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Aerobic biodegradation characteristics and metabolic products of quinoline by a *Pseudomonas* strain

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ABSTRACT

A bacterial strain, BW003, which utilized quinoline as its sole C, N and energy source, was isolated and identified as *Pseudomonas* sp. BW003 degraded 192–911 mg/l quinoline within 3–8 h with removal rates ranging from 96% to 98%. The optimum conditions for the degradation were 30 °C and pH 8. In the process of biodegradation, at least 43% of quinoline was transformed into 2-hydroxyquinoline, then 0.69% of 2-hydroxyquinoline was transformed into 2,8-dihydroxyquinoline, and then, presumably, into 8-hydroxycoumarin. Meanwhile, at least 48% of the nitrogen in quinoline was directly transformed into ammonia-N. An extra carbon source enhanced the nitrogen transformation from ammonia-N. Further experiments showed that, besides cell synthesis, BW003 transformed less than 6% of ammonia-N into nitrate through heterotrophic nitrification. In addition, BW003 contained a large plasmid, which may be involved in quinoline metabolism. The study indicates that quinoline and its metabolic products can be eliminated from wastewater by controlling the C/N ratio using BW003 as the bioaugmentation inoculum.

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1. Introduction

Quinoline occurs widely in coal tar, oil shale, and petroleum, and serves as an intermediate and solvent in many industries (Fetzner, 1998; Shukla, 1986). Due to 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 helps to reveal the metabolic mechanism of quinoline, but also benefits the bio-treatment 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 being able to degrade quinoline. These include *Rhodococcus* sp. (O'Loughlin et al., 1996), *Desulfobacterium indolicum* (Licht et al., 1997), *Burkholderia pickettii* (Wang et al., 2004), *Comamonas* sp. (Cui et al., 2004), and white rot fungus (Zhang et al., 2007). Previous studies of quinoline-degrading strains mainly focused on two points:

(i) The mechanism of transformation of quinoline. Although different genera of bacteria may produce different intermediates, almost all of them transform quinoline into 2-hydroxyquinoline in the first step (Fetzner, 1998; Kaiser et al., 1996).

(ii) Bioaugmentation for pollution treatment. The decomposition of quinoline and its derivatives have recently been enhanced either by using free cells or immobilized cells of degrading bacteria (Chen et al., 2003; Tian et al., 2006; Wang et al., 2002).

Pseudomonas plays an important role in the quinoline biodegradation. Previous studies focused on the biodegradation pathway (Brockman et al., 1989; Shukla, 1986, 1989) and degradation genes (Bläse et al., 1996; Carl and Fetzner, 2005). Although the metabolic pathway in strains of *Pseudomonas* has been studied intensively, the quantitative characteristics of intermediates and final products during the biodegradation were vague; especially the N transformation from quinoline remained unclear. In this study, a quinoline-degrading bacteria strain, *Pseudomonas* sp. BW003, was isolated from the activated sludge in a coking wastewater treatment plant. Its biodegradation characteristics in different conditions were studied, and the amounts of organic intermediates and inorganic products were analyzed by means of products analysis and genetic characterization. This will benefit to acquire the basic information and optimum conditions for the treatment of quinoline-containing wastewater by the bioaugmentation technology.

2. Methods

2.1. Chemicals

Quinoline was from AccuStandard Inc., USA. 2-hydroxyquinoline and 2,8-dihydroxyquinoline were from Sigma–Aldrich Inc.,

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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 used were of analytical grade.

2.2. Media

The 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, 0.2 $MgSO_4 \cdot 7H_2O$, 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 C, N, and energy source of the bacteria. When required, glucose solution was also added to the MSM as an extra C source. Solid medium contained 1.9% (w/v) agar. All media were sterilized at 121 °C for 20 min before use.

In order to study the pathway of nitrogen transformation, 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. The initial C/N ratios of the three media were all kept at 20:1.

