Biodegradation (2010) 21:335–344
DOI 10.1007/s10532-009-9304-9

ORIGINAL PAPER

Quinoline biodegradation and its nitrogen transformation pathway by a *Pseudomonas* sp. strain

Yaohui Bai · Qinghua Sun · Cui Zhao · Donghui Wen · Xiaoyan Tang

Received: 23 June 2009 / Accepted: 13 October 2009 / Published online: 24 October 2009

Abstract A *Pseudomonas* sp. strain, which can utilize quinoline as its sole carbon, nitrogen and energy source, was isolated from activated sludge in a coking wastewater treatment plant. Quinoline can be degraded via the 8-hydroxycoumarin pathway. We quantified the first two organic intermediates of the biodegradation, 2-hydroxyquinoline and 2,8-dihydroxyquinoline. We tracked the transformation of the nitrogen in quinoline in two media containing different C/N ratios. At least 40.4% of the nitrogen was finally transformed into ammonium when quinoline was the sole C and N source. But addition of an external carbon source like glucose promoted the transformation of N from NH_3 into NO_3^-, NO_2^-, and then to N_2. The product analysis and gene characteristics indicated that the isolate accomplished heterotrophic nitrification and aerobic denitrification simultaneously. The study also demonstrated that quinoline and its metabolic products can be eliminated if the C/N ratio is properly controlled in the treatment of quinoline-containing wastewater.

Nucleotide sequence accession number The accession numbers of the isolates 16S rRNA gene, *nirS*, and *nosZ* on GenBank are EU266621, FJ393272 and FJ393273.

Y. Bai · Q. Sun · C. Zhao · D. Wen (✉) · X. Tang
College of Environmental Sciences and Engineering,
Peking University, Beijing 100871,
People's Republic of China
e-mail: dhwen@pku.edu.cn

Keywords *Pseudomonas* sp. · Quinoline · Biodegradation · Nitrification · Denitrification

Introduction

Quinoline and its derivates are found widely in coal tar, oil shale, and petroleum, and serve as intermediates and solvents in many industries (Shukla 1986; Fetzner 1998). Because of its toxicity and nauseating odor, discharging quinoline-containing waste does great damage to human health and environmental quality. The study of quinoline-degrading bacteria not only provides a better understanding of the metabolic degradation of quinoline, but also benefits the biotreatment of quinoline-containing wastewater. Since *Moraxella* sp. (Grant and Al-Najjar 1976) and *Pseudomonas* sp. (Shukla 1986) were isolated from soil, many bacteria and fungi have been reported to degrade quinoline, such as *Rhodococcus* sp. (O'Loughlin et al. 1996), *Burkholderia pickettii* (Wang et al. 2004), *Comamonas* sp. (Cui et al. 2004), *Desulfobacterium indolicum* (Licht et al. 1997), and white rot fungus (Zhang et al. 2007).

Previous studies of quinoline-degrading strains mainly focused on two points: (1) The metabolic pathways of quinoline. Although different genera of bacteria may have different intermediates, almost all of them transform quinoline into 2-hydroxyquinoline in the first step (Kaiser et al. 1996; Fetzner 1998). (2) Bioaugmentation for pollution treatment. The

decomposition of quinoline and its derivatives has recently been enhanced either by using free cells or immobilized cells (Wang et al. 2002; Chen et al. 2003; Tian et al. 2006).

Pseudomonas acts an important role in quinoline biodegradation. Previous studies mainly focused on the biodegradation pathway (Shukla 1986, 1989; Brockman et al. 1989) and degradation genes (Bläse et al. 1996; Carl and Fetzner 2005). Although the metabolic pathway by strains of *Pseudomonas* has been studied intensively, the quantitative characteristics of the intermediates and the N transformation from quinoline remain unclear. For denitrification, all molecular studies on the ecology of denitrifying bacteria are based on functional genes and their products (Bothe et al. 2000). *nirS* and *nosZ* genes were often used as functional markers to identify the denitrifying bacteria (Scala and Kerkhof 1998; Hallin and Lindgren 1999; Rich et al. 2003). NIR enzyme encoded by the *nirS* gene could transfer NO_2^- to NO and NOS enzyme encoded by the *nosZ* gene could transfer N_2O to N_2 (Wallenstein et al. 2006). Both genes had been proved to be broadly present in different strains of *Pseudomonas* (Cutruzzola et al. 2001; Rösch et al. 2002; Arai et al. 2003; Rich et al. 2003).

In this study, a quinoline-degrading strain, *Pseudomonas* sp. BC001, was isolated from the activated sludge in a coking wastewater treatment plant. We investigated the characteristics of the quinoline biodegradation, and the heterotrophic nitrification and aerobic denitrification corresponding to the N of quinoline. In addition, the amounts of organic intermediates and inorganic products were analyzed. This basic information will improve the treatment of quinoline-containing wastewater using bioaugmentation technology.

Materials and methods

Chemicals

Quinoline was from AccuStandard, Inc., USA. 2-hydroxyquinoline and 2,8-dihydroxyquinoline were from Sigma–Aldrich, Inc., USA. NH_3–N, NO_2^-–N, and NO_3^-–N were from the China Research Center of Certified Reference Materials. Tryptone and yeast extract were from Oxoid Ltd., UK. Solvents for HPLC analysis and GC/MS analysis were of chromatographic grade. All other chemicals were of analytical grade.

