Microbial Degradation of Quinoline: Kinetics Study With *Burkholderia picekttii*[1]

JIAN-LONG WANG[#,2], WEI-ZHONG WU[※], AND XUAN ZHAO[#]

[#]*Laboratory of Environmental Technology, INET, Tsinghua University, Beijing 100084, China;*
[※]*School of Environmental Science, Peking University, Beijing 100871, China*

Objective To investigate the kinetics of quinoline biodegradation by *Burkholderia pickttii,* a Gram negative rod-shaped aerobe, isolated in our laboratory. **Methods** HPLC (Hewlett-Packard model 5050 with an UV detector) was used for the analysis of quinoline concentration. GC/MS method was used to identify the intermediate metabolites of quinoline degradation. **Results** The biodegradation of quinoline was inhibited by quinoline at a high concentration, and the degradation process could be described by the Haldane model. The kinetic parameters based on Haldane substrate inhibition were evaluated. The values were ν_{max}= 0.44 h^{-1}, K_S=166.7 mg/L, K_i= 650 mg/L, respectively. The quinoline concentration to avoid substrate inhibition was inferred theoretically and determined to be 329 mg/L. **Conclusion** The biodegradation of quinoline conforms to the Haldane inhibition model and the main intermediate metabolite of quinoline biodegradation is 2-hydroxy-quinoline.

Key words: Quinoline; Microbial degradation; *Burkholderia pickttii*; Kinetics; Substrate inhibition; Intermediate metabolite

INTRODUCTION

Quinoline, a heterocyclic compounds, is found in coal tar, mineral oil and bone oil. In the chemical industry it serves as a solvent and is the starting material for the synthesis of quinoline dyes and pharmaceuticals. Quinoline and some of its derivatives were reported to be toxic, carcinogenic and mutagenic[1,2]. The widespread use of quinoline and its derivatives entails that these compounds, together with many other environmental chemicals, are distributed in the environment, thus polluting soil and water.

Previous research on quinoline biodegradation has mostly focused on the isolation of quinoline-degrading microorganisms. There have been several reports on the bacterial degradation of quinoline[3-6]. Investigations on the taxonomy of the quinoline-degrading bacteria showed that in most cases they were members of the genus *Pseudomonas*. *Pseudomonas* is characterized by their ability to use a wide range of organic compounds as sole sources of carbon and energy. Among them are also aromatic and heterocyclic compounds, which occur naturally or are generated as synthetic products.

However, the ability to degrade quinoline does not appear to be confined to

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[2]Correspondence should be addressed to Dr. Jian-Long WANG, Tel:86-10-62784843. Fax:86-10-62771150. E-mail: wangjl@tsinghua.edu.cn

Biographical note of the first author: Jian-Long WANG, born in 1964, Ph. D. degree, professor of Tsinghua University, majoring in the field of water pollution control as well as environmental biotechnology.

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Pseudomonas. A *Moraxella* species, also a Gram-negative, aerobic bacterium, and a *Nocardia* species were described showing these degrading properties[5,6]. In our laboratory, several strains of quinoline-degrading microorganisms were isolated from activated sludge of coke-plant's wastewater treatment plant by enrichment and acclimation shaking cultivation. One of them was identified as *Burkholderia pickttii*, an aerobic, rod shaped and Gram-negative bacterium. The characteristics of quinoline biodegradation in batch culture experiment were investigated[7-10].

Information on kinetics of quinoline biodegradation is very limited[11]. Investigation of the kinetics of biodegradation is important for the evaluation of the persistence of organic pollutants and the design of biodegradation facilities. The object of this study was to investigate the kinetics of biodegradation of quinoline and to analyze the main intermediates of microbial metabolism by GC/MS method.

MATERIALS AND METHODS

Microorganism

The microorganism used in this study was a pure strain of quinoline-degrading microbe, which was isolated from the activated sludge of wastewater treatment plant by enrichment shaking culture at 30℃. The strain was purified by successive streak transfers agar-plate medium and maintained as slant cultures on tryptone-glucose extract agar. It was identified as *Burkholderia pickttii*.

Medium

The mineral salt medium (SMW) for the growth of the microorganism and degradation contained (in grams per liter) 4.26 Na_2HPO_4, 2.65 KH_2PO_4, 0.2 $MgSO_4 \cdot 7H_2O$, 0.02 $CaCl_2$, 0.002 $MnSO_4 \cdot 7H_2O$, and 1 mL trace element solution.

The trace element solution contained (in grams per liter) 1.5 $FeCl_2 \cdot 4H_2O$, 0.024 $NiCl_4 \cdot 6H_2O$, 0.19 $CoCl_2 \cdot 6H_2O$, 0.002 $CuCl_2 \cdot 2H_2O$, 0.1 $MnSO_4 \cdot 7H_2O$, 0.024 $Na_2MoO_4 \cdot 2H_2O$, 0.07 $ZnCl_2$, 0.006 H_3BO_3. The pH of the medium was adjusted to 7.0.

Quinoline was used as the sole source of carbon and nitrogen in all experiments.

