 176 hr. The degradation of 2-methylquinoline by *E. aerogenes* TJ-D could be well described by the Haldane model ($R^2 > 0.91$). During the degradation period of 2-methylquinoline (initial concentration 100 mg/L), nitrate was almost completely consumed (the removal efficiency was 98.5%), while nitrite remained at low concentration (< 0.62 mg/L) during the whole denitrification period. 1,2,3,4-Tetrahydro-2-methylquinoline, 4-ethyl-benzenamine, N-butyl-benzenamine, N-ethyl-benzenamine and 2,6-diethyl-benzenamine were metabolites produced during the degradation. The degradation pathway of 2-methylquinoline by *E. aerogenes* TJ-D was proposed. 2-Methylquinoline is initially hydroxylated at C-4 to form 2-methyl-4-hydroxy-quinoline, and then forms 2-methyl-4-quinolinol as a result of tautomerism. Hydrogenation of the heterocyclic ring at positions 2 and 3 produces 2,3-dihydro-2-methyl-4-quinolinol. The carbon-carbon bond at position 2 and 3 in the heterocyclic ring may cleave and form 2-ethyl-N-ethyl-benzenamine. Tautomerism may result in the formation of 2,6-diethyl-benzenamine and N-butyl-benzenamine. 4-Ethyl-benzenamine and N-ethyl-benzenamine were produced as a result of losing one ethyl group from the above molecules.

Key words: biodegradation; *Enterobacter aerogenes* TJ-D; 2-methylquinoline; denitrifying conditions; activated sludge

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Introduction

Quinolines are typical N-heterocyclic aromatic compounds widely existing in oil shale, coal processing, creosote wood preservation and fossil fuel facilities (Pereira et al., 1983; Godsy et al., 1992; Mundt and Hollender, 2005; Padoley et al., 2008; Qiao and Wang, 2010). Moreover, they are widely used as solvents in chemical industrial processes such as the synthesis of quinoline dyes and pharmaceuticals (Fetzner et al., 1998). Because of the lone-pair electrons on N-atoms in the ring system, quinolines and other N-heterocyclic compounds are more polar than homocyclic analogues, and thus the water solubility of wastes from these industries are markedly higher, resulting in a lower tendency to be absorbed on organic constituents of soil and aquifer materials. Quinolines have already been documented as toxic pollutants by the United States Environmental Protection Agency (Richards and Shieth, 1986) due to their carcinogenic and mutagenic properties (Wang et al., 2002; Neuwoehner et al., 2009; Bai et al., 2010). Therefore, it is important to understand the transport and fate of quinolines in the environment.

Considerable efforts have been invested in the isolation of microorganisms capable of degrading quinolines. Under aerobic conditions, Shukla (1984) isolated *Pseudomonas* sp. from sewage to degrade quinoline, and found that 8-hydroxycoumarin was an important intermediate in degradation by *Pseudomonas* sp. Aislabie et al. (1989) successfully isolated an *Acinetobacter* strain from oil- and creosote-contaminated soils to degrade isoquinoline. Later, Aislabie et al. (1990) found that *Pseudomonas aeruginosa* QP and *Pseudomonas putida* QP hydroxylated a limited number of methylquinolines, but could not degrade them, nor could they transform 2-methylquinoline, isoquinoline, and pyridine. Moreover, they found *Pseudomonas* sp. MQP could degrade 2-methylquinoline. *Pseudomonas aeruginosa* QP was able to degrade or transform quinoline and a few methylquinolines in a complex heterocyclic nitrogen-containing fraction of a shale oil. Sutton et al. (1996) isolated *Pseudomonas putida* QP from

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the consortium, which could utilize 4-methylquinoline as the carbon source and energy source. During the degradation of 4-methylquinoline by the isolated strain, they found two important intermediates: 2-hydroxy-4-methylquinoline and hydroxy-4-methylcoumarin. Kilbane et al. (2000) isolated *Pseudomonas ayucida* IGTN9m from petroleum-contaminated soil and water samples utilizing quinoline as the sole nitrogen source but not carbon source. They found that *Pseudomonas ayucida* IGTN9m converted quinoline to 2-quinolinone and subsequently to 8-hydroxycoumarin. Moreover, they suggested *Pseudomonas ayucida* IGTN9m was useful in petroleum biorefining for selective removal of organically-bound nitrogen from petroleum. In another study, Sugaya et al. (2001) isolated *Comamonas* sp. TKV3-2-1 that utilized quinoline as the sole carbon and nitrogen source. The strain degraded quinoline to 2-hydroxyquinoline and finally to water-soluble substances. *Rhodococcus* sp. QL2 was another strain in activated sludge of a coke plant wastewater treatment process (Zhu et al., 2008). It utilized quinoline as the sole source of carbon, nitrogen and energy. The quinoline degradation pathway by *Rhodococcus* sp. QL2 was unique, via 5, 6-dihydroxy-1H-2-oxoquinoline and 8-hydroxycoumrain pathways simultaneously. A *Pseudomonas* strain from coking wastewater treatment plant also degraded quinoline (Sun et al., 2009) with at least 43% of quinoline transformed into 2-hydroxyquinoline, of which 0.69% was transformed into 2,8-dihydroxyquinoline, and then presumably into 8-hydroxy-coumarin. Meanwhile, at least 48% of the nitrogen in quinoline was directly transformed into ammonia-N. Addition of another carbon source enhanced nitrogen transformation from ammonia-N. The study also indicated that quinoline and its metabolic products could be eliminated from wastewater by controlling the COD/NO_3^--N ratio. Hund et al. (1990) and Bauer et al. (1994) used *Arthrobacter* sp. Rü61a to degrade 2-methylquinoline, whereas Dembek et al. (1989) and Bott et al. (1990) used *Pseudomonas putida* 33/1 to degrade 2-methylquinoline. They found a similar degradation pathway: 2-methylquinoline was first transformed to 2-methyl-4(1H)-quinolinone, and then to 3-hydroxy-2-methyl-4(1H)quinolinone, N-acetylanthranilic acid and anthranilic acid, and finally to catechol. Under anaerobic conditions, Johansen et al. (1997) reported the transformation of quinoline, isoquinoline, and 3-, 4-, 6-, 8-methylquinoline by *Desulfobacterium indolicum* (DSM 3383), while Reineke et al. (2008) observed the transformation of 3-, 4-, 6-, 7- and 8-methylquinoline. They both indicated that 2-methylquinoline was not degradable under anaerobic conditions. However, our recent studies (Li et al., 2010; Wang et al., 2010) suggested that 2-methylquinoline could be transformed by acclimated activated sludge under denitrifying conditions.

