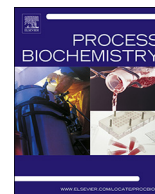




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Competition for molecular oxygen and electron donor between phenol and quinoline during their simultaneous biodegradation

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ABSTRACT

It is common that phenol and quinoline co-exist in the same industrial wastewater, such as coking wastewater. For both biodegradations, the initial steps are mono-oxygenation reactions, which require two co-substrates: molecular oxygen (O₂) and an intercellular electron donor (2H). Competition for O₂ and 2H was investigated using a vertical baffled bioreactor (VBBR) with a biofilm acclimated to phenol and quinoline biodegradation. Batch experiments documented mutual inhibition between phenol and quinoline, which competed for O₂, 2H, or both during simultaneous biodegradation. Low DO was a limiting factor for phenol and quinoline biodegradations, as both rates slowed significantly for DO ≤ 3 mg/L, compared to DO ≥ 5 mg/L. A DO concentration of 0.5 mg/L led to 89% and 65% slower removal kinetics for phenol and quinoline, respectively. Although adding succinate as an exogenous electron donor was able to alleviate competition when the DO was 4 ~ 5 mg/L, it had no benefit for a DO ≤ 3 mg/L. Thus, significant DO limitation could not be overcome by addition of more donor. The results imply that a strategy that involves adding or creating an exogenous electron donor may be effective only when DO is not significantly rate limiting for the initial oxygenation reactions.

1. Introduction

Phenol and quinoline are important raw materials for the chemical industry [1,2], but are also components in industrial wastewater, such as from the coking industry [3], and they are found in natural waters [4]. Because phenol and quinoline are carcinogens, teratogens, and mutagens, their discharge to natural waters creates human-health risks [5,6]. Many methods have been employed to remove phenol and quinoline from wastewater [7–12], but biodegradation usually is the most efficient strategy for industrial wastewater treatment [13–15]. While biodegradation mechanisms have been researched for phenol and quinoline as separate compounds [16,17], information of their interactions during simultaneous biodegradation is scarce, despite the frequency of their co-occurrence [18].

An important common feature for phenol and quinoline biodegradations is that the initial steps are mono-oxygenation reactions [19,20], during which a hydroxyl group is inserted in phenol ring or pyridine ring by the action of a mono-oxygenase that requires molecular oxygen (O₂) and intercellular electron donor (2H) as co-substrates. Their biodegradation pathways, shown in Fig. 1, illustrate how the mono-oxygenase reactions begin the biotransformation and what

are the mono-oxygenation products [4,21].

When phenol and quinoline are biodegraded simultaneously, it is likely that the first two steps of each pathway compete for O₂ and 2H. In addition, the third step of quinoline biodegradation is a hydrogenation that requires 2H, which means that this step also should compete for 2H. Several previous studies addressed competition for electron donor. Cao et al. [22] discussed interaction between reductive dechlorination of 2,4,6-trichlorophenol and nitrate/nitrite denitrification; Jiang et al. [23] explored interactions between phthalic acid di-oxygenation and nitrate denitrification for co-existing aerobic and anoxic conditions; and Xu et al. [24] explored the competition for electrons between pyridine and quinoline mono-oxygenations in fully aerobic conditions. All the studies documented that competition for 2H slowed the rate of biotransformation of one or both substrates and that addition of an exogenous electron donor could relieve competition and speed both reactions.

None of the prior works discussed competition for O₂, although O₂ is one of co-substrates for the oxygenations. Supplying O₂ in biological treatment is a major expense, often more than 50% of the total operating costs. Furthermore, insufficient O₂-delivery capacity and high organic loading can lead to a low concentration of dissolved O₂.

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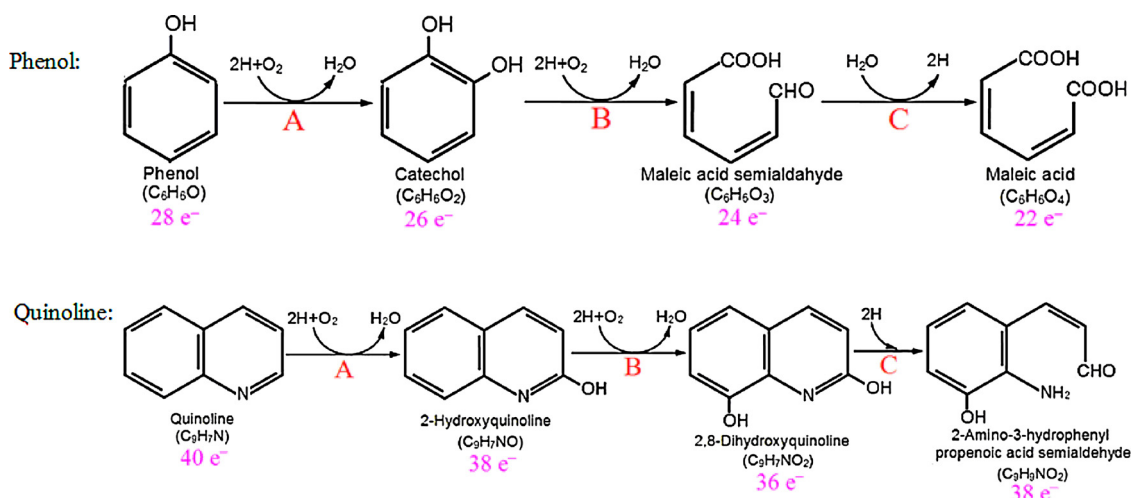


Fig. 1. Initial steps of biodegradation for phenol (top) and quinoline (bottom). The first two steps are mono-oxygenations that require O_2 and $2H$.