Bacterial strain

A quinoline-degrading bacterium was isolated from the activated sludge of the coking wastewater treatment plant of the Capital Iron and Steel Group, Beijing, China. Based on physico-chemical and 16S rRNA characterization, the quinoline-degrading bacterium was identified as a gram-negative, aerobic, and motile strain and named as *Pseudomonas* sp. BC001. This strain is a rod-shaped bacterium with average dimensions of 1.9 μm in length and 0.6 μm in width. A culture of the strain has been deposited in the China General Microorganism Culture Center (CGMCC; accession number 2223).

Media

Two kinds of media were used to assess quinoline biodegradation. Luria-Bertani (LB) medium (Sambrook and Russell 2001) was used for bacterial enrichment and maintenance. Mineral salt medium (MSM), described by Wang et al. (2004) was modified and used in the biodegradation experiments. Each liter of MSM contained (in grams) 4.26 Na_2HPO_4, 2.65 KH_2PO_4, 0.20 $MgSO_4{\cdot}7H_2O$, 0.006 $CaCl_2$, and 1 ml trace elements solution, pH 7.0. Quinoline solution filtered with 0.2 μm membrane was added into the MSM as the sole carbon, nitrogen, and energy source for the bacteria. When required, glucose solution was also added to the MSM as an extra carbon source. 1.9% (w/v) agar was added to the medium to solidify it when agar plates were needed. All media were sterilized at 121°C for 20 min before use.

Inoculum enrichment for biodegradation

The inoculum for all experiments was prepared by inoculating the strain BC001 in LB medium with 500 mg/l of quinoline and incubating at 30°C, and 180 rpm on a rotary shaker until the bacteria grew into the logarithmic phase. The bacterial cells were harvested by centrifuging at $3000\times g$ for 5 min. The cells were washed three times with 20 ml of MSM. The bacterial pellet was resuspended by vortex, and

diluted with MSM to an optical density of 1–2 at 602 nm (OD_{602}) (Shimadzu UV2401, Japan). This bacterial suspension was used immediately as the inoculum in the biodegradation experiments.

Biodegradation of quinoline

The experiments were conducted using a series of 500-ml Erlenmeyer flasks, each containing 200 ml of MSM with a specific concentration of quinoline and the same initial amount of the inoculum. All flasks were sealed with sealfilm and shaken at 30°C, 180 rpm, and sampled periodically. For GC/MS, quinoline, NH_3–N, NO_3^-–N, and NO_2^-–N analysis, a portion of samples was filtered through a 0.22 μm membrane. OD_{602} values were also measured against time.

Qualitative analysis for metabolic products

Possible metabolites were analyzed with GC/MS (Agilent 6890 N GC/5973 MSD, DB-5MS narrow bore column, 30 m × 0.25 mm × 0.25 μm), and the samples were extracted with dichloromethane and dried over anhydrous Na_2SO_4. Helium was used as the carrier gas. The oven temperature was programmed at 40°C for 2 min, followed by a linear increase of 6°C min^{-1} to 280°C, holding at 280°C for 3 min. MS analysis was performed at electron energy of 70 eV. The structures of metabolites were confirmed from the fragmentation patterns of the mass spectra, through comparison with those predicted for known compounds.

The Ammonium-Testkit QUANTOFIX (Sigma–Aldrich, Germany) was used to determine the presence of NH_3 and the approximate range of its concentration during the biodegradation.

Above qualitative results of metabolic products was verified by PCR amplification of specific degradation gene fragments that encode quinoline degradation. Based on the characteristics and PCR primers of quinoline-degrading gene fragments of *Pseudomonas putida* 86 (Rosche et al. 1997; Carl et al. 2004), the *qorL* and *oxoO*, *oxoR* were amplified from the total DNA of strain BC001. The primer pairs were:

qorL-f: GCTCTAGAAGGATTTCCCCTTCACC AAC
qorL-r: GCTCTAGATGGATCACCACATCGC TG
oxoO-f: GCTCTAGACTACCGACGAAGACCGC
oxoO-r: GCTCTAGAGCTTGAAGATCAGGC TGG
oxoR-f: GCTCTAGAAAGCGTTGTTCAGCCTGG
oxoR-r: GCTCTAGATCAGTGGCTGGCGACAA

The PCR thermal program was set as 94°C for 2 min, followed by 30 cycles at 94°C for 40 s, 62°C for 40 s, and 72°C for 40 s, followed by a final extension performed at 72°C for 7 min and then kept at 4°C. Takara *Taq* hot-start polymerase (Takara, Japan) was used for the PCR reaction. After purified, PCR products were sequenced directly on both strands. The sequences were edited by the software BioEdit and assembled by the software Vector NTI suite 7, then analyzed for similarity to other published sequences by using BLAST program in the GenBank database.

Quantitative analysis of metabolic products

After qualitative analysis, quinoline and its organic intermediates, 2-hydroxyquinoline and 2,8-dihydroxyquinoline, were analyzed during the quinoline biodegradation process by a high performance liquid chromatography (HPLC) system (Shimadzu LC10 AD_{VP}, $SPD10A_{VP}$ UV–Vis Detector; Rheodyne 7725i manual injector; Diamonsil C_{18} reverse-phase column, 250 mm × 4.6 mm, 5 μm). For quinoline detection, the mobile phase was methanol solution at a volume ratio of 4:1 (methanol:water) at the flow rate of 1.0 ml min^{-1}, and quinoline was detected at 275 nm (high concentration) or 230 nm (low concentration) wavelength. For 2-hydroxyquinoline and 2, 8-dihydroxyquinoline detection, the mobile phase was methanol solution with the volume ratio of 35:65 (methanol:water) at the flow rate of 1.5 ml min^{-1}, and the wavelength was 254 nm.

