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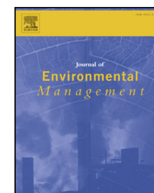

Research article

Simultaneous anaerobic and aerobic transformations of nitrobenzene

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ABSTRACT

Aerobic biodegradation of nitrobenzene (NB) produces nitrophenol (NP), which has stronger toxicity than NB. Anaerobic biodegradation of NB produces aniline (AN), which has weaker toxicity, but is a dead-end product in anaerobic conditions. Accumulation of AN should be overcome by coupling anaerobic and aerobic transformations: NB is transformed to AN in an anaerobic zone of the bioreactor, and AN is then transformed in an aerobic zone. A vertical baffled bioreactor (VBBR) was employed for NB biodegradation with a controlled dissolved oxygen (DO) concentration. NB biodegradation was accelerated by simultaneous anaerobic and aerobic transformations, since AN was biotransformed by a mono-oxygenase reaction. Adding exogenous electron donor (acetate) enhanced NB removals when the DO concentration was ~0.5 mg/L, because the donor accelerated mono-oxygenations of NB and AN. Coupling anaerobic and aerobic transformations can be a valuable strategy for biodegrading organic compounds that undergo aerobic and anaerobic biotransformations.

1. Introduction

Nitrobenzene (NB) is an important organic intermediate for producing dyes, pesticides, fluorescent brighteners, and medicines (Huang et al., 2012; Mu et al., 2009; Haderlein et al., 2011a,b; Wang et al., 2011), and it is found in wastewaters from all of these industries. NB is harmful to fish and algae (Liu et al., 2018), and it is biodegraded slowly in natural environments (Yang et al., 2015). Removing NB from wastewaters can be accomplished by physical and chemical methods (Lipczynska-Kochany, 1991; Li et al., 2007; Zhang et al., 2007; Qin et al., 2007; Carlos et al., 2010), but biological treatment would be the most cost-effective if it were reliable (Kulkarni and Chaudhari, 2007; Gurevich et al., 1993; Huang et al., 2012).

NB biodegradation is initiated by a mono-oxygenation reaction in an aerobic pathway or by a series of reductions in an anaerobic pathway (Nishino and Spain, 1995; Peres et al., 1998; Haderlein and Schwarzenbach, 1995; Haigler and Spain, 1991). The initial steps of both routes are shown in Fig. 1, and they generate distinctly different products: NB is transformed to nitrophenol (NP) in the aerobic pathway and to aniline in the anaerobic pathway (Haigler and Spain, 1991; Yang et al., 2015). NP is more inhibitory to microorganism than NB (Sponza and Kuscu, 2011; Yen et al., 2002; Donlon et al., 1995), and its accumulation will decelerate NB biodegradation (Yang et al., 2015). One way to avoid NP formation is to utilize anaerobic transformation to aniline (AN), which is less inhibitory than NB (Lin et al., 2009; Yen et al., 2002). However, AN is a dead-end product in anaerobic conditions (Field, 2002), because ring opening at a non-trivial rate requires molecular oxygen (O_2) (Cheng et al., 2015; Huang et al., 2012; Sun et al., 2015), as shown in Fig. 1.

The dilemmas that come from accumulation of NP or AN could be overcome by coupling anaerobic and aerobic transformations: NB is transformed to aniline in an anaerobic zone of the bioreactor, and aniline is then transformed in an aerobic zone (Pan et al., 2014; Peres et al., 1998). In this work, we employed a vertical baffled bioreactor (VBBR) (Cao et al., 2017) for NB biodegradation, because the VBBR can provide anaerobic and aerobic zones simultaneously in one system: Biofilm at the bottom of the cylinder is aerobic, while biofilm at the top of cylinder is anaerobic (visualized by its black color, as shown in Fig. S1(b) of Supplementary Information, SI) due to gradual depletion of dissolved oxygen (DO) along the flow path. During the experiments, we controlled the DO in the VBBR to realize differing degrees of aerobic and anaerobic conditions.

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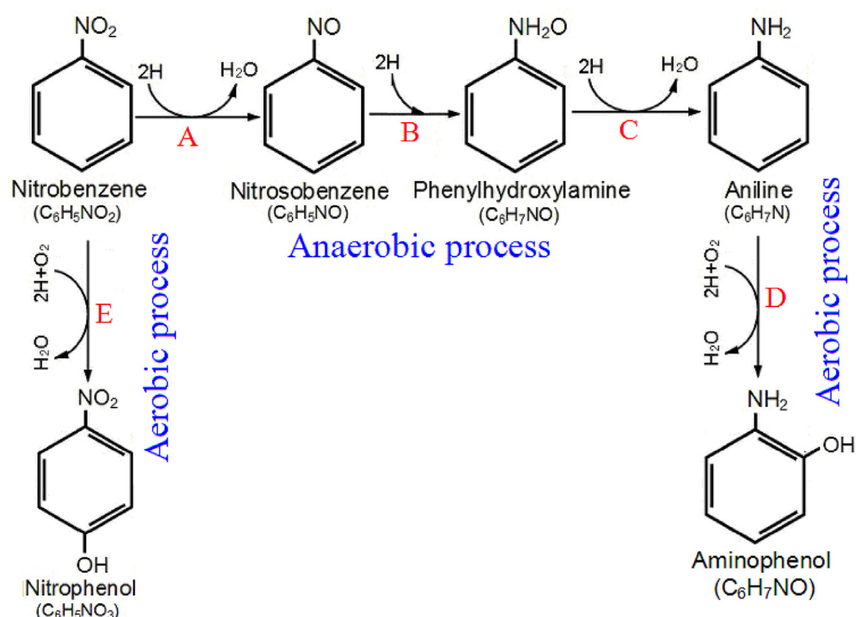