Analytical Method

Quinoline concentrations of all samples in this study were analyzed by HPLC system (Hewlett-Packard model 5050 with an UV detector). Samples were prepared by centrifugation and filtration, 20 μL of which was injected. Separation was carried out in a C_{18} reverse-phase column, 250 × 4.6 mm, 5 μm (Hewlett-Packard Zorbax SB-C_{18}, USA). The elution solvent, which consisted of a mixture of methanol and water (60:40, v/v), was introduced to the column at a flow rate of 1 mL/min. Quinoline was detected at 275 nm wavelength.

Degradation of Quinoline

The experiments of quinoline biodegradation were carried out in a series of 250-mL sterile Erlenmeyer flasks. Each flask contained 100-mL sterile mineral salt medium with various quinoline concentrations, and inoculated with pre-cultured biomass, grown on basic mineral salt medium with quinoline as the sole carbon/energy source. The initial biomass concentration in each flask was equal. The biodegradation was carried out in a shaker at 200

rpm and the temperature was 30℃.

RESULTS AND DISCUSSION

Quinoline-degrading Microbe and Its Characteristics

A quinoline-degrading microbe was isolated from the activated sludge of wastewater treatment plant by enrichment shaking culture at 30℃. The strain was purified by successive streak transfers agar-plate medium and maintained as slant cultures on tryptone-glucose extract agar. The experiment showed that this quinoline degrader was capable of using quinoline as the sole source of carbon, nitrogen and energy. It was identified as *Burkholderia pickettii* according to the report of Biolog Microstation System (ID=0.733). It was a Gram-negative rod-shaped aerobe (6 μm long and 2 μm wide). Colonies were mucoid and gray when grown on solid quinoline-MSM (mineral salts medium).

Quinoline Degradation at Different Initial Concentrations

The effect of the initial quinoline concentration on degradation was studied at the initial concentration of 50 mg/L, 100 mg/L, 200 mg/L, 500 mg/L and 750 mg/L using the pre-cultured biomass. The biodegradation of quinoline at different initial concentrations is demonstrated in Fig. 1.

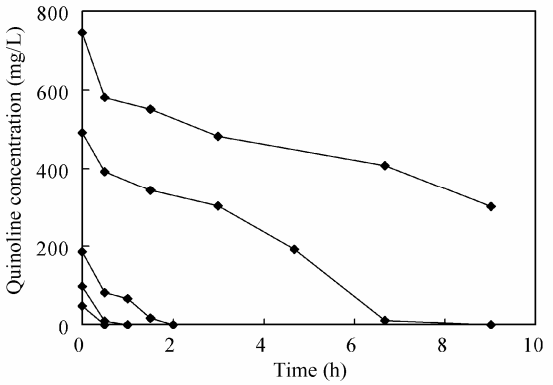

FIG. 1. Biodegradation of quinoline at different initial concentrations.

It can be seen from Fig. 1 that when the initial concentration of quinoline was relatively low, the pre-cultured biomass could degrade the quinoline very quickly without the lag-phase duration. For example, at the initial concentration of 100 mg/L and 200 mg/L, the biomass could degrade quinoline within 1 and 2 h, respectively. Five hundred mg/L of quinoline could be degraded within 7 h. The degradation rate was rather high in comparison with the reported research findings.

Kinetics Analysis

In the quinoline biodegradation experiments, an equal amount of pre-cultured biomass was inoculated into solutions containing different concentrations of quinoline. Assuming that within 0.5 h of initial degradation, the increase of the biomass could be ignored. Moreover, the biodegradation rate of quinoline within this period was proportional to the initially inoculated biomass concentration, that is, the degradation of quinoline followed the

first-order reaction kinetic equation. Therefore, the specific initial biodegradation rate of quinoline could be expressed as follows:

$$v = \frac{dS/dt}{X} = \frac{S_0 - S_i}{X\Delta\Delta} \tag{1}$$

Where,

ν: Specific degradation rate of quinoline (h^{-1})

X: Biomass concentration (mg/L)

S: Quinoline concentration (mg/L)

t: Degradation time (h)

According to this equation, the initial degradation rate of quinoline at different concentrations could be calculated. Then a plot of ν *versus* S could be depicted. The plot of ν *versus* S for quinoline degradation is shown in Fig. 2.

It can be seen from Fig. 2 that the specific initial degradation rate of quinoline increased with the increase of quinoline concentration when the initial concentration was low. When the initial quinoline concentration was higher than 200 mg/L, the specific initial degradation rate of quinoline began to decrease. Therefore, the biodegradation of quinoline followed the Haldane equation, and a substrate inhibition model proposed by Haldane could be used in this case.

