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Nitrate-dependent biodegradation of quinoline, isoquinoline, and 2-methylquinoline by acclimated activated sludge

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ABSTRACT

The anaerobic degradation of quinoline, isoquinoline and 2-methylquinoline was investigated under nitrate-reducing conditions with acclimated activated sludge. Quinoline was completely transformed during degradation with an optimum COD/NO_3–N ratio of 7. Isoquinoline and 2-methylquinoline were also completely transformed; however, nitrate consumption was much lower with the optimum COD/NO_3–N ratios being in the ranges of 83–92 and 21–26, respectively. GC-MS analyses showed that during degradation, quinoline and isoquinoline were transformed by hydroxylation into 2(1H)-quinolinone and 1(2H)-isoquinolinone, respectively. While quinoline was completely mineralized, only 92% of isoquinoline was mineralized, and 1(2H)-isoquinolinone remained in the effluent. 2-Methylquinoline was transformed by hydrogenation to 1,2,3,4-tetrahydro-2-methyl-quinoline, and further degradation resulted in cleavage of the heterocyclic ring leaving 4-ethyl-benzenamine. Both the metabolites remained in the effluent, resulting in the low mineralization of 2-methylquinoline (58%). This is the first time that 2-methylquinoline is observed degradable under denitrifying conditions, and its metabolites are identified.

1. Introduction

Quinolinic compounds, including quinoline, isoquinoline, and methylquinoline, occur in many products such as coal tar, oil, creosote, pharmaceuticals, pesticides and dyes [1–3]. As many of these compounds are considered toxic, carcinogenic and mutagenic [4,5], it is essential to understand their biodegradability to assess their fate in the environment and in the wastewater treatment plants (WWTP). Most quinolinic compounds were considered recalcitrant under anaerobic conditions [6,7]. In recent years however, this view is changing as the anaerobic biodegradation of quinolines has been observed under nitrate-reducing, sulfate-reducing, and methanogenic conditions [8–13].

Most research on the biodegradation of quinolines has used pure cultures [6,7,12–17], but a few studies used soil microcosms to degrade quinolines [9,10,18]. Under aerobic conditions, biodegradation pathways for the microbial transformation of quinoline and isoquinoline involve hydroxylation, ring cleavage of the homocyclic and heterocyclic rings, and carboxylation [7,19]. Under anaerobic conditions, the degradation of quinolines involves the initial hydroxylation at position 2 [9–12,20,21]. Johansen et al. [12] proposed that the sulfate-reducing bacterium *Desulfobacterium indolicum*, after this initial hydroxylation, transforms quinoline to 3,4-dihydro-2(1H)-quinolinone, which is further transformed into unidentified products. 6- and 8-methylquinoline were converted to 6- and 8-methyl-3,4-dihydro-2(1H)-quinolinone by this microorganism, whereas 3- and 4-methyl-2(1H)-quinolinone were not degraded. Isoquinoline and 2-methylquinoline were not degraded by *D. indolicum* in their study. Reineke et al. [21] investigated the degradation of methylquinolines under nitrate-, sulfate- and iron-reducing conditions in microcosms with aquifer material of a former coke manufacturing site. The degradation activities were only revealed under sulfate-reducing conditions, and the hydroxylated methylquinolines, with the exception of 4-methyl-2(1H)-quinolinone, were recalcitrant. Little data is available on the metabolism of quinolinic compounds under denitrifying conditions. Johansen et al. [11] found that quinoline and 3-, 4-, 6-, 8-methylquinoline were transformed by hydroxylation into their 2-hydroxyquinoline analogues, and isoquinoline was transformed to 1-hydroxyisoquinoline. The hydroxylated metabolites of isoquinoline and quinolines methylated at the heterocylic ring were not transformed further, whereas metabolites of quinoline and quinolines methylated at the homocyclic ring were hydrogenated at position 3 and 4, and the resulting 3,4-dihydro-2-quinolinone analogues accumulated. They also found that 2-methylquinoline was not degraded at all.

Wastewaters from coal-coking and pharmaceutical industries usually contain relatively high concentrations of ammonia and

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N-heterocyclic compounds [22,23]. In WWTPs, ammonia is usually removed by nitrification under aerobic conditions followed by denitrification. Therefore, during the biological treatment of these wastewaters, N-heterocyclic compounds will also be exposed to nitrate-reducing conditions. Previous studies by Li et al. [8,23] indicated that a nitrate containing anaerobic reactor is effective in eliminating most N-heterocyclic compounds, including quinolines. However, detailed information about the degradation pathways, rates, optimal conditions, etc. is limited.

As denitrification is a process for both organic compound degradation and nitrate reduction, and neither organics nor nitrate is desired to be released into the environment during wastewater treatment or in situ remediation, it is also of interest to investigate the optimum C/N ratio for both the degradation of quinolines and denitrification. The optimum C/N ratio in this paper is defined as the COD/NO_3–N ratio at which COD is completely degraded and nitrate or nitrite is completely reduced.

In the present study, batch experiments were conducted to investigate the degradation potential of quinoline, isoquinoline and 2-methylquinoline under nitrate-reducing conditions with the three quinolines as sole carbon sources, respectively. The optimum COD/NO_3–N ratios for quinoline degradation and denitrification were determined, and the degradation metabolites of the three quinolines were also analyzed. These studies have important engineering implications for the treatment of wastewater containing quinolinic compounds.