Fig. 1. Initial steps of aerobic and anaerobic pathways for nitrobenzene biodegradation (Haigler and Spain, 1991; Yang et al., 2015; Peres et al., 1998; Cao et al., 2004; Lyons et al., 1984; Marvin-Sikkema and Bont, 1994).

2. Materials and methods

2.1. Chemicals and preparation of nitrobenzene, aniline and nutrient solutions

All analytical reagents, as well as NB and AN, were purchased from Shanghai Sinopharm Chemical Reagent Co. Ltd. in China. 0.83 mL NB and 0.98 mL AN were separately diluted in 1000-mL ultrapure deionized water (18 MΩ, prepared with a Millipore Milli-Q water purifier, USA), and the solutions were stirred for 24 h to obtain NB and AN stocks of 1000 mg/L, which was storied at 4 °C. A mineral-element solution was prepared by diluting 0.4 g of $CaCl_2$, 0.2 g of $MgSO_4{\cdot}7H_2O$, and 0.12 g of $MnSO_4{\cdot}H_2O$ in 1 L of the Milli-Q water. For all experiments, the stock solutions were diluted in tap water to obtain specified initial NB and AN concentrations, and 1 mL mineral-element solution was added per 1 L.

2.2. Acclimation of sludge

Activated sludge was taken from the Changqiao Wastewater Treatment Plant in Shanghai. The sludge was acclimated to NB in two stages. In the first-stage, the sludge was fed with sodium acetate as the sole organic substrate for two weeks. In the second stage, sodium acetate was gradually replaced with NB to obtain NB-degrading bacteria: The NB concentration was stepwise increased to 25 mg/L (0.20 mM) over four weeks, during which 2 mM acetate also was provided in the NB solution. In both stages, fresh medium was provided daily after sedimentation for 30 min and decanting the supernatant. Acclimation was carried out in batch flasks at 30 °C, pH 7.5, and DO of 0.1–0.5 mg/L. After six weeks of acclimation, the biomass had a highly diverse bacterial community (described in SI) that was able to biodegrade 200 μM NB within 30 h.

2.3. Bioreactor

A vertical baffled bioreactor (VBBR) (Cao et al., 2017) was used for NB and aniline biodegradation. A schematic of the VBBR is provided in the Supplemental Information (SI). The VBBR had a total liquid volume of 350 mL that was divided into a top cylinder of 130 mL and bottom water tank of 220 mL. A DO probe was immersed in the water tank.

Acclimated sludge was fed into the reactor to immerse all D-shaped plates for 2 h; some biomass adsorbed to colonize a biofilm. Before biodegradation of NB, the acclimated sludge was fed the VBBR to initiate a biofilm, which was further acclimated for two weeks. Fresh medium containing 10 mg/L NB (0.08 mM) was added every day after the previous day's medium has been drained. During biofilm acclimation, the medium was circulated between the top cylinder and bottom water tank; the DO concentration in the water tank was between 0.2 and 0.5 mg/L. After one week, the biofilm was visible to the eye and able to biodegrade NB and aniline.

2.4. NB-biodegradation experiments

NB-biodegradation experiments were conducted in three sets, all at 30 °C. The first set of experiments was carried out in two 250-mL flasks agitated at 120 rpm on a shaker. One flask was sealed and kept anaerobic; the other was anaerobic for the first 30 h, but then opened and made aerobic for another 34 h. The second set was carried out in the VBBR with the DO set either at ~0.5 mg/L or ≤0.2 mg/L; for the latter, the medium was sparged with N_2 gas. The third set of experiments was carried out in the VBBR by adding different initial concentrations of acetate to investigate the effect of adding extra electron donor on NB and AN biodegradation kinetics with DO of 0.5 mg/L. For all experiments, samples were taken at time intervals to measure the progression of NB, AN, and NP concentrations, as well as soluble chemical oxygen demand (sCOD).

2.5. Analytical methods

NB, AN, and NP were measured with an HPLC (model: Ultimate 3000, USA) equipped with a diode-array detector (DAD) set at a wavelength of 295 nm and a ZORBAX SB-C18 column (5 μm, 4.6 × 150 mm, Agilent). The mobile phase was methanol:water (60:40, V/V) at a flow rate of 1 mL/min. The DO was measured with a DO meter (model: HACH, HQ 30d, USA), with the sensor immersed in the VBBR's water tank. COD was measured using potassium dichromate oxidation according to standard procedures (APHA, 2001). All samples were filtered through a 0.22-μm membrane filter before measurement.

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