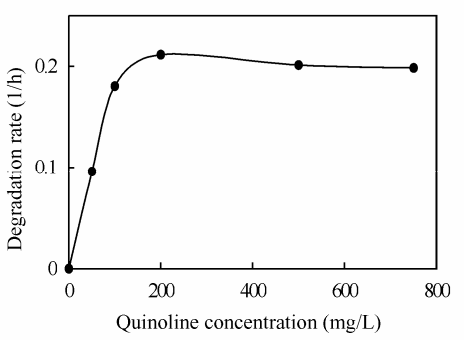

FIG. 2. Kinetics of quinoline biodegradation.

$$v = \frac{v_{max}}{1 + \frac{K_S}{S} + \frac{S}{K_i}} \tag{2}$$

Where

S: Quinoline concentration (mg/L)

ν: Specific degradation rate of quinoline (h^{-1})

ν_{max}: Maximum specific degradation rate of quinoline (h^{-1})

K_i : Inhibition constant for quinoline (mg/L)

K_s: Saturation constant for quinoline (mg/L)

Equation (2) can be rearranged to the following form:

$$\frac{1}{v} = \frac{1}{v_{max}} + \frac{K_S}{v_{max}} \cdot \frac{1}{S} + \frac{1}{v_{max} K_i} \cdot S \tag{3}$$

Equation (3) can be simplified by assuming $K_i >> S$ and the linearization leads to

$$\frac{1}{v} = \frac{1}{v_{max}} + \frac{K_S}{v_{max}} \cdot \frac{1}{S} \tag{4}$$

According to the equation (4), a plot of $\frac{1}{\nu}$ *versus* $\frac{1}{S}$ should be linear. Application of the data shown in Fig. 2 to the equation (4), the kinetic parameters, that is, the half saturation coefficient and the maximum specific degradation rate could be determined, which are K_S= 166.7 mg/L, ν_{max}=0.44 h^{-1}, respectively. The inhibition coefficient, K_i could be obtained through the square differences analysis, that is K_i=650 mg/L.

Therefore the kinetic equation of quinoline degradation can be expressed as follows:

$$\nu = \frac{0.4395}{1 + \frac{166.7}{S} + \frac{S}{650}} \tag{5}$$

Minimum Inhibition Substrate Concentration

From equation (2), by setting the first derivative of Haldane model equal to zero,

$$\frac{d\nu}{dS} = 0 \tag{6}$$

Then the following relation is obtained

$$S^* = \sqrt{K_i K_S} \tag{7}$$

which gives a quinoline concentration at which the highest specific substrate degradation could be attained.

The specific substrate degradation rate can be calculated by

$$\nu = \nu_{max} \cdot \frac{1}{1 + \sqrt{K_S / K_i}} \tag{8}$$

If we substitute the value of K_s =166.7 mg/L into equation (7), the value of S* can be calculated to be 329 mg/L. The calculated results suggested that the reactor should be operated at a quinoline concentration below 329 mg/L to avoid substrate inhibition. This result indicated that the isolated microbe was highly tolerant to quinoline and it was potential to be used in the practical treatment of quinoline-containing wastewater.

Analysis of Intermediates

The intermediates of quinoline biodegradation were analyzed using HPLC. The results are shown in Fig. 3. It can be seen from Fig. 3 that with the falling of quinoline peak at retention time (RT) 5.9 min, another peak appeared at RT 3.9 min. It could be inferred that some intermediates accumulated in the course of quinoline biotransformation. The first intermediate was extracted and analyzed by GC-MS, which was verified to be 2-hydroxyquinoline (2-OH-Q). The mass spectrum of 2-OH-Q was characterized by a M^+ peak at m/z 145, a base peak at m/z 117(M-CO), and other peaks at m/z 90 (M-CO, -HCN) and 89($C_7H_5^-$). The data of tested intermediates were identical to the data obtained with the reference compound 2-OH-Q (Fig. 3). Therefore it can be concluded that 2-OH-Q is the first intermediate during quinoline biotransformation.

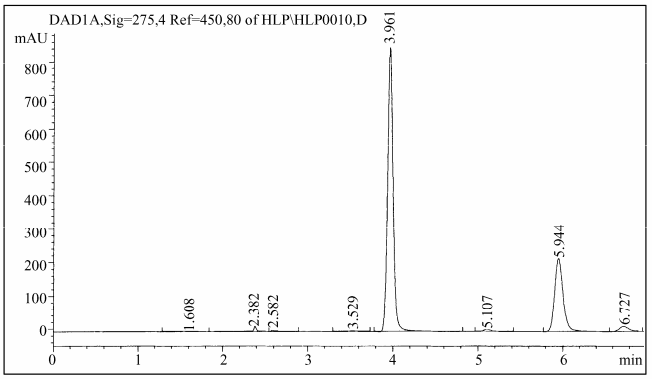

FIG. 3. HPLC analysis of intermediates of quinoline biodegradation.

CONCLUSIONS

An aerobic microorganism capable of using quinoline as the sole source of carbon, nitrogen and energy has been isolated and identified as *Burkholderia pickettii*. The kinetics of quinoline degradation could be described by the Haldane model. Kinetic parameters based on Haldane substrate inhibition are ν_{max}=0.44 h^{-1}, K_S=166.7 mg/L, K_i=650 mg/L, respectively. The quinoline concentration to avoid substrate inhibition has been inferred theoretically to be 329 mg/L. The GC-MS analysis reveals that 2-hydroxy-quinoline is the main intermediate metabolite of quinoline biodegradation by *Burkholderia pickettii*.

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