In this article, bacterial strain *Enterobacter aerogenes* TJ-D capable of degrading 2-methylquinoline was isolated from activated sludge that had already been acclimated under denitrifying conditions for more than one year. It was identified based on 16S rDNA sequencing analysis. The degradation of 2-methylquinoline by the isolated strain was investigated under denitrifying conditions and as effected by temperature and pH. At the optimal temperature and pH conditions, nitrate and nitrite were traced during the degradation of 2-methylquinoline simultaneously. Moreover, we identified metabolites during the degradation of 2-methylquinoline under denitrifying conditions.

1 Materials and methods

1.1 Chemicals and culture medium

2,6-Diethyl-benzenamine was purchased from Chem Service, Inc. (USA). 4-Ethyl-benzenamine, N-ethyl-benzenamine and N-butyl-benzenamine were purchased from Aldrich (USA). 1,2,3,4-Tetrahydro-2-methylquinoline was purchased from Tokyo Chemical Industry Co., Ltd. (Japan). The above chemicals were used as standards for the identification of metabolites produced during the biodegradation of 2-methylquinoline. 2-Methylquinoline used for bacterial growth and degradation experiments was purchased from Sigma Chemical Co. (USA).

The strain was grown on mineral salt medium prepared with distilled water and 2-methylquinoline as the sole carbon and energy source under denitrifying conditions. One liter of mineral salt medium (DMSM) contained 2.65 g KH_2PO_4, 4.26 g $Na_2HPO_4{\cdot}12H_2O$, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.02 g $FeSO_4{\cdot}7H_2O$, 1.0 g NaCl and 1 mL of trace element stock solution (0.2 g $CaCl_2$, 0.002 g $MnSO_4{\cdot}7H_2O$, 0.002 g $ZnSO_4{\cdot}7H_2O$, 0.03 g $CuSO_4{\cdot}7H_2O$, 0.09 g $CoCl_2{\cdot}6H_2O$ and 0.06 g $Na_2MoO_4{\cdot}\ 2H_2O$ per liter of distilled water). $NaNO_3$ was added according to the input concentration of 2-methylquinoline, as the COD/NO_3^--N was controlled at 25. The solid medium contained 1.5% (*W/V*) agar. All media were sterilized at 121°C for 20 min before use.

1.2 Isolation of bacterial strain

The inoculant was obtained from an anoxic bioreactor under denitrifying conditions (mixed liquor suspended solid = 3150 mg/L) which had been operated for more than one year with synthetic wastewater containing 2-methylquinoline as the sole carbon source and nitrate as the nitrogen source (Wang et al., 2010). The initial enrichment culture was established in an Erlenmeyer flask (500 mL) by inoculating 8 g activated sludge (wet weight) with 250 mL sterilized DMSM containing 20 mg/L of 2-methylquinoline on a rotary shaker at 30°C (150 r/min). The Erlenmeyer flask was then sealed and the oxygen in it was purged with high purity nitrogen for 20 min. After the fourth day of incubation, 2-methylquinoline contained

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in the Erlenmeyer flask was degraded, and portions were taken to fresh DMSM to continue the incubation. After six more transfers at 96 hr intervals, the culture was purified by serial streak plating onto solidified DMSM containing 2-methylquinoline of 20 mg/L. Finally, a pure strain was obtained and named TJ-D.

1.3 16S rDNA sequencing and phylogenetic tree analysis

The DNA of the isolate was extracted using a commercial genomic DNA extraction kit (BioTeke Corp., China). The isolated genomic DNA was stored at –20°C for further experiments. Afterward, this genomic DNA was used for 16S rDNA analysis. 16S rDNA of the strain was amplified from the bacterial genomic DNA by a polymerase chain reaction (PCR) using universal primers of 5′-GAGCGGATAACAATTTCACACAGG-3′ and 5′-CGCCAGGGTTTTCCCAGTCACGAC-3′. The PCR amplification condition was as follows: each PCR mixture (50 μL) contained 1 μL of primers, 25 μL of PCR premix, 1 μL of 5 units DNA polymerase, and 23 μL of DNA-free grade water. The PCR was performed in a TaKaRa Thermal Cycler Dice TP600 (TaKaRa BIO Inc., USA) and consisted of a denaturation step at 94°C for 5 min, 30 amplification cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1.5 min, followed by a final extension at 72°C for 5 min. The PCR products were visualized on 1.0% agarose gels and the products were excised with TaKaRa Agarose Gel DNA Purification Kit Ver. 2.0 following the manufacturer's instructions. The PCR products were cloned in an ABI PRISM^{TM} 3730XL DNA Sequencer (Applied Biosystems, USA) following the manufacturer's instructions.

The sequences of the strain TJ-D were compared with other related sequences available in National Center for Biotechnology Information (NCBI) databases using the BLAST program (Altschul et al., 1997). Furthermore, the sequences of the strain TJ-D were aligned with closely related sequences found in NCBI using CLUSTAL X, and then calculated using the MEGA 4.0 program. Phylogenetic tree analysis was performed using neighbor-joining methods (Saitou and Nei, 1987).

1.4 Effects of temperature and initial pH on the biodegradation of 2-methylquinoline by the isolate

Effects of temperature and initial pH on the biodegradation of 2-methylquinoline by the isolate were investigated using 500 mL Erlenmeyer flasks. For the degradation test using anoxic isolate (the initial optical density at 600 nm (OD_{600}) of the inoculants was 0.018), 380 mL DMSM containing 50 mg/L of 2-methylquinoline was added to each flask. The flasks were sealed with Teflon-coated silicone septa and oxygen was purged with nitrogen. At the initial pH of 7.0, the effect of temperature on the biodegradation of 2-methylquinoline was examined at 20, 25, 30, 35, and 40°C. Then at the optimal temperature, the effect of the initial pH was investigated at 5.0, 6.0, 7.0, 7.5, 8.0 and 9.0.

After the optimal temperature and initial pH were determined, the degradation rates for 2-methylquinoline by TJ-D were measured under denitrifying conditions. 2-Methylquinoline was spiked into the flasks with the initial concentrations of 20, 40, 60, 80 and 100 mg/L, respectively.