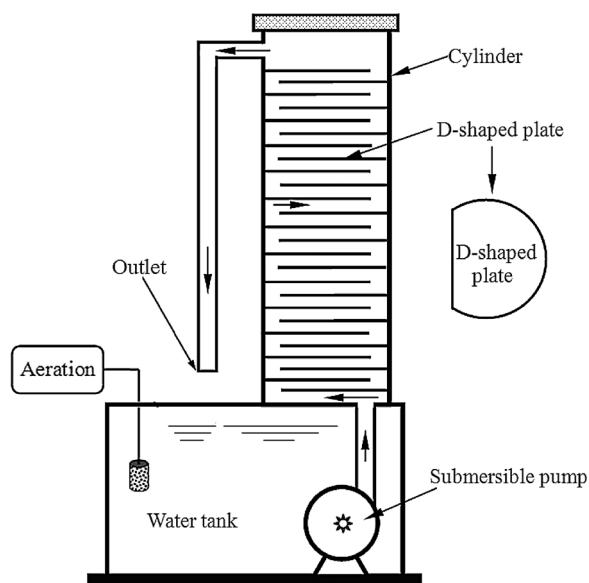


Fig. 2. Diagrammatic sketch of vertical baffled bioreactor (VBRR).

Therefore, competition of O_2 is of widespread practical importance, along with being of fundamental interest for understanding the biodegradation of organic pollutants whose biodegradation involves mono-oxygenations.

In this work, we test the hypothesis that phenol and quinoline compete for O_2 and $2H$ during their simultaneous biodegradation. We focus on competition for O_2 , as this aspect has not been studied in the past. We also address whether or not the addition of donor can compensate for a low concentration of dissolved O_2 .

2. Materials and methods

2.1. Chemical agents and solution preparation

All chemicals were analytical reagent grade and were purchased from Shanghai Sinopharm Group Co., Ltd. To make the stock substrate solution, 9.4 g phenol was dissolved in 1 L deionized water with 18.2 M Ω conductivity (produced by a MILLIPORE (USA), model: Elix[®]) to obtain a 100-mM stock solution of phenol; 5.9 mL quinoline was diluted in 1 L of deionized water for a 50-mM stock solution of quinoline; and 1.652 g succinate was dissolved in 1 L the same water for a 14-mM succinate solution. All stock solutions were kept in refrigerator at 4 $^{\circ}C$

before use. To prepare the trace-element solution, 0.19 g $CoCl_2 \cdot 6H_2O$, 0.07 g $ZnCl_2$, 0.006 g H_3BO_3 , 0.024 g $NiCl_2 \cdot 6H_2O$, 0.024 g $Na_2MoO_4 \cdot 2H_2O$, 1.5 g $FeCl_2 \cdot 4H_2O$, 0.061 g $MnSO_4 \cdot 7H_2O$, and 0.002 g $CuCl_2 \cdot 2H_2O$ were diluted in 1 L deionized water to produce the trace-element solution. The buffer solution contained 4.26 g KH_2PO_4 and 21.75 g K_2HPO_4 diluted in 1 L of deionized water, and it maintained a pH of 6.5–7.0.

2.2. Acclimation of phenol- and quinoline-degrading bacteria

An inoculum of activated sludge was taken from the underflow of a secondary clarifier at Changqiao wastewater treatment plant (Shanghai). The activated sludge was first washed with tap water to remove suspended contaminants. For the washing, 300 mL of sludge was mixed with 700 mL of tap water in a 1-L cylinder by aeration for 10 min and then settled for another 30 min. After that, 700 mL of supernatant was poured out. The washing process was repeated three times to get the washed sludge, which was the retained settled solids.

For acclimation, 700 mg glucose and 7 mL trace element solution were fed into 300 mL of activated sludge, and then tap water was supplemented to 1000 mL. The acclimation was operated with aeration at $30 \pm 2^{\circ}C$ for one week, during which time the nutrient solution was replaced every day. After one week, glucose was gradually decreased, and phenol was increased from zero to 3.2 mM for another week, and then phenol decreased gradually from 3.2 mM to 2 mM, with the quinoline concentration increased from zero to 1 mM over one week. For the all phases of acclimation, substrates in fresh nutrient solution were replaced every day. At the end of two weeks, we had an acclimated sludge that could degrade phenol and quinoline simultaneously.

2.3. Reactor and biofilm formation

We employed a vertical baffled bioreactor (VBRR) for simultaneous biodegradation of phenol and quinoline. The VBRR, shown schematically in Fig. 2, was similar to that used by Cao et al. [22], but modifications included aeration of the water tank (to control the DO concentration in the VBRR) and raising the outlet above the water level of the water tank. The VBRR had a total volume of 800 mL: 550 mL were in the water tank at the bottom, and 250 mL were in the cylinder above the water tank; the cylinder had a height of 180 mm and 20 D-shaped plates of 40 mm diameter. D-shaped plates were installed in the cylinder with staggered arrangement and were the biofilm substrata. A submersible pump was installed in the water tank to circulate the medium between the cylinder and the tank, and an aerator was fitted into the water tank to control the dissolved oxygen (DO) concentration

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