The inorganic products including NH_3–N, NO_3^-–N, and NO_2^-–N were measured during the biodegradation process in two media, MSM and MSM plus glucose. NH_3–N concentrations were analyzed by the salicylate-hypochlorous acid method, NO_2^-–N by *N*-1-naphthyl-ethylenediamine method, and NO_3^-–N by UV-spectrophotometric determination (State Environmental Protection Administration of China 1989; USEPA 2003). Sometimes, the concentration of NO_3^-–N was also determined by a HACH DR/890

colorimeter with NitraVer Test N Tube^{TM} for nitrate (Hach Company, USA).

The growth of the bacterial strain was monitored by OD_{602}. Cell dry weight (CDW, g/l) was determined gravimetrically by drying harvested cells in an oven at 105°C for 24 h after centrifugation and washing with sterilized ddH_2O. A linear equation was found between the OD_{602} value and the corresponding CDW. So, biomass was determined by converting the OD_{602} value to CDW according to this equation.

Nitrification and denitrification potential

Three kinds of media were used. The MSM + NH_4Cl + glucose medium was used for the determination of nitrification potential. MSM + KNO_3 + glucose and MSM + KNO_2 + glucose were used to assess the denitrification potential. A series of 500-ml Erlenmeyer flasks, each containing 200 ml of one of the three media was used. The initial C/N ratios of the three media were all kept at 20:1. NH_3–N, NO_3^-–N and NO_2^-–N were measured in the MSM + NH_4Cl + glucose medium; NO_3^-–N and NO_2^-–N were measured in the MSM + KNO_3 + glucose medium; and NO_2^-–N was measured in the MSM + KNO_2 + glucose medium.

From the total DNA of strain BC001, two gene fragments encoding cytochrome cd_1-containing nitrite reductase (*nirS*) and nitrous oxide reductase (*nosZ*), which participate in denitrification, were amplified with the following primers (Rösch et al. 2002):

nirS-F: 5′ CACGG**Y**GT**B**CTGCGCAAGGGCGC 3′
nirS-R: 5′ CGCCACGCGCGG**Y**TC**S**GGGTGGTA 3′
nosZ-F: 5′ CGYTGTTC**M**TCGACAGCCAG 3′
nosZ-R: 5′ CATGTGCAG**N**GC**R**TGGCAGAA 3′

The Takara *Taq* hot-start polymerase (Takara, China) was used for the PCR reaction. The PCR program was set as described by Rösch et al. (2002), except that the denaturation temperature was set at 94°C. Negative controls without DNA template were performed at the same time. The PCR products were separated by 1% agarose gel electrophoresis and stained by SYBR Safe DNA gel stain (Molecular Probes, USA). The target DNA fragments were purified and cloned into pGEM-T Easy vectors. The recombinant plasmids were transfected into competent *E. coli* TOP10 and sequenced. The *nirS* and *nosZ* DNA sequences were analyzed for similarity to other published sequences in the GenBank database by using BLAST program.

Results

Qualitative analysis of metabolic products

GC/MS analysis of metabolic products

GC/MS analysis detected two metabolic intermediates, which were more than 90% identical to the standard MS chromatogram of authentic 2-hydroxyquinoline and 8-hydroxycoumarin, during the biodegradation.

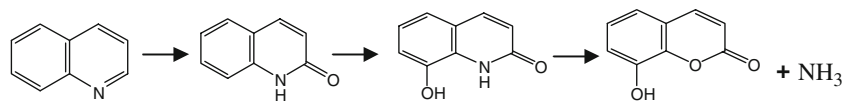
PCR amplification of gene fragments encoding degradation

After sequencing, a 368 bp *qorL* fragment, a 271 bp *oxoO* fragment, and a 267 bp *oxoR* fragment were amplified from the total DNA of BC001. The lengths of PCR products were similar to the PCR gene fragment lengths of the *Pseudomonas putida* 86-derived PCR products from the same primer pairs (Carl et al. 2004). From the GenBank BLAST program, the *qorL*, *oxoO*, and *oxoR* fragments were 98%, 99%, and 100% identical to those of *P. putida* 86, respectively. The *QorL* protein catalyses the transformation from quinoline to 2-hydroxyquinoline, and the *OxoO* and *OxoR* proteins catalyse the transformation from 2-hydroxyquinoline to 2,8-dihydroxyquinoline. These results indicated that the metabolic intermediates of quinoline by BC001 included 2-hydroxyquinoline and 2,8-dihydroxyquinoline.

NH_4^+ determination

The concentration of NH_4^+ increased along with the biodegradation of quinoline, and finally reached a stable level. Hence, NH_3 was identified as a final metabolic product of quinoline by BC001.

From above results, the biodegradation pathway of quinoline by BC001 can be described as follows:



Quantitative analysis for metabolic products

Organic intermediates

The 2-hydroxyquinoline and 2,8-dihydroxyquinoline concentrations increased during the catabolism of quinoline (Fig. 1). The yield of 2-hydroxyquinoline was much higher than that of 2,8-dihydroxyquinoline, indicating that 2-hydroxyquinoline was the initial product. From the results of the BC001 growth, the biomass continued to increase rapidly when the biodegradation of quinoline nearly ended, because that BC001 can continuously utilize the 2-hydroxyquinoline and 2,8-dihydroxyquinoline. When 2,8-dihydroxyquinoline decreased completely, the growth of the BC001 entered the stationary phase (32 h). That is why the time point of the quinoline biodegradation was not identical to the bacterial growth.