1.5 Analytical methods

To determine the concentration of 2-methylquinoline and the growth of microorganisms, 10 mL of the suspension was taken from each flask at the sampling time using a sterilized syringe. Seven milliliters of the sample was transferred to a gas-tight centrifuge tube and centrifuged at 10000 r/min for 10 min. The supernatant was filtered through a 0.45 μm membrane and 6 mL of the filtrate was transferred to another gas-tight tube for extraction with dichloromethane. The extractions were conducted using 2 mL dichloromethane each time, and the organic phase from all three exactions withdrawn using a syringe was mixed. The extract was then analyzed with a gas chromatograph (GC, Agilent 6890N). The remaining 3 mL of the suspension sample was used for determining the growth of organisms, which was measured by monitoring OD at 600 nm using a spectrophotometer (UV-2600A, Unico, USA). Nitrite and nitrate measurements were performed according to Li et al. (2010).

To trace metabolites during the degradation of 2-methylquinoline, samples (20 mL) were withdrawn periodically (sampling times were 26, 72, 116 and 176 hr under denitrifying conditions) and extracted with dichloromethane three times (6, 7 and 7 mL dichloromethane each time). The extracts were then dried using high purity nitrogen, dissolved in 0.5 mL dichloromethane, and finally analyzed using a Thermo Focus DSQ gas chromatograph coupled with a mass spectrometer (GC-MS). The method of GC-MS analysis was the same as Wang et al. (2010). The mass spectral library database of the National Institute of Standards and Technologies was used to identify compounds from the mass fragment patterns obtained from the GC-MS analysis. Fragment patterns were verified using commercially available standard chemicals.

2 Results and discussion

2.1 Identification of the isolated strain TJ-D degrading 2-methylquinoline

The isolated strain TJ-D was a gram-negative and rod-shaped species. Based on the 16S rDNA sequence amplification and compared with sequences published in the GenBank database, the strain TJ-D was 100% similar to *Enterobacter aerogenes* CTSP48 (EU855221.1). The phylogenetic tree of TJ-D was constructed in **Fig. 1**. The final sequence of 16S rDNA from *Enterobacter aerogenes*

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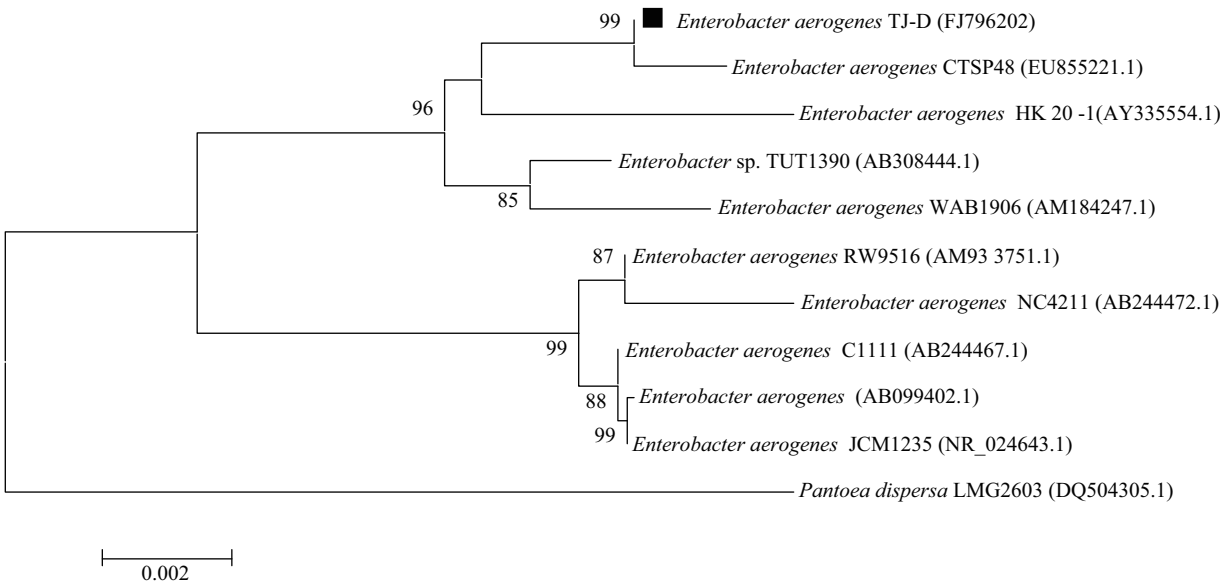

Fig. 1 Phylogenetic tree based on the 16S rDNA sequence of *Enterobacter aerogenes* TJ-D with other reference sequences. The numbers at the forks indicate the bootstrap values in percentage. Bars indicate the nucleotide difference per sequence position. The accession numbers of the sequences are given in parentheses.

TJ-D was submitted to the GenBank under the accession number assigned FJ796202.

2.2 Effect of temperature and initial pH on the biodegradation of 2-methylquinoline by TJ-D

Figure 2a shows the effect of temperature on the biodegradation of 2-methylquinoline by *E. aerogenes* TJ-D under denitrifying conditions. When the temperature was in the range of 20°C–40°C, 2-methylquinoline was well transformed under denitrifying conditions. It needed 126, 116, 96, 80 and 88 hr to remove 95% of 2-methylquinoline when the temperature was at 20°C, 25°C, 30°C, 35°C, and 40°C, respectively. The corresponding degradation rate was 0.0304, 0.0344, 0.041, 0.0509 and 0.0454 hr^{-1} when the temperature was at 20°C, 25°C, 30°C, 35°C, and 40°C, respectively. The results revealed that *E. aerogenes* TJ-D was mesophilic bacteria. The optimal temperature was 35°C for *E. aerogenes* TJ-D. This result is consistent with other researchers (Zhu et al., 2008), who concluded that mesophilic bacteria had advantages in degrading quinolines.

