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Removal of triclosan in nitrifying activated sludge: Effects of ammonia amendment and bioaugmentation

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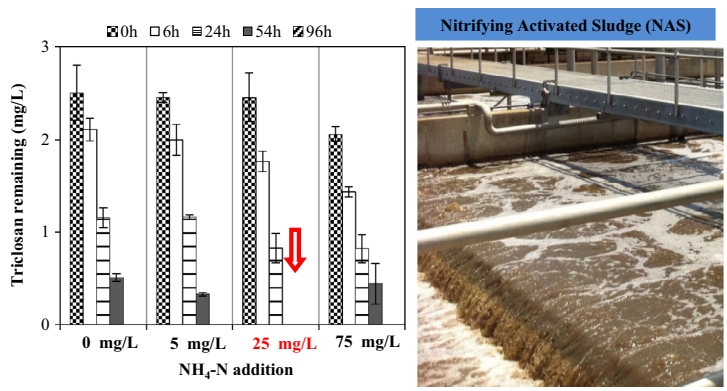
HIGHLIGHTS

- Faster triclosan removal was highly related to higher ammonia oxidation in NAS.
- NAS showed slower triclosan removal when ammonia oxidation was inhibited.
- Bioaugmentation with strain KCY1 did not enhance triclosan removal in NAS.
- This study indicated the important role of unknown triclosan degraders in NAS.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated two possible strategies, increasing ammonia oxidation activity and bioaugmenting with triclosan-degrader *Sphingopyxis* strain KCY1, to enhance triclosan removal in nitrifying activated sludge (NAS). Triclosan (2 mg L^{-1}) was removed within 96-h in NAS bioreactors amended with 5, 25 and 75 mg L^{-1} of ammonium (NH_4–N). The fastest triclosan removal was observed in 25 mg NH_4–N L^{-1} amended-bioreactors where high ammonia oxidation occurred. Inhibition of ammonia oxidation and slower triclosan removal were observed in 75 mg NH_4–N L^{-1} amended-bioreactors. Triclosan removal was correlated to the molar ratio of the amount of nitrate produced to the amount of ammonium removed. Bioaugmentation with strain KCY1 did not enhance triclosan removal in the bioreactors with active ammonia oxidation. Approximately 36–42% and 59% of triclosan added were removed within 24-h by ammonia-oxidizing bacteria and unknown triclosan-degrading heterotrophs, respectively. The results suggested that increasing ammonia oxidation activity can be an effective strategy to enhance triclosan removal in NAS.

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1. Introduction

Numerous household and personal care products contain triclosan, a broad-spectrum antimicrobial agent (Schweizer, 2001). The presence of triclosan in the environment could pose a potential public health risk by promoting antibiotic-resistant microorganisms (Braoudaki and Hilton, 2004). Triclosan is also known as an endocrine-disrupting compound (Gee et al., 2008). Moreover, upon exposure to sunlight or UV light, environmental triclosan can be transformed into more toxic compounds such as chlorinated dioxins (Latch et al., 2005).

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Wastewater is one major source of environmental triclosan. Approximately 72–94% of triclosan in wastewater can be removed by activated sludge systems in wastewater treatment plants (WWTPs) (Singer et al., 2002; Roh et al., 2009). However, a considerable amount of triclosan still remains in the treated wastewater effluent, which is subsequently discharged into receiving waters. As a result, triclosan has been frequently detected in surface water, marine environment, soils, sediments, and even in humans (Singer et al., 2002; Allmyr et al., 2006). To minimize the occurrence of environmental triclosan, activated carbon treatment systems and nanofiltration/reverse osmosis membranes could be used to effectively remove triclosan (Westerhoff et al., 2005; Nghiem and Coleman, 2008; Hernandez-Leal et al., 2011). However, these methods are neither cost-effective nor destructive for triclosan. Accordingly, an inexpensive and destructive method such as biological degradation for triclosan during wastewater treatment is needed.

The findings of two previous studies (Lee et al., 2012; Roh et al., 2009) have implicated two possible treatment strategies to enhance triclosan removal by biological treatment processes. Roh et al. (2009) demonstrated that ammonia monooxygenase (AMO) for the oxidation of ammonia to nitrite in ammonia-oxidizing bacteria (AOB), known as an important group of autotrophic bacteria in nitrifying activated sludge (NAS), is responsible for triclosan degradation in NAS. This study suggested that it might be possible to enhance triclosan removal through improving ammonia oxidation activity during wastewater treatment. Lee et al. (2012) reported an effective wastewater triclosan-degrading isolate, *Sphingopyxis* strain KCY1 (hereafter referred as strain KCY1). The strain KCY1 can rapidly and completely dechlorinate triclosan, remove the androgenicity of triclosan and retain its triclosan degradation ability when grown in complex nutrient medium with ambient triclosan concentrations reported in wastewater. Bioaugmentation is an effective treatment strategy to enhance the removal of specific compounds in natural or engineered systems when effective degradative microorganisms are added (Van Limbergen et al., 1998). Thus, strain KCY1 could be an ideal candidate as a bioaugmenting agent to enhance triclosan removal in NAS.