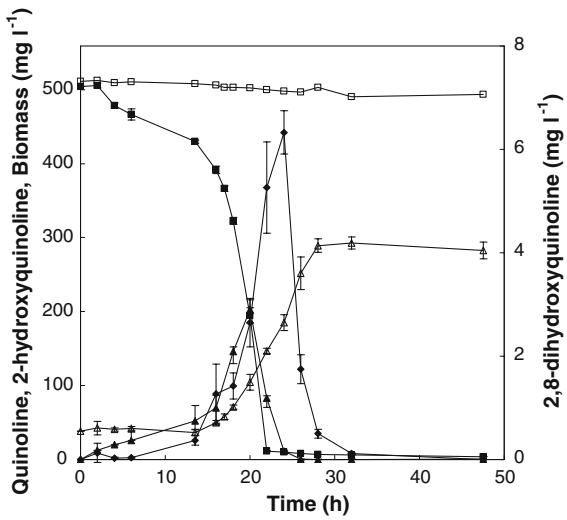


Fig. 1 Quantitative analysis of the organic intermediates during the biodegradation of quinoline by BC001. (*square*) Quinoline (sterile control), (*filled square*) Quinoline, (*filled triangle*) 2-hydroxyquinoline, (*filled diamond*) 2,8-dihydroxyquinoline, (*triangle*) Biomass

Inorganic products

This experiment focused on the variation of NH_3–N transformed from quinoline under different C/N ratios (g/g) of two media, MSM and MSM plus glucose (Fig. 2). In MSM alone (Fig. 2a), the

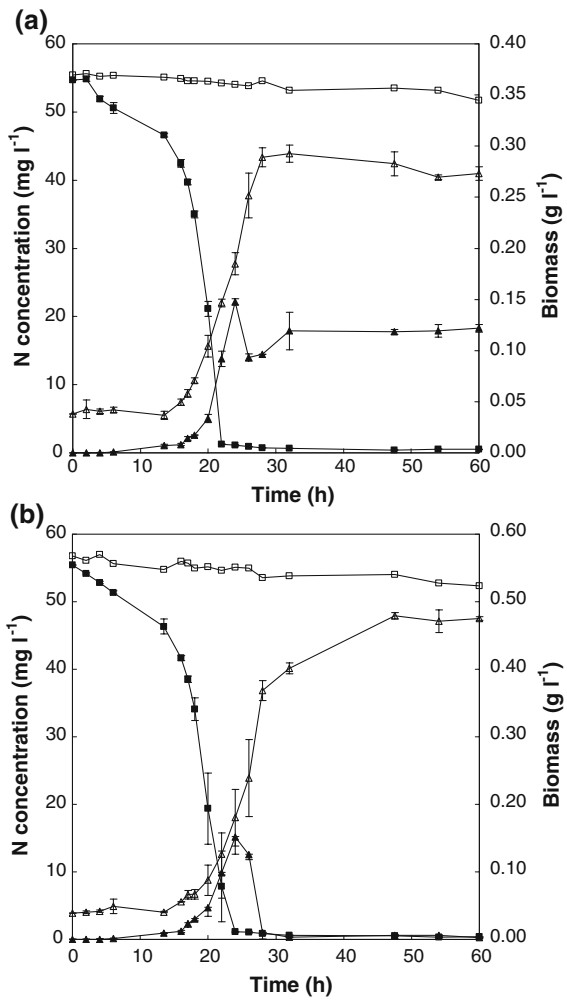


Fig. 2 The variation of NH_3–N in two media. **a** Single substrate-505 mg/l quinoline. **b** Substrate mixture-511 mg/l quinoline + 1874 mg/l glucose. (*square*) Quinoline-N (sterile control), (*filled square*) Quinoline-N, (*filled triangle*) NH_3–N, (*triangle*) Biomass

concentration of NH_3–N increased continuously during the biodegradation of quinoline. When the biodegradation entered the slow phase, the concentration of NH_3–N continued to increase since the 2-hydroxyquinoline was still available for use by BC001. Finally, the concentration of NH_3–N reached a stable level. At least 40.4% of the quinoline-N was transformed into NH_3–N.

In MSM plus glucose (Fig. 2b), the concentration of NH_3–N also increased during the biodegradation of quinoline. After reaching a maximum of 15.2 mg/l, the concentration of NH_3–N dropped quickly. The production of NH_3–N was lower than that in MSM alone. Since quinoline is an energy-deficient substrate as its C/N ratio is 7.7, the extra NH_3–N was not utilized sequentially by BC001 when the available carbon source was exhausted in MSM. So, the metabolism of the bacteria stopped when the C source in MSM was exhausted. However, in MSM plus glucose, glucose supplied enough carbon source and energy to the bacteria. The ratio of C/N in the solution was 20. The biodegradation exhibited was a co-metabolic process of quinoline and glucose. The bacteria utilized carbon not only from quinoline but also from glucose. The lower yield of NH_3–N in the solution of MSM plus glucose was probably caused by a greater proportion of the quinoline-N being synthesized by the bacterial cells due to the addition of glucose. In addition, although glucose did not increase the biodegradation rate, the bacterial growth was markedly improved, as reflected by the growth rate and final biomass. The results also demonstrated that the NH_3–N transformed from quinoline was utilized by BC001.

During the biodegradation in above two media, NO_3^-–N and NO_2^-–N were also determined. The concentration of NO_2^-–N ranged from 0 to 0.1 mg/l, and the concentration of NO_3^-–N varied from 0 to 1 mg/l during the biodegradation in both media.