Figure 2b shows the effect of initial pH on the biodegradation of 2-methylquinoline by *E. aerogenes* TJ-D under denitrifying conditions. It was found that the proper initial pH range was 6-8 for 2-methylquinoline degradation by *E. aerogenes* TJ-D under denitrifying conditions. When the initial pH was 6, 7, 7.5 and 8, the removal efficiencies of about 96% were reached at the reaction times of 106, 80, 88 and 96 hr, respectively. At the initial pH of 5 and 9, 156 and 126 hr was required to remove 90% of the 2-methylquinoline. These results are similar to other studies, in which pure cultures were used (Kilbane et al., 2000; Zhu et al., 2008; Sun et al., 2009). The optimal pH was found to be between 6.5 and 7 when quinoline was degraded by *Pseudomonas ayucida* IGTN9m (Kilbane et al., 2000) or 6–8 when it was degraded by *Rhodococcus* sp. (Zhu et al.,

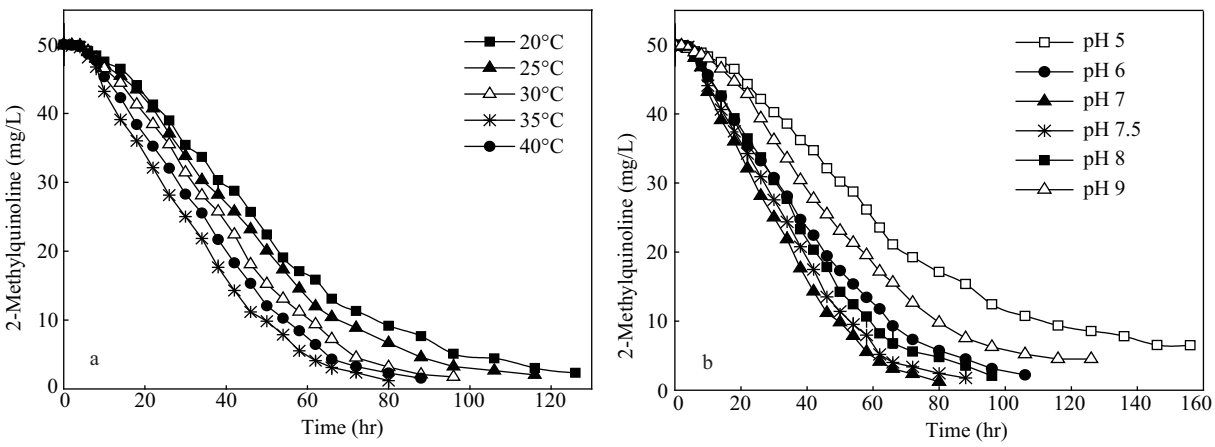

Fig. 2 Effect of temperature (a) and initial pH (b) on the biodegradation of 2-methylquinoline by *E. aerogenes* TJ-D under denitrifying conditions.

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2008) or *Pseudomonas* (Sun et al., 2009).

2.3 Biodegradation of 2-methylquinoline at optimal temperature and initial pH

Degradation of 2-methylquinoline by *E. aerogenes* TJ-D at the optimal temperature and initial pH is shown in **Fig. 3a**. The isolate started with a lag due to adapting to the new environment, quickly followed by rapid degradation of 2-methylquinoline. The degradation rate by *E. aerogenes* TJ-D was slow. Under denitrifying conditions, *E. aerogenes* TJ-D degraded 2-methylquinoline of 20, 40, 60, 80 and 100 mg/L in 42, 66, 96, 126 and 176 hr, respectively. The removal efficiencies were 95.1%, 97.5%, 97.6%, 98.6% and 98.6% for these initial concentrations, respectively.

The growth of the isolate was also determined during the degradation of 2-methylquinoline (**Fig. 3b**). The biomass increased with decreasing concentration of 2-methylquilonline under denitrifying conditions. At higher 2-methylquinoline concentrations, the lag phases were also extended, demonstrating a positive correlation between biomass and 2-methylquinoline degradation. The initial biomass of *E. aerogenes* TJ-D was very low, and increased slowly at first. After a period of acclimation, it grew exponentially. The results also indicated that the maximum biomass concentration rose with the increase of the substrate concentration. When the initial concentration of 2-methylquinoline was 100 mg/L, OD_{600} of the maximum biomass concentration was 0.584 under denitrifying conditions.

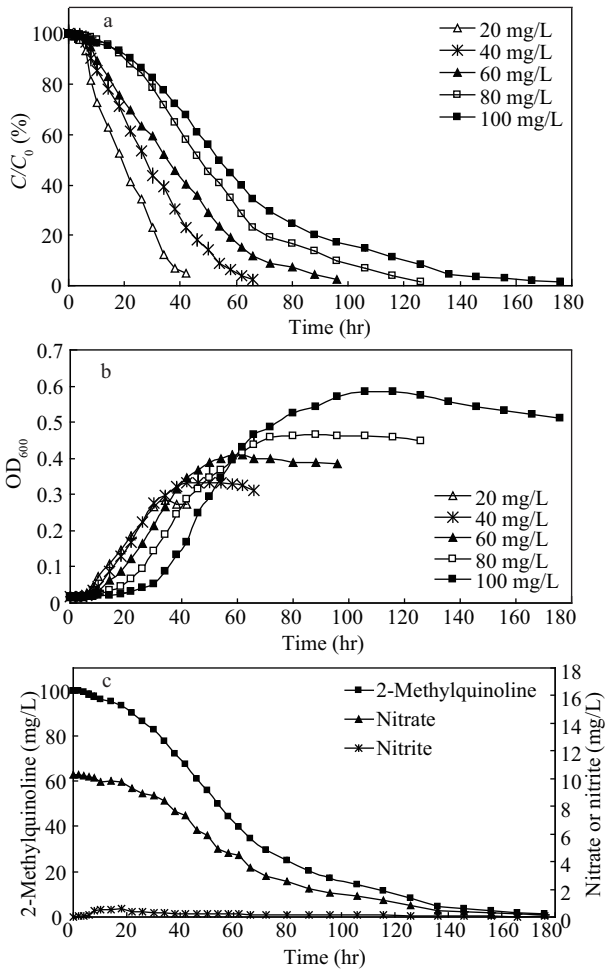

Fig. 3 Degradation of 2-methylquinoline (a) and the variation of growth of *E. aerogenes* (b), 2-methylquionoline, nitrate and nitrite (c) under denitrifying conditions. *C* is actual 2-methylquinoline concentration, and C_0 is initial 2-methylquinoline concentration. Experimental condition: temperature 35°C; pH 7; initial 2-methylquinoline concentration 100 mg/L.

It appeared that *E. aerogenes* TJ-D under denitrifying conditions had strong degradation potential for 2-methylquinoline. Haldane inhibition kinetics was used to describe the substrate degradation:

$$r = \frac{r_{max} S}{K_s + S + (S^2/K_i)} \quad (1)$$

where, r (mg/(L·hr)) is the substrate degradation rate; r_{max} (mg/(L·hr)) is the maximum substrate degradation rate; S (mg/L) is the substrate concentration; K_s (mg/L) is the half saturation constant; K_i (mg/L) is the substrate inhibition constant.