This study investigated two strategies for enhancing triclosan removal in NAS: (i) by increasing the activity of ammonia oxidation and (ii) bioaugmenting with strain KCY1. To test these strategies, ammonium in the range of 0–75 mg NH_4–N L^{-1} and/or strain KCY1 were amended into a series of laboratory-scale bioreactors containing NAS and triclosan. Removal of triclosan in the bioreactors was evaluated and correlated to changes in ammonia oxidation, strain KCY1, AOB, and *amoA* genes (encodes the active site of AMO for ammonia oxidation).

2. Materials and methods

2.1. Chemicals

Triclosan (97% pure) and allylthiourea (ATU; 98% pure) were purchased from Sigma–Aldrich Inc. (St. Louis, MO). Triclosan stock solution of 10 g L^{-1} was prepared in acetone. N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) were purchased from Pierce Biotechnology Inc. (Rockford, IL). FastDNA SPIN kit and FastDNA SPIN kit for soil were purchased from MP Biomedicals (Solon, OH). Primers and probes for real-time PCR assays were purchased from Sigma–Aldrich Inc. (St. Louis, MO). Quanti-Tect probe PCR supermix and SYBR Green I were purchased from Qiagen (Valencia, CA).

2.2. Bacterial strains and culture conditions

Strain KCY1, a wastewater triclosan-degrading bacterium isolate (Lee et al., 2012), is available in house and was pregrown in a flask containing 20% R2A medium (Teknova, Hollister, CA) and 2 mg L^{-1} of triclosan. The flask was incubated aerobically on a rotary shaker at 160 rpm at 30 °C for 2 days. The cells in the late exponential phase (OD_{600} = 0.8–1.0) were harvested by centrifugation at 10,000*g* for 5 min for bioaugmentation use.

2.3. Nitrifying activated sludge (NAS)

Nitrifying activated sludge (NAS) was collected from a local wastewater treatment plant (WWTP) in College Station, TX, in November 2011 and May 2012. The WWTP uses single-sludge nitrification process to treat 9.5 million gallon of wastewater per day. Operating with a solid retention time (SRT) of 7–8 days, the WWTP was able to achieve 97–98% BOD removal and 95% ammonia removal. The NAS from this WWTP was used for bioreactor experiments to examine the effects of ammonium addition and bioaugmentation on triclosan removal. The genomic DNA of the NAS was used as a template to quantify the total microbial population, strain KCY1, AOB and *amoA* gene (Hermansson and Lindgren, 2001; Roh and Chu, 2011).

2.4. Bioreactor setup and experimental approach

A total of twenty batch bioreactors (1-L glass flasks) were constructed and divided into two sets: twelve in Set A and eight in Set B (Table S3). The NAS collected in November 2011 was used for Set A and the NAS collected in May 2012 was used for Set B. Eight bioreactors in Set A (referred as #A0, #A5, #A25 and #A75; duplicate) were amended with four different ammonium concentrations (0, 5, 25 and 75 mg NH_4–N L^{-1}) to determine the effect of ammonia oxidation by ammonium amendment on triclosan removal. Ammonium stock solution was prepared from ammonium sulfate ($(NH_4)_2SO_4$) for the bioreactor setup. The range of ammonium concentrations added in the bioreactors was based on the typical ammonia concentration (from 12 to 45 mg NH_4–N L^{-1}) in domestic wastewater (Tchobanoglous et al., 2003).

The bioreactors in Set A (#A25-KCY1 and #A25-KCY1*; duplicate) were amended with 25 mg NH_4–N L^{-1} and strain KCY1 (the initial average concentration of 2.2×10^9 16S rRNA gene copies mL^{-1}) to investigate the combined effects of ammonia oxidation and bioaugmentation on triclosan removal. The bioreactor #A25-KCY1* received 10 mg L^{-1} of allylthiourea (ATU), an inhibitor of ammonia-oxidizing bacteria (AOB) activity (Rasche et al., 1991), to determine the effect of bioaugmentation with strain KCY1 in NAS where promoted AOB activity with the addition of 25 NH_4–N mg L^{-1} was absent (the amount of ammonium addition was experimentally determined in this study).

Four bioreactors in Set B (referred as #B0-KCY1 and B0-KCY1*; duplicate) were inoculated with strain KCY1 to examine the effect of bioaugmentation on triclosan removal in NAS with no amendment of ammonium. Two types of live controls were used; one without ATU (#B0) and the other one with ATU (#B0*) (Table S3). The initial average concentration of strain KCY1 in #B0-KCY1 and B0-KCY1* was 8.7×10^8 16S rRNA gene copies mL^{-1}. The bioreactor B0-KCY1* containing strain KCY1 and ATU was used to assess the role of strain KCY1 in triclosan removal while AOB activity was absent.

Activated sludge samples were aerated for 1 day to reduce its dissolved organic matter content before use for the bioreactor setup. Each bioreactor was