2.3. Bacteria cultivation and isolation

Samples of activated sludge were obtained from the coking wastewater treatment plant of the Wuhan Iron and Steel Corporation, Hubei Province, China. 200 ml of samples were put into a 500-ml Erlenmeyer flask. In order to deflocculate and mix thoroughly, two drops of 0.01% sodium pyrophosphate and several glass beads were added, then the mixture was shaken at 30 °C, 180 rpm for 30 min. The mixture was centrifuged at 3000×*g* for 10 min. 1 g of the centrifuged deposition was transferred into the MSM containing 300 mg/l quinoline and incubated at 30 °C, 180 rpm. When quinoline was removed completely from the medium, 5 ml of the culture was transferred to 95 ml of the fresh medium. After three times of successive transfers, the serial dilution of suspensions (10^{-1}–10^{-7}) were spread onto the MSM plates. Colonies of quinoline-degrading bacteria were purified through three cycles of single colony isolation. Finally, pure colonies were transferred to quinoline-containing liquid MSM to investigate their ability of metabolize quinoline under the aerobic condition. For preservation, these strains were cultivated aerobically with the LB liquid medium containing 500 mg/l quinoline, and then preserved in 15% glycerol at −70 °C in an ultra-low temperature freezer (Sanyo MDF-382E, Japan).

2.4. Bacterium identification by 16S rRNA sequence

A TIANamp Bacteria DNA Kit (TianGen, China) was used to extract DNA from bacteria grown overnight in LB liquid medium. Partial fragments of the 16S rRNA gene were amplified with the following primer pair (Devereuv and Wilkinson, 2004):

Forward 27F: AGAGTTTGATCCTGGCTCAG,
Backward 1492R: TACGGTTACCTTGTTACGACTT.

PCR was performed in a PCR thermocycler (Takara, Japan) with an initial denaturation of the template DNA at 94 °C for 2 min, followed by 30 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, followed by a final extension performed at 72 °C for 15 min and then kept at 4 °C. The PCR product was separated by agarose (0.8%) gel electrophoresis. The target DNA fragment was purified by a Qiaquick gel extraction kit (Qiagen, Germany) and cloned into the pGEM-T Easy vector (Promega, USA). The recombinant plasmid was transformed into competent *Escherichia coli* TOP10, and then the sequence of the 16S rRNA gene insert of the plasmid was determined. The sequence data were compared with known 16S rRNA gene sequences using the BLAST program of the National Center for Biotechnology Information (NCBI) database.

2.5. Inoculum enrichment for biodegradation

The BW003 strain was inoculated in LB medium with 500 mg/l of quinoline and incubated at 30 °C/180 rpm on a rotary shaker until the bacteria grew into the logarithmic phase. The bacterial cells were harvested by centrifuging at 3000×*g* for 5 min. Then the cells were washed three times with 40 ml of MSM. The bacterial deposition was resuspended by vortexing, and diluted with MSM to an optical density of 1–2 at 602 nm (OD_{602}) (Shimadzu UV2401, Japan). The bacterial suspension was used immediately as the inoculum in the biodegradation experiments.

2.6. Biodegradation and biotransformation of quinoline

The biodegradation experiments were performed under different initial quinoline concentrations (192–911 mg/l), temperatures (20–35 °C), and initial pH values (3.0–10.0). The experiments were conducted using a series of 250-ml Erlenmeyer flasks. Each flask contained 100 ml of MSM with a specific concentration of quinoline and a constant amount of inoculum. These flasks were sealed with sealfilm, shaken at 30 °C/180 rpm, and sampled periodically. For analyzing the concentration of quinoline, a portion of sample was filtered with 0.22 μm membrane. OD_{602} values were also measured against time.

The biotransformation of quinoline-N were studied using a series of 500-ml Erlenmeyer flasks, each containing 200 ml of one of the three media, MSM + NH_4Cl + glucose, MSM + KNO_3 + glucose, and MSM + KNO_2 + glucose. The experimental process was the same as the biodegradation experiments. Ammonia-N, nitrate and nitrite were measured in the MSM + NH_4Cl + glucose medium; nitrate and nitrite were measured in the MSM + KNO_3 + glucose medium; and nitrite was measured in the MSM + KNO_2 + glucose medium.

2.7. Gene-based techniques

Based on the literatures (Carl et al., 2004; Rösche et al., 1997), the quinoline-degrading gene fragments – *qorL*, *oxoO*, and *oxoR*, which control the transformation of quinoline into 2-hydroxyquinoline, and then into 2,8-dihydroxyquinoline, were amplified from the DNA of BW003. The primer pairs were:

qorL-f: GCTCTAGAAGGATTTCCCCTTCACCAAC
qorL-r: GCTCTAGATGGATCACCACATCGCTG
oxoO-f: GCTCTAGACTACCGACGAAGACCGC
oxoO-r: GCTCTAGAGCTTGAAGATCAGGCTGG
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. The PCR products were purified, and then sequenced directly with double strands. The sequences were edited by the software BioEdit and assembled by the software Vec-

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