Nitrification potential

In order to demonstrate the nitrification ability of BC001, MSM + NH_4Cl + glucose was used as the growth medium. As shown in Fig. 3a, when the initial NH_3–N concentration was 74.87 mg/l, BC001 reduced it by 98.7% within 10 h. But when the initial concentration was increased to 170.53 mg/l, 67.4% was reduced within 12 h, and then the biodegradation

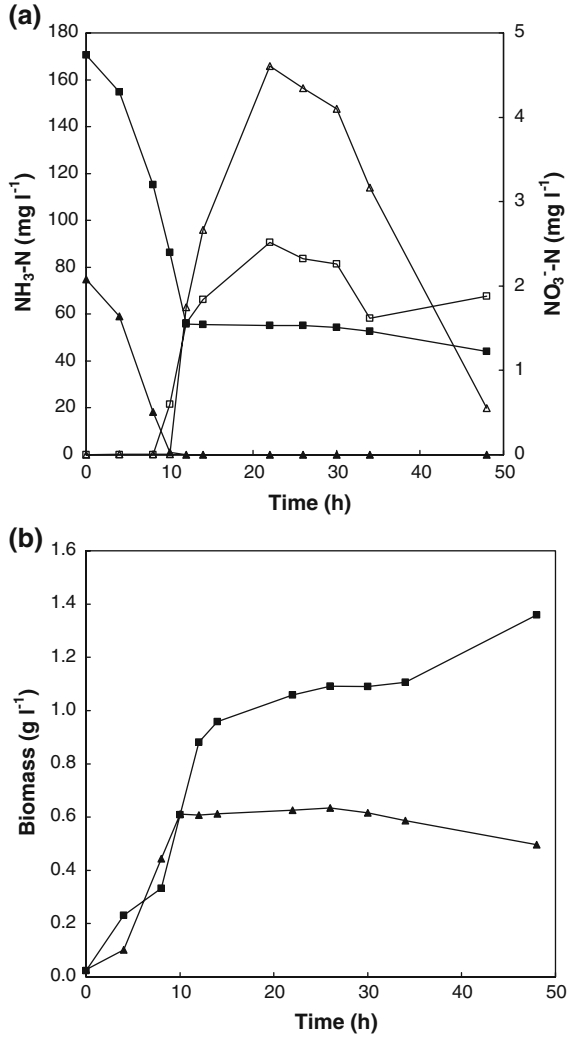


Fig. 3 Utilization of NH_4^+ with two different initial concentrations, 170.53 and 74.87 mg/l (NH_3–N), by BC001 (glucose is the carbon source). **a** The transformation of NH_3–N (*filled square)* 170.53 mg/l and (*filled triangle*) 74.87 mg/l; and the product of NO_3^- (*square*) 170.53 mg/l and (*triangle*) 74.87 mg/l. **b** The growth of BC001 (*filled square*) 170.53 mg/l and (*filled triangle*) 74.87 mg/l

entered the stagnant phase, which indicated that the intermediate inhibited growth.

During the biodegradation, BC001 transformed NH_4^+ into NO_3^- (Fig. 3a). Up to a maximum of 4.6 mg/l NO_3^- was detected in MSM +74.87 mg/l NH_3–N + glucose, and 2.5 mg/l NO_3^- was detected in MSM + 170.53 mg/l NH_3–N + glucose. Moreover, the NO_3^- was produced after a lag, not at the beginning of biodegradation, indicating that the NO_2^- was the initial product. We also found that a little

NO_2^- was produced. Up to 0.05 mg/l NO_2^- was detected in the medium containing 74.87 mg/l NH_3–N, and up to 0.09 mg/l NO_2^- was detected in the medium containing 170.53 mg/l NH_3–N. Each experiment was repeated twice, and similar results were obtained.

BC001 grew rapidly while the concentration of NH_4^+ decreased (Fig. 3b), which indicated that BC001 utilized glucose and NH_4^+ as C and N sources for its growth.

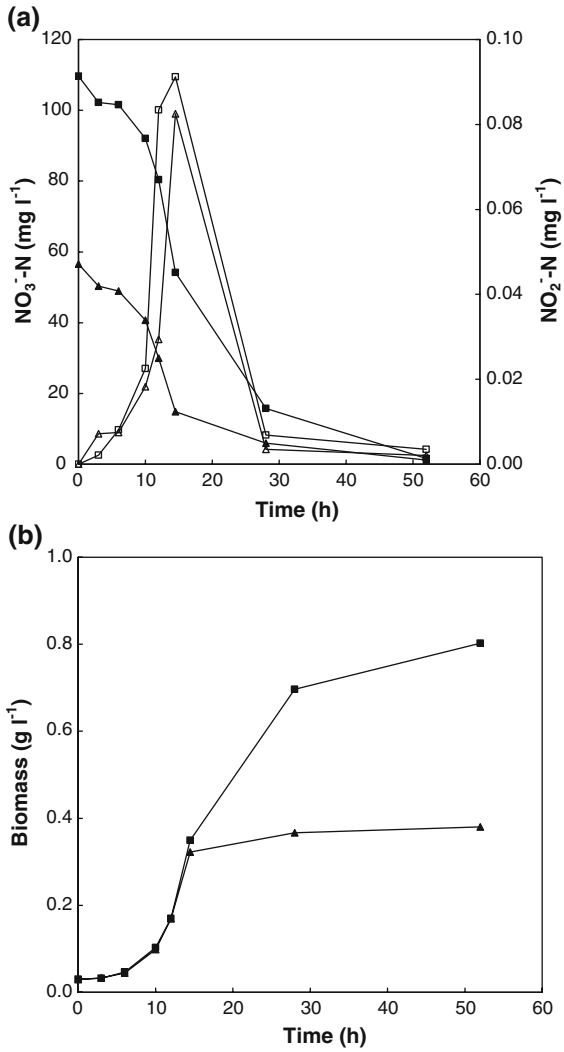


Fig. 4 Utilization of NO_3^- with two different initial concentrations, 109.69 and 56.57 mg/l (NO_3^-–N), by BC001 (glucose is the carbon source). **a** The transformation of NO_3^-–N (*filled square*) 109.69 mg/l and (*filled triangle*) 56.57 mg/l; and the product of NO_2^- (*square*) 109.69 mg/l and (*triangle*) 56.57 mg/l. **b** The growth of BC001 (*filled square*) 109.69 mg/l and (*filled triangle*) 56.57 mg/l

Based on the above results, most of the NH_4^+ was utilized by BC001 for its growth, and a little was transformed into NO_2^- and NO_3^-.