Table 1 shows the kinetic parameters simulated using the experimental data. It appeared that the experimental results could be well described by the Haldane model ($R^2 > 0.92$).

From former research (Li et al., 2010), we found that COD/NO_3^--N influenced the degradation of 2-methylquinoline, and the optimal COD/NO_3^--N ratio was between 21 and 26. Therefore, the COD/NO_3^--N ratio was controlled at 25. When 2-methylquinoline was at 20, 40, 60, 80 and 100 mg/L, the initial nitrate was 2.10, 4.17, 6.23, 8.25, and 10.54 mg/L, respectively. It was found that at the optimal COD/NO_3^--N ratio, nitrate reduction had no relationship with the initial concentration of 2-methylquinoline. Taking the initial concentration of 2-methylquionoline of 100 mg/L as an example, concentration profiles of 2-methylquinoline, nitrate and nitrite are illustrated in **Fig. 3c**. Along with the degradation of 2-methylquinoline, nitrate was almost completely consumed (the removal efficiency was 98.5%), while nitrite remained at low level (<0.62 mg/L) during the denitrification period. This is likely a result of the low initial nitrate concentration (10.54 mg/L) in the experiments.

2.4 Identification of metabolites of 2-methylquinoline by TJ-D

In order to trace the metabolites during the degradation of 2-methylquinoline by *E. aerogenes* TJ-D under denitrifying conditions, a much higher concentration of 2-methylquinoline (150 mg/L) was spiked initially in the experiment. However, *E. aerogenes* TJ-D was inhibited, and 2-methylquinoline could not be degraded. Therefore, 2-methylquinoline was spiked at 100 mg/L in the experiments. Several metabolites were detected in the sample

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taken at 116 hr using GC-MS and the total ion chromatogram (TIC) is shown in **Fig. 4a**. Based on the National Institute of Standards and Technologies Library, they were identified as 1,2,3,4-tetrahydro-2-methylquinoline, 2,6-diethyl-benzenamine and its isomer N-butyl-benzenamine, 4-ethyl-benzenamine and its isomer N-ethyl-benzenamine. These compounds were confirmed by comparison with standard chemicals under the same analytical conditions. **Figure 4b, c** shows the mass spectrum of metabolites N-butyl-benzenamine and N-ethyl-benzenamine produced during the degradation and their corresponding authentic standards. N-butyl-benzenamine was characterized by an M^+, peak at *m/z* 149, a base peak at *m/z* 106 (M-CH_3CHO) and the other main peak at 77 ($C_6H_5^-$). The mass spectrum of N-ethyl-benzenamine was characterized by an M^+, peak at *m/z* 121, a base peak at *m/z* 106 (M-CH_3), and other main peaks at *m/z* 91 (M-$2CH_3$) and 77 ($C_6H_5^-$). The mass spectrum of the other three metabolites (1,2,3,4-tetrahydro-2-methylquinoline, 2,6-diethyl-benzenamine and 4-ethyl-benzenamine) and their authentic standards were published previously (Wang et al., 2010). However, N-butyl-benzenamine and N-ethyl-benzenamine were not observed in the previous studies.

Table 1 Kinetic parameters of the Haldane inhibition model for the biodegradation of 2-methylquinoline using *E. aerogenes* TJ-D

2-Methylquinoline concentration (mg/L)	*E. aerogenes* TJ-D Haldane's model			
	r_{max} (mg/(L·hr))	K_s (mg/L)	K_i (mg/L)	R^2
20	0.035	31.63	135.58	0.9459
40	0.031	32.48	128.84	0.9211
60	0.029	32.67	143.44	0.9662
80	0.031	30.83	137.16	0.9188
100	0.033	27.66	130.27	0.9607

Figure 5 shows the peak area in TIC of the above metabolites during the time course for 2-methylquinoline degradation by *E. aerogenes* TJ-D under denitrifying conditions. The concentration of N-butyl-benzenamine was low during the entire degradation period. It was almost undetectable in the final effluent at 176 hr, indicating its rapid transformation. 4-Ethyl-benzenamine and its isomer N-ethyl-benzenamine appeared soon after the degradation began, and reached a high level at 72 hr; they could be well degraded in the subsequent degradation period. Johansen et al. (1997) found 3,4-dihydro-6-methylquinolinone and 3,4-dihydro-8-methylquinolinone when using *Desulfobacterium indolicum* (DSM3383) to degrade 6- and 8-methylquinoline, respectively; but they did not find the degradation of 2-methylquinoline. They found 2-ethyl-benzenamine was the metabolite of quinoline. In our previous studies (Li et al., 2010; Wang et al., 2010) we found the isomer 4-ethyl-benzenamine

