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Fate and toxicity of melamine in activated sludge treatment systems after a long-term sludge adaptation

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ABSTRACT

Melamine is a nitrogen-rich (67% nitrogen by mass) heterocyclic aromatic compound that could significantly increase effluent total nitrogen concentrations. In this study, we investigated the degradation of melamine and its impact on activated sludge operations by employing two common activated sludge processes, namely the Modified Ludzack-Ettinger (MLE) process and the continuous stirred tank reactor (CSTR) process. Melamine was dosed continuously from day 125 in both activated sludge treatment systems at an influent concentration of 3 mg/L for about 100 days. Even after such a long period of sludge adaptation, melamine appeared not to be easily biodegradable. The average melamine removal efficiencies in the CSTR and MLE systems were 14 ± 10% and 20 ± 15%, respectively. There was no significant difference in melamine removal between the two different activated sludge processes. The long-term input of melamine resulted in a decrease in the nitrifying bacterial activities (by 82 ± 8%) and population in both systems. Short-term microtiter assay results also showed that melamine reduced activated sludge growth by 80% when supplied at a concentration of 75.6 mg/L. These results suggest that sludge adaptation plays a minimal role in melamine degradation, as the enzymes responsible for hydrolytic deamination of melamine in activated sludge are not easily induced. The insignificant biodegradation of melamine is also attributed to bacterial growth inhibition under long-term dosing conditions with melamine, resulting in a significant decrease in effluent water quality.

1. Introduction

Melamine ($C_3H_6N_6$), chemically known as 1, 3, 5-triazine-2, 4, 6-triamine, is a nitrogen-rich (67% nitrogen by mass) heterocyclic aromatic compound commonly used to make plastic for food containers and flame retardants (Costa and Camino, 1988; Salaün et al., 2011). Incidents of pet-food contamination by melamine and a 2008 Chinese milk scandal raise concerns about the impact of melamine on wastewater treatment operations and effluent water quality. With more stringent nutrient discharge limits for wastewater, it is important to understand the fate and toxicity of melamine in wastewater treatment systems.

Although it is not carcinogenic, melamine is well known to cause urinary stones and acute renal failure in human and animals. A combination of melamine and cyanuric acid (one of the melamine degradation byproducts) in a diet may lead to acute kidney failure (Dobson et al., 2008; Puschner et al., 2007). Long-term exposure to melamine may also result in sperm DNA damage and abnormalities (Zhang et al., 2011). The

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toxicity of melamine to microorganisms is, however, rarely reported. Polyvinylalcohol (PVA) gel plate studies showed that melamine inhibited yeast growth at a concentration of 500 mg/L (Nishimura et al., 2002). Toxicity to activated sludge and nitrifying bacteria (*Nitrosomonas*) was not observed after a short-term (<2 h) exposure at melamine concentrations of 1992 mg/L and 100 mg/L, respectively (Hockenbury and Grady, 1977; UNEP, 2002). However, the impact on microbial growth after long-term activated sludge exposure to melamine is largely unknown.

To date, only a few soil bacteria have been isolated that are capable of degrading melamine via stepwise hydrolytic deamination reactions producing ammeline, ammelide, and cyanuric acid, sequentially (Boundy-Mills et al., 1997; Cook and Hütter, 1981; El-Sayed et al., 2006; Shelton et al., 1997). Melamine can also be hydrolyzed by melamine deaminase (TriA) from *Acidovorax avenae* subsp. *citrulli* strain NRRL B-12227 (Seffernick et al., 2001, 2000). Cyanuric acid is further subject to hydrolytic ring cleavage, producing CO_2 and NH_4^+–N via hydrolysis of biuret and allophanate (Cheng et al., 2005; Cook, 1987; Nenner and Schulz, 1975).

Sludge acclimation or adaptation generally improves degradation rates of recalcitrant organic compounds in the environment (Hu et al., 2005a, 2005b). Adaptations contribute to the fitness and plasticity of microorganisms in response to environmental stress and chemical exposure. There are several interrelated adaptation mechanisms including (i) selective enrichment of microorganisms, (ii) induction and/or depression of specific enzymes, and (iii) genetic changes resulting in new metabolic capabilities (Leahy and Colwell, 1990; Rittman and McCarty, 2001). Hence, a selection and concentration of specialized bacteria during acclimation improve biodegradation rates of synthetic organic chemicals such as nitrobenzoate and chlorophenol (Hu et al., 2005a, 2005b).