Denitrification potential

Product analysis

In order to demonstrate the denitrification potential of BC001, MSM + KNO_3 + glucose and MSM + KNO_2 + glucose were used as media for bacterial growth.

At 52 h, 98.5% NO_3^- was reduced when the initial NO_3^- concentration was 109.69 mg/l (NO_3^-–N), and 97.9% was reduced when the initial concentration was 56.57 mg/l (NO_3^-–N) (Fig. 4a). Some NO_2^- (0–0.1 mg/l) was produced in both media during the biodegradation. The concentration of NO_2^-–N in both media were increased at first, and then decreased. BC001 utilized NO_3^- as the sole N source for its rapid growth (Fig. 4b).

Table 1 Utilization of NO_2^- by BC001

NO_2^-–N (mg/l)		Biomass (g/l)	
0 day	5 days	0 day	5 days
50.93 ± 0.52	0.25 ± 0.04	0.053 ± 0.001	0.376 ± 0.016

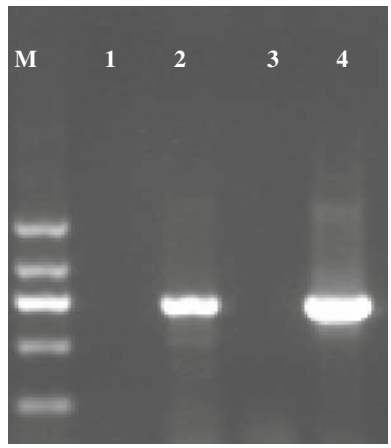


Fig. 5 PCR amplification of the fragments of *nirS* and *nosZ* genes from the *Pseudomonas* sp. BC001. M, the molecular size markers, with the size of 300, 500, 700, 900, 1200 bp from *bottom* to *top*; *lane 1*, negative control of *nirS* gene; *lane 2*, *nirS* gene; *lane 3*, negative control of *nosZ* gene; *lane 4*, *nosZ* gene

The above results show that most of the NO_3^- was utilized by BC001 for its growth, and a little of it was transformed into NO_2^-.

We also found that BC001 utilized NO_2^- as the sole N source for its growth (Table 1). Since detection of N_2 in minimal amount is difficult, we applied molecular biology to determine whether BC001 can transform NO_2^- into N_2.

PCR amplification

From the above results, we suspected that BC001 is a denitrifying bacterium. Using published primers (Rösch et al. 2002), a 702 bp *nirS* fragment and a 701 bp *nosZ* fragment were amplified from the BC001 genetic template (Fig. 5). The lengths of *nirS* and *nosZ* were close to the expected lengths (700 bp). Using the GenBank BLAST program, we found that the *nirS* fragment from BC001 is 85% identical to that of *Pseudomonas aeruginosa* (X16452), and the *nosZ* fragment from BC001 is 80% identical to that of *P. aeruginosa* (X65277). These results indicated that BC001 had the potential ability to reduce NO_2^- to NO, and then to N_2.

Discussion

Our main finding was that 2-hydroxyquinoline, 2,8-dihydroxyquinoline, and 8-hydroxycoumarin are organic intermediates during the biodegradation of quinoline by BC001. This pathway was partly described in previous studies of quinoline-degrading *Pseudomonas* strains (Shukla 1986, 1989; Kilbane et al. 2000; Carl et al. 2004). According to Shukla (1986), 8-hydroxycoumarin is subsequently transformed into 2,3-dihydroxyphenylpropionic acid, and finally to CO_2 and H_2O; also, quinoline-N is transformed into NH_3–N. The latter result has been reported in other genera of bacteria (O'Loughlin et al. 1996; Sugaya et al. 2001).

So far, few reports have focused on the variation of intermediates' concentration during quinoline biodegradation. Our quantitative analysis of the two intermediates revealed that most of the quinoline was first transformed into 2-hydroxyquinoline. Since a low level of 2,8-dihydroxyquinoline was accompanied by a rapid increase in NH_3–N, we conclude that 2-hydroxyquinoline was converted to 2,8-dihydroxyquinoline, which was immediately and rapidly transformed into 8-hydroxycoumarin and ammonium.

In addition, few studies have considered the complete transformation of N in quinoline. Our study demonstrated that the final product was mainly NH_3–N in quinoline biodegradation in MSM alone, and its concentration remained much higher when the available organic matter was degraded completely at last. When glucose was added, the yield of NH_3–N was reduced notably. Therefore, if optimal C/N ratio is provided, NH_3–N as well as quinoline and its metabolic products can be completely eliminated. Although the production of NH_3–N was recognized in previous studies, other nitrogenous intermediates were seldom considered. In this study, the appearance of NO_2^- and NO_3^- indicated that some NH_3–N was transformed into NO_2^- and NO_3^-. The results of product analysis, when using NH_4^+ as sole N source, also support this argument. Some strains of *Pseudomonas* are heterotrophic nitrifiers (Robertson et al. 1989; Wehrfritz et al. 1996; Jetten et al. 1997; Nemergut and Schmidt 2002), which can heterotrophically transfer NH_3 to NH_2OH, and then to NO_2^- by ammonia monooxygenase (AMO) and hydroxylamine oxidase (HAO). Heterotrophic nitrification may be linked to aerobic denitrification, i.e., the nitrate produced though nitrification is converted to N_2 via nitric oxide and nitrous oxide (Wehrfritz et al. 1993; Crossman et al. 1997). The product analysis showed that BC001 utilized NO_3^- as a N source for growth, and transformed some NO_3^- into NO_2^-. Further, NO_2^- can also be converted by BC001. Our PCR analysis showed that some NO_2^- was finally