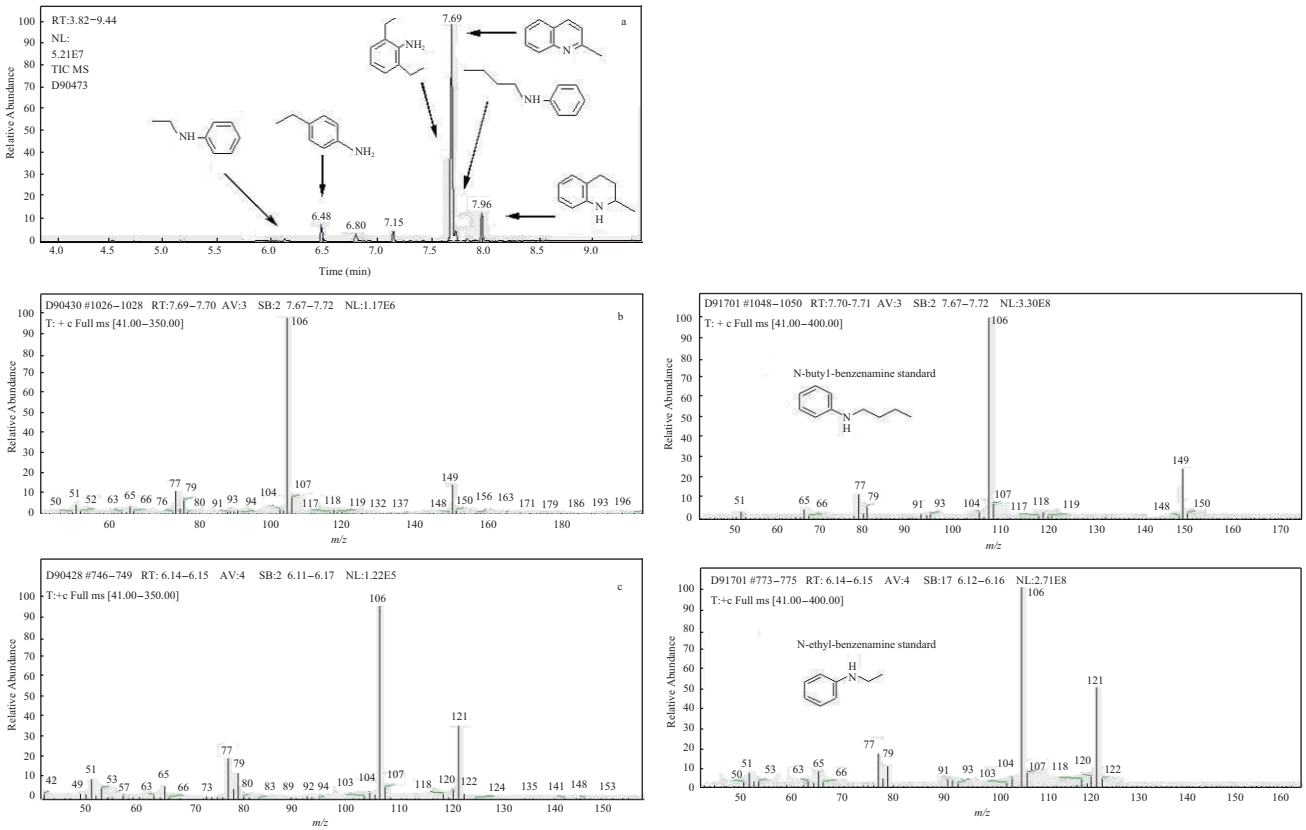

Fig. 4 (a) TIC of the sample taken at 116 hr during the degradation of 2-methylquinoline by *E. aerogenes* TJ-D under denitrifying conditions, (b) and (c) comparisons of mass spectrum data of metabolites during degradation of 2-methylquinoline with authentic standards.

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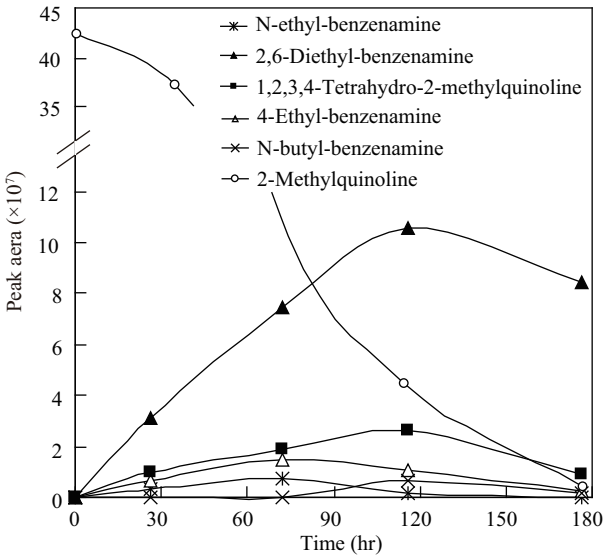

Fig. 5 Peak area of 2-methylquinoline and its metabolites by *E. aerogenes* TJ-D under denitrifying conditions during degradation. The initial concentration of 2-methylquinoline was 100 mg/L.

as the metabolite of 2-methylquinoline for the first time. In this study, we detected another isomer, N-ethyl-benzenamine, as a metabolite of 2-methylquinoline. These results indicate that ethyl-benzenamines may be common metabolites of quinolines. Of all the metabolites, 2,6-diethyl-benzenamine and 1,2,3,4-tetrahydro-2-methylquinoline accumulated to the highest level at 116 hr, and then decreased afterwards. However, they remained in the final effluent, suggesting their persistency in the environment. The recalcitrant characteristics of 1,2,3,4-tetrahydro-2-methylquinoline were consistent with our previous studies (Li et al., 2010; Wang et al., 2010), as it was also detected in the final effluent when 2-methylquinoline was completely degraded by activated sludge. However, our previous research (Wang et al., 2010) found that 2,6-diethyl-benzenamine could be further transformed and did not accumulate in the final effluent. That might be due to some other strains capable of degrading 2,6- diethyl-benzenamine in the activated sludge.

Hund et al. (1990) and Bauer et al. (1994) detected 2-methyl-4-quinolinol when using *Arthrobacter* sp. Rü61a to degrade 2-methylquinoline. A number of other researchers such as Pereira et al. (1983), Johansen et al. (1997), and Reineke et al. (2008) found that 3-, 4-, 6-, 7- and 8-methylquinoline were also transformed to 2-hydroxy-methylquinoline. Thus, methylquinolinol was an important metabolite during the degradation of methylquinolines. In our previous research (Wang et al., 2010) on the degradation of 2-methylquinoline by activated sludge, we also traced 2-methyl-4-quinolinol. However, in this study, it was not detected. One possible reason is that the further transformation of 2-methyl-4-quinolinol to other metabolites by the pure strain was more rapid than by activated sludge. Another reason might be that the sampling volume (20 mL) collected from the Erlenmeyer flask was much less than that collected from the bioreactor (100 mL) with activated sludge, and thus the concentration of metabolites after extraction was too low to be detected. Nevertheless, we believe that 2-methylquinoline was initially hydroxylated at C-4 to form 2-methyl-4-quinolinol. Hund et al. (1990) and Bauer et al. (1994) found that 2-methyl-4-quinolinol further underwent hydroxylation at C-3 to form 3-hydroxy-2-methyl-4-quinolinol. However, in our study, it seems 2-methyl-4-quinolinol was not further oxidized. Furthermore, the reduced forms such as 1,2,3,4-tetrahydro-2-methylquinoline were detected. This was consistent with our previous studies (Li et al., 2010; Wang et al., 2010). It appeared that 2-methyl-4-quinolinol was further hydrogenated at positions 1, 2, 3 and 4, resulting in 1,2,3,4-tetrahydro-2-methylquinoline. Afterwards, the N-heterocyclic ring was cleaved and some alkyl-benzenamines such as 2,6-diethyl-benzenamine and N-butyl-benzenamine were produced. Other studies (Hund et al.,1990; Bauer et al., 1994; Kilbane et al., 2000; Sun et al., 2009) also indicated that the heterocyclic ring was degraded prior to the benzene ring during the degradation of 2-methylquinoline. Moreover, Frerichs-Deeken et al. (2003) and Griese et al. (2006) purified key enzymes involved in the oxidation and cleavage of the N-heterocyclic ring of quinoline. So far, the pathway from 1,2,3,4-tetrahydro-2-methylquinoline to these two alkyl-benzenamine isomers was unclear, and remained to be further investigated. The 4-ethyl-benzenamine and N-ethyl-benzenamine were most likely formed from 4-butyl-benzenamine and N-butyl-benzenamine by losing an ethyl group.