2. Materials and methods

2.1. Synthetic wastewaters

Quinoline, isoquinoline and 2-methylquinoline were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Synthetic wastewaters were prepared using tap water supplemented with minimal media containing KH_2PO_4 (0.6 g/L), $CaCl_2$ (0.1 g/L), and $MgSO_4$ (0.1 g/L). One of the three quinolines was spiked and sodium nitrate was supplemented at desired COD/NO_3–N ratio. The COD for quinoline, isoquinoline and 2-methylquinoline was theoretically estimated based on the following reactions:

$$C_9H_7N + 10O_2 \rightarrow 9CO_2 + 2H_2O + NH_3 \quad (1)$$

$$C_{10}H_9N + \tfrac{23}{2}O_2 \rightarrow 10CO_2 + 3H_2O + NH_3 \quad (2)$$

where 1.0 g of quinoline or isoquinoline is equivalent to 2.48 g of COD; 1.0 g of 2-methylquinoline is equivalent to 2.57 g of COD.

2.2. Acclimation of inocula

The seed sludge was obtained from a coal-coking wastewater treatment facility in Baoshan Steel Factory (Shanghai, China). This facility has been in operation for more than 10 years, and consists of an anoxic and aerobic treatment system. The seed sludge was obtained from the anoxic tank and to avoid impurities such as ammonia, nitrate and N-heterocyclic compounds, the seed sludge was washed using tap water and separated by static sedimentation three times in the laboratory. After decanting the supernatant, the mixed liquor suspended solid (MLSS) was 12 g/L. This was evenly divided into three cylindrical plexiglass sequencing batch reactors (SBR) and filled the top with synthetic wastewaters containing quinoline, isoquinoline and 2-methylquinoline, respectively. The working volume of each SBR was 8 L. The reactors were sealed with plexiglass lids. In each reactor, mixing was achieved by a motor-driven mixer with two four-blade propellers (at 1/3 and 2/3 of the reactor height). It was set to a speed of 60–70 rpm that was sufficient to completely mix the wastewater and activated sludge, but not to shear the flocs.

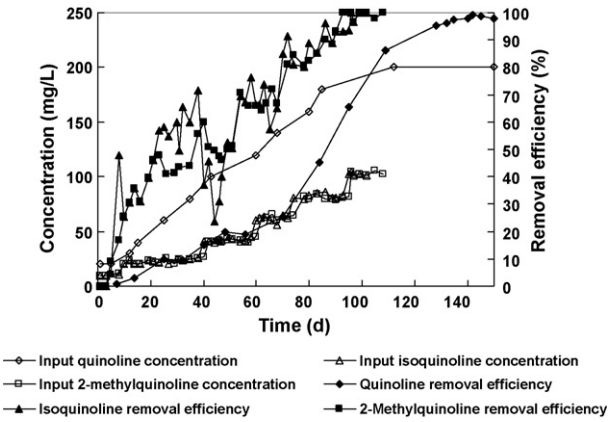

Fig. 1. Input concentrations and removal efficiencies of the three quinolines during activated sludge acclimation.

The reactors were incubated at a temperature-controlled room at around 25 °C.

When quinoline was used as the sole carbon source, the SBR was operated in a cycle time of 24 h, and each cycle consisted of 5 phases: fill (0.25 h), react (21 h), settle (2.0 h), draw (0.25 h) and idle (0.5 h). When isoquinoline and 2-methylquinoline were used as the sole carbon source, respectively, the SBR cycle time was 30 h with the fill, react, settle, draw and idle time of 0.25 h, 27 h, 2.0 h, 0.25 h and 0.5 h, respectively. Initially, the MLSS in each reactor was about 7000 mg/L, and concentrations of quinoline, isoquinoline and 2-methylquinoline were 20 mg/L, 10 mg/L, and 10 mg/L, respectively. When quinoline removals and denitrification rate were stable, quinoline, isoquinoline and 2-methylquinoline concentrations were gradually increased to 200 mg/L, 100 mg/L, and 100 mg/L, respectively (steps of 10–20 mg/L). Nitrate was amended to a COD/NO_3–N ratio of 5. Fig. 1 shows the concentrations and removal efficiencies for the three quinolines during acclimation. After 3–4 months, the removal efficiencies of all the three quinolines were greater than 95% and MLSS concentrations in the three reactors had reached steady state. The final MLSS concentrations in the reactors were 3250 mg/L, 3080 mg/L and 2940 mg/L when quinoline, isoquinoline and 2-methylquinoline were used as the sole carbon source, respectively.

2.3. Determination of the optimum COD/NO_3–N ratio

Optimum COD/NO_3–N ratios for the degradation of quinolines were investigated using 1-L Erlenmeyer flasks. Acclimated sludge was removed from the SBRs, and was washed using tap water to eliminate residual quinolines. After gravity separation and decanting the supernatant, the sludge was re-suspended in the minimal media. About 600 mL of the mixed liquor containing 1.8 g of MLSS was transferred to each flask. The number of the flasks depended on the COD/NO_3–N ratios tested. To determine the optimum COD/NO_3–N ratio for both quinoline degradation and denitrification, the flasks were spiked with quinoline concentration in the range of 40–150 mg/L, and nitrate was added for COD/NO_3–N ratios of 3, 5, 7, 8, and 10, respectively. When isoquinoline or 2-methylquinoline was used as the sole carbon source, as only small amounts of nitrate were consumed, a wider range of COD/NO_3–N ratio was tested. The flasks were capped with butyl rubber stoppers after spiking, and their headspace was purged with nitrogen for 20 min. Mixing was accomplished using a magnetic stir bar (~90 rpm).