$$\text{Quinoline-N} \xrightarrow{?} \text{NH}_3 \xrightarrow{\text{AMO}} \text{NH}_2\text{OH} \xrightarrow{\text{HAO}} \text{NO}_2^-, \text{NO}_3^- \xrightarrow{\text{NIR}} \text{NO}, \text{N}_2\text{O} \xrightarrow{\text{NOS}} \text{N}_2$$

Fig. 6 Proposed transformation pathway of the nitrogen from quinoline by BC001. *Note*: 1. Most NH_3–N was reserved when biodegradation occurs with quinoline as sole substrate. On the contrary, NH_3–N was transformed into the following compounds if extra carbon source was supplied. 2. The abbreviations above the *arrows* represent the necessary enzymes relative to the transformation

transformed into N_2, since the *nirS* and *nosZ* genes were found in BC001.

These results indicated that BC001 can transform a portion of NH_3 into NO_3^- and NO_2^-, then to N_2 at last. Compared with another quinoline-degrading bacterium of *Pseudomonas* sp. in our laboratory which has only nitrification potential (Sun et al. 2009), the strain BC001 accomplished heterotrophic nitrification and aerobic denitrification simultaneously. The BC001 could utilize quinoline-N thoroughly if proper extra C source was provided. The aerobic biodegradation pathway of quinoline-N by BC001 could be described as Fig. 6. The enzyme and functional gene of the first step is still obscure, but other enzymes and genes involved in the transformation of NH_3 and following products are clearly detected.

In addition, previous studies found that the quinoline degradation genes may locate in the plasmids of the strains (Aislabie et al. 1990; Sun et al. 2009). But this study demonstrated that the degradation genes were all located in the chromosome as no plasmid was observed in the strain BC001.

Pseudomonas strains can utilize a large number of organics as its carbon or nitrogen source, since it is an important genus among the environmental microorganisms. In the bioaugmentation experiment carried out in our laboratory, the addition of BC001 to coking wastewater containing 200 mg/l quinoline in a sequence batch reactor (SBR), proved that the quinoline was removed efficiently (data not shown). This implies that the quinoline pollution can be solved by applying the degrading-bacteria in the treatment system.

Acknowledgments This study was supported by an exploration project grant from the National High-tech Research and Development Program of China (863 Program, Grant No. 2006AA06Z336) and a general Project grant from the National Natural Science Foundation of China (Grant No. 50878001). We would like to express our appreciation to Prof Yi Li and Mr Mian Xia in the Beijing Weiming Kaituo Agrobiotechnology Ltd., for assistance with the molecular biology. We also thank Prof. Iain Bruce in Zhejiang University School of Medicine for critical checking and revising the paper's English.

References

State Environmental Protection Administration of China (1989) Monitoring and analysis method of water and wastewater. China Environmental Science Press, Beijing (In Chinese)

Aislabie J, Bej AK, Hurst H, Rothenburger S, Atlas RM (1990) Microbial degradation of quinoline and methylquinolines. Appl Environ Microbiol 56(2):345–351

Arai H, Mizutani M, Igarashi Y (2003) Transcriptional regulation of the *nos* genes for nitrous oxide reductase in *Pseudomonas aeruginosa*. Microbiology-SGM 149:29–36

Bläse M, Bruntner C, Tshisuaka B, Fetzner S, Lingens F (1996) Cloning, expression, and sequence analysis of the three genes encoding quinoline 2-oxidoreductase, a molybdenum-containing hydroxylase from *Pseudomonas putida* 86. J Biol Chem 271(38):23068–23079

Bothe H, Jost G, Schloter M, Ward BB, Witzel KP (2000) Molecular analysis of ammonia oxidation and denitrification in natural environments. FEMS Microbiol Rev 24(5):673–690

Brockman FJ, Denovan BA, Hicks RJ, Fredrickson JK (1989) Isolation and characterization of quinoline-degrading bacteria from subsurface sediments. Appl Environ Microbiol 55(4):1029–1032

Carl B, Fetzner S (2005) Transcriptional activation of quinoline degradation operons of *Pseudomonas putida* 86 by the AraC/XylS-type regulator *OxoS* and cross-regulation of the P*qor*M promoter by XylS. Appl Environ Microbiol 71(12):8618–8626

Carl B, Arnold A, Hauer B, Fetzner S (2004) Sequence and transcriptional analysis of a gene cluster of *Pseudomonas putida* 86 involved in quinoline degradation. Gene 331:177–188

Chen FZ, Cui MC, Fu JM, Sheng GY, Sun GP, Xu MY (2003) Biodegradation of quinoline by freely suspended and immobilized cells of *Comamonas* sp. strain Q10. J Gen Appl Microbiol 49(2):123–128

Crossman LC, Moir JWB, Enticknap JJ, Richardson DJ, Spiro S (1997) Heterologous expression of heterotrophic nitrification genes. Microbiology-UK 143:3775–3783

Cui MC, Chen FZ, Fu JM, Sheng GY, Sun GP (2004) Microbial metabolism of quinoline by *Comamonas* sp. World J Microbiol Biotechnol 20(6):539–543