The degradation pathway of 2-methylquinoline by *E. aerogenes* TJ-D is proposed in **Fig. 6**. 2-Methylquinoline is initially hydroxylated at C-4 to form 2-methyl-4-hydroxy-quinoline, and then forms 2-methyl-4-quinolinol as a result of tautomerism. Hydrogenation of the heterocyclic ring at positions 2 and 3 produces 2,3-dihydro-2-methyl-4-quinolinol. The formation of 1,2,3,4-tetrahydro-2-methylquinoline may result from the further hydrogenation at the C=O bond and its subsequent cleavage. The carbon-carbon bond at position 2 and 3 in the heterocyclic ring may cleave and form 2-ethyl-N-ethyl-benzenamine. Tautomerism may result in the formation of 2,6-diethyl-benzenamine and N-butyl-benzenamine. 4-Ethyl-benzenamine and N-ethyl-benzenamine were produced as a result of losing one ethyl group from the above molecules. The degradation pathway presented here is also very similar to the 2-methylquinoline degradation pathway by activated sludge presented in our previous study (Wang et al., 2010). This further confirmed that the bacterial strain *E. aerogenes* TJ-D was the priority species in the activated sludge responsible for the degradation of 2-methylquinoline under denitrifying conditions.

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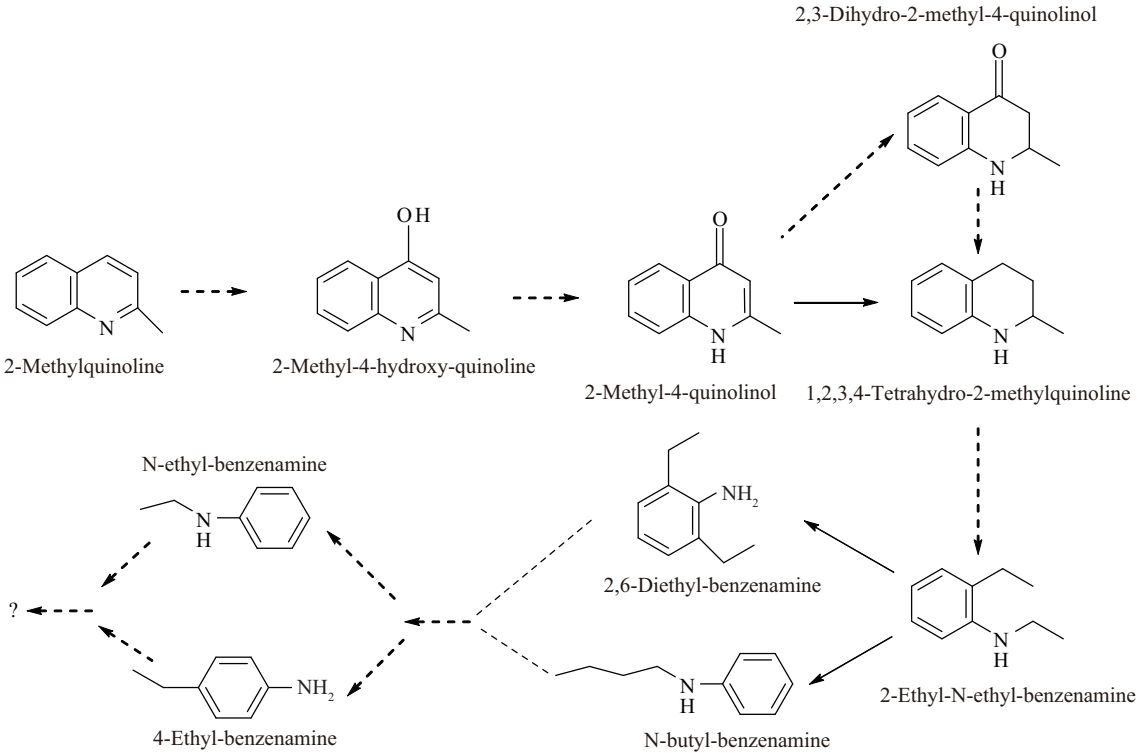

Fig. 6 Proposed degradation pathway of 2-methylquinoline by *E. aerogenes* TJ-D.

3 Conclusions

A bacterial strain degrading 2-methylquinoline under denitrifying conditions was isolated from activated sludge in an anoxic bioreactor, and was identified as *E. aerogenes* TJ-D. The optimal temperature and pH was 35°C and 7.0 for *E. aerogenes* TJ-D. At the optimal temperature and pH, 2-methylquinoline of 100 mg/L was degraded by *Enterobacter aerogenes* TJ-D under denitrifying conditions within 176 hr. The degradation of 2-methylquinoline by *E. aerogenes* TJ-D could be well described by the Haldane model. The optimal kinetic parameters calculated from the experimental data were as follows: r_{max} = 0.029–0.035 mg/(L·hr), K_s = 27.66–32.67 mg/L and K_i = 128.84–143.44 mg/L. Along with the degradation of 2-methylquinoline (initial concentration 100 mg/L), nitrate was almost completely consumed (the removal efficiency was 98.5%), while nitrite remained at low level (< 0.62 mg/L) during the denitrification period. 1,2,3,4-Tetrahydro-2-methylquinoline, 4-ethyl-benzenamine, N-butyl-benzenamine, N-ethyl-benzenamine and 2,6-diethyl-benzenamine were detected during the degradation of 2-methylquinoline under denitrifying conditions.

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References

Aislabie J, Bej A K, Hurst H, Rothenburger S, Atlas R M, 1990. Microbial degradation of quinoline and methylquinolines. *Applied and Environmental Microbiology*, 56(2): 345–351.

Aislabie J, Rothenburger S, Atlas R M, 1989. Isolation of microorganisms capable of degrading isoquinoline under aerobic conditions. *Applied and Environmental Microbiology*, 55(12): 3247–3249.

Altschul S F, Madden T L, Schäffer A A, Zhang J H, Zhang Z, Miller W et al., 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research*, 25(17): 3389–3402.