Earlier studies have shown that the inherent biodegradability of melamine by unacclimated activated sludge is very low, ranging from 0 to 16% after continuous aeration at 24 °C in the dark for 28 days (UNEP, 2002). The objective of this study was to determine the fate and toxicity of melamine in activated sludge systems and to evaluate whether long-term sludge adaptation can improve melamine degradation.

2. Materials and methods

2.1. Bioreactor setup and operation

Two lab-scale activated sludge systems were operated in parallel by employing two commonly used activated sludge treatment processes during this study. Each system had a working volume of 7.4 L. For the bioreactor using the Modified Ludzack-Ettinger (MLE) process, the system was composed of sequential anoxic and aerobic chambers separated by a glass baffle. The effective volumes for the anoxic, aerobic, and internal settling chambers were 1.9, 3.8 and 1.7 L, respectively. There was a recirculation from the aerobic chamber to the anoxic chamber in the MLE system at a flow rate equal to the influent flow rate. The continuous stirred tank reactor (CSTR) system was a completely mixed bioreactor with aeration and settling chamber effective volumes of 5.7 and 1.7 L, respectively. For each bioreactor, a fine bubble diffuser and a magnetic stirrer provided mixing and aeration in the aeration chamber.

The synthetic wastewater primarily contained nonfat dry milk powder with a target chemical oxygen demand (COD) concentration of 500 mg/L, 50 mg/L total N, 30 mg/L NH_4^+–N and 6 mg/L PO_4^{3-}–P. The synthetic wastewater also contained the following micronutrients per liter: 44 mg·$MgSO_4$, 14 mg·$CaCl_2 \cdot 2H_2O$, 2 mg·$FeCl_2 \cdot 4H_2O$, 3.4 mg·$MnSO_4 \cdot H_2O$, 1.2 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.8 mg·$CuSO_4$, 0.3 mg·$NiSO_4 \cdot 6H_2O$, and 1.8 mg·$Zn(NO_3)_2 \cdot 6H_2O$ (Sigma Aldrich, St Louis, MO) (Liang et al., 2010a, 2010b).

The two bioreactors were operated with a hydraulic retention time (HRT) of 0.75 d and target solids retention time (SRT) of 15 d. At the beginning of reactor operation, a total of 2000 mL of activated sludge taken from the aeration basin of a local municipal wastewater treatment plant (WWTP) (Columbia, MO) was added as an inoculum to each bioreactor. The bioreactors were operated and monitored for 227 days, which was divided into two phases. Phase I consisted of the first 124 days of operation before melamine dosing. Phase II started on day 125, with continuous melamine dosing at an influent concentration of 3 mg/L. This concentration was chosen considering some industrial wastewater streams containing melamine and its derivatives (e.g., melamine formaldehyde) at a concentration of about 30 mg/L (Othman, 2012) and the fact of dilution with other wastewater prior to entering the WWTP.

2.2. Effect of long-term melamine dosing on bioreactor performance

Melamine (99%) was purchased from Acros Organics. From day 125 onwards, a melamine stock solution with a concentration of 122.8 mg/L was fed separately into each bioreactor at a flow rate of 0.172 L/d. This was mixed with influent synthetic wastewater to reach an influent nominal concentration of 3 mg/L in each bioreactor. The change in HRT due to melamine addition was negligible because the flow rate of melamine stock was much lower than the influent (6.9 L/d). Wastewater effluent from each bioreactor was collected and analyzed for melamine, NH_4^+–N, NO_3^-–N, NO_2^-–N, and COD following the standard methods (APHA, 1998).

Aliquots of mixed liquor were periodically taken from the aeration zone to determine the nitrifying bacterial activities. These were inferred from specific oxygen uptake rate (SOUR) measurements using extant respirometry (Hu et al., 2002). Briefly, 120 ml aliquots of biomass sample (for duplicate measurements) were collected from the aeration chamber of each bioreactor and poured into two 50 ml respirometric bottles. After 3 min of aeration with pure oxygen gas, the respirometric bottles were tightly capped with no air space. MOPs (3-(N-morpholino) propanesulfonic acid) (Sigma Aldrich, St Louis, MO) were added to reach a final concentration of 20 mM to maintain a constant pH of 7.5. At predetermined times, an aliquot of substrate (10 mg N/L NH_4^+–N) was injected using a 10-μL glass syringe. The decrease of the dissolved oxygen (DO) level in the respirometric bottles due to substrate oxidation was measured with a DO probe (YSI