Cutruzzola F, Brown K, Wilson EK, Bellelli A, Arese M, Tegoni M, Cambillau C, Brunori M (2001) The nitrite reductase from *Pseudomonas aeruginosa*: essential role of two active-site histidines in the catalytic and structural properties. P Natl Acad Sci USA 98(5):2232–2237

Fetzner S (1998) Bacterial degradation of pyridine, indole, quinoline, and their derivatives under different redox conditions. Appl Microbiol Biotechnol 49(3):237–250

Grant DJ, Al-Najjar TR (1976) Degradation of quinoline by a soil bacterium. Microbios 15(61–62):177–189

Hallin S, Lindgren PE (1999) PCR detection of genes encoding nitrite reductase in denitrifying bacteria. Appl Environ Microbiol 65(4):1652–1657

Jetten MSM, deBruijn P, Kuenen JG (1997) Hydroxylamine metabolism in *Pseudomonas* PB16: involvement of a novel hydroxylamine oxidoreductase. Antonie Van Leeuwenhoek 71(1–2):69–74

Kaiser JP, Feng YC, Bollag JM (1996) Microbial metabolism of pyridine, quinoline, acridine, and their derivatives under aerobic and anaerobic conditions. Microbiol Rev 60(3):483–498

Kilbane JJ, Ranganathan R, Cleveland L, Kayser KJ, Ribiero C, Linhares MM (2000) Selective removal of nitrogen

from quinoline and petroleum by *Pseudomonas ayucida* IGTN9m. Appl Environ Microbiol 66(2):688–693

Licht D, Johansen SS, Arvin E, Ahring BK (1997) Transformation of indole, quinoline by *Desulfobacterium indolicum* (DSM 3383). Appl Microbiol Biotechnol 47(2):167–172

Nemergut DR, Schmidt SK (2002) Disruption of *narH*, *narJ*, and *moaE* inhibits heterotrophic nitrification in *Pseudomonas* strain M19. Appl Environ Microbiol 68(12):6462–6465

O'Loughlin EJ, Kehrmeyer SR, Sims GK (1996) Isolation, characterization, and substrate utilization of a quinoline-degrading bacterium. Int Biodeter Biodegr 38(2):107–118

Rich JJ, Heichen RS, Bottomley PJ, Cromack K, Myrold DD (2003) Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. Appl Environ Microbiol 69(10):5974–5982

Robertson LA, Cornelisse R, Devos P, Hadioetomo R, Kuenen JG (1989) Aerobic denitrification in various heterotrophic nitrifiers. A Van Leeuw J Microb 56(4):289–299

Rösch C, Mergel A, Bothe H (2002) Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. Appl Environ Microbiol 68(8):3818–3829

Rosche B, Tshisuaka B, Hauer B, Lingens F, Fetzner S (1997) 2-oxo-1, 2-dihydroquinoline 8-monooxygenase: Phylogenetic relationship to other multicomponent nonheme iron oxygenases. J Bacteriol 179(11):3549–3554

Sambrook J, Russell D (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Scala DJ, Kerkhof LJ (1998) Nitrous oxide reductase (*nosZ*) gene-specific PCR primers for detection of denitrifiers and three *nosZ* genes from marine sediments. FEMS Microbiol Lett 162(1):61–68

Shukla OP (1986) Microbial transformation of quinoline by a *Pseudomonas* sp. Appl Environ Microbiol 51(6):1332–1442

Shukla OP (1989) Microbiological degradation of quinoline by *Pseudomonas stutzeri*: the coumarin pathway of quinoline catabolism. Microbios 59(238):47–63

Sugaya K, Nakayama O, Hinata N, Kamekura K, Ito A, Yamagiwa K, Ohkawa A (2001) Biodegradation of quinoline in crude oil. J Chem Technol Biotechnol 76(6):603–611

Sun QH, Bai YH, Zhao C, Xiao YN, Wen DH, Tang XY (2009) Aerobic biodegradation characteristics and metabolic products of quinoline by a *Pseudomonas* strain. Bioresour Technol 100(21):5030–5036

Tian S, Qian C, Yang XS (2006) Biodegradation of biomass gasification wastewater by two species of *Pseudomonas* using immobilized cell reactor. Appl Biochem Biotechnol 128(2):141–147

USEPA (2003) EPA test methods. http://www.epa.gov/

Wallenstein MD, Myrold DD, Firestone M, Voytek M (2006) Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods. Ecol Appl 16(6):2143–2152

Wang JL, Quan XC, Han LP, Qian Y, Hegemann W (2002) Microbial degradation of quinoline by immobilized cells of *Burkholderia pickettii*. Water Res 36(9):2288–2296

Wang JL, Wu WZ, Zhao X (2004) Microbial degradation of quinoline: Kinetics study with *Burkholderia pickttii*. Biomed Environ Sci 17(1):21–26

Wehrfritz JM, Reilly A, Spiro S, Richardson DJ (1993) Purification of hydroxylamine oxidase from *Thiosphaera pantotropha*. Identification of electron acceptors that couple heterotrophic nitrification to aerobic denitrification. FEBS Lett 335(2):246–250

Wehrfritz JM, Carter JP, Spiro S, Richardson DJ (1996) Hydroxylamine oxidation in heterotrophic nitrate-reducing soil bacteria and purification of a hydroxylamine-cytochrome c oxidoreductase from a *Pseudomonas* species. Arch Microbiol 166(6):421–424

Zhang XY, Yan KL, Ren DJ, Wang HX (2007) Studies on quinoline biodegradation by a white rot fungus (*Pleurotus ostreatus* BP) in liquid and solid state substrates. Fresen Environ Bull 16(6):632–638