Bai Y H, Sun Q H, Xing R, Wen D H, Tang X Y, 2010. Removal of pyridine and quinoline by bio-zeolite composed of mixed degrading bacteria and modified zeolite. *Journal of Hazardous Materials*, 181(1-3): 916–922.

Bauer I, de Beyer A, Tshisuaka B, Fetzner S, Lingens F, 1994. A Novel Type of oxygenolytic ring cleavage: 2,4-oxygenation and decarbonylation of 1H-3-hydroxy-4-oxoquinaldine and 1H-3-hydroxy-4-oxoquinoline. *FEMS Microbiology Letters*, 117(3): 299–304.

Bott G, Schmidt M, Rommel T O, Lingens F, 1990. Microbial metabolism of quinoline and related compounds. V. Degradation of 1*H*-4-oxoquinoline by *Pseudomonas putida* 33/l. *Biological Chemistry Hoppe-Seyler*, 371(10): 999–1003.

Dembek G, Rommel T, Lingens F, Hartmut H, 1989. Degradation of quinaldine by *Alcaligenes* sp. and by *Arthrobacter* sp. *FEBS Letters*, 246(1-2): 113–116.

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Fetzner S, Tshisuaka B, Lingens F, Kappl R, Huttermann J, 1998. Bacterial degradation of quinoline and derivatives–Pathways and their biocatalysts. *Angewandte Chemie International Edition*, 37(5): 576–597.

Frerichs-Deeken U, Goldenstedt B, Gahl-Janßen R, Kappl R, Hüttermann J, Fetzner S, 2003. Functional expression of the quinoline 2-oxidoreductase genes (qorMSL) in *Pseudomonas putida* KT2440 pUF1 and in *P. putida* 86-1Δqor pUF1 and analysis of the Qor proteins. *European Journal of Biochemistry*, 270(7): 1567–1577.

Godsy E M, Goerlitz D F, Grbie-Galic D, 1992. Methanogenic biodegradation of creosote contaminants in natural and simulated ground-water ecosystems. *Ground Water*, 30(2): 232–242.

Griese J J, Jakob R P, Schwarzinger S, Dobbek H, 2006. Xenobiotic reductase A in the degradation of quinoline by *Pseudomonas putida* 86: physiological function, structure and mechanism of 8-hydroxycoumarin reduction. *Journal of Molecular Biology*, 361(1): 140–152.

Hund H, Beyer A, Lingens F, 1990. Degradation of quinaldine by *Arthrobacter* sp. *Biological Chemistry Hoppe-Seyler*, 371(2): 1005–1008.

Johansen S S, Licht D, Arvin E, Mosbaek H, Hansen A B, 1997. Metabolic pathways of quinoline, indole and their methylated analogs by *Desulfobacterium indolicum* (DSM 3383). *Applied and Environmental Microbiology*, 47(3): 292–300.

Kilbane J J, Ranganathan R, Cleveland L, Kayser K J, Ribiero C, Linhares M M, 2000. Selective removal of nitrogen from quinoline and petroleum by *Pseudomonas ayucida* IGTN9m. *Applied and Environmental Microbiology*, 66(2): 688–693.

Li Y M, Wang L, Liao L S, Sun L J, Zheng G H, Luan J F et al., 2010. Nitrate-dependent biodegradation of quinoline, isoquinoline, and 2-methylquinoline by acclimated activated sludge. *Journal of Hazardous Materials*, 173(1-3): 151–158.

Mundt M, Hollender J, 2005. Simultaneous determination of NSO-heterocycles, homocycles and their metabolites in groundwater of tar oil contaminated sites using LC with diode array UV and fluorescence detection. *Journal of Chromatography A*, 1065(2): 211–218.

Neuwoehner J, Reineke A K, Hollender J, Eisentraeger A, 2009. Ecotoxicity of quinoline and hydroxylated derivatives and their occurrence in groundwater of a tar-contaminated field site. *Ecotoxicology and Environmental Safety*, 72(3): 819–827.

Padoley K V, Mudliar S N, Pandey R A, 2008. Heterocyclic nitrogenous pollutants in the environment and their treatment options–an overview. *Bioresource Technology*, 99(10): 4029–4043.

Pereira W E, Rostad C E, Garbarino J R, Hult M F, 1983. Groundwater contamination by organic bases derived from coal-tar wastes. *Environmental Toxicology and Chemistry*, 2: 283–294.

Qiao L, Wang J L, 2010. Biodegradation characteristics of quinoline by *Pseudomonas putida*. *Bioresource Technology*, 101(19): 7683–7686.

Reineke A K, Preiss A, Elend M, Hollender J, 2008. Detection of methylquinoline transformation products in microcosm experiments and in tar oil contaminated groundwater using LC-NMR. *Chemosphere*, 70(11): 2118–2126.

Richards D J, Shieth W K, 1986. Biological fate of organic priority pollutants in the aquatic environment. *Water Research*, 20(9): 1077–1090.

Saitou N, Nei M, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4(4): 406–425.

Shukla O P, 1984. 8-Hydroxycoumarin: an intermediate in the microbial transformation of quinoline. *Current Science*, 53: 1145–1147.

Sugaya K, Nakayama O, Hinata N, Kamekura K, Ito A, Yamagiwa K et al., 2001. Biodegradation of quinoline in crude oil. *Journal of Chemical Technology and Biotechnology*, 76(6): 603–611.

Sun Q H, Bai Y H, Zhao C, Xiao Y N, Wen D H, Tang X Y, 2009. Aerobic biodegradation characteristics and metabolic products of quinoline by a *Pseudomonas* strain. *Bioresource Technology*, 100(21): 5030–5036.

Sutton S D, Pfaller S L, Shann J R, Warshawskii D, Kinkle B K, Vestal J R, 1996. Aerobic biodegradation of 4-methylquinoline by a soil bacterium. *Applied and Environmental Microbiology*, 62(8): 2910–2914.

Wang J L, Quan X C, Han L P, Qian Y, Hegemann W, 2002. Microbial degradation of quinoline by immobilized cells of *Burkholderia pickettii*. *Water Research*, 36(9): 2288–2296.

Wang L, Li Y M, Yang D H, 2010. Biodegradation and metabolites of 2-methylquinoline by acclimated activated sludge under aerobic and denitrifying conditions. *Process Biochemistry*, 45(6): 919–928.

Zhu S N, Liu D Q, Fan L, Ni J R, 2008. Degradation of quinoline by *Rhodococcus* sp. QL2 isolated from activated sludge. *Journal of Hazardous Materials*, 160(2-3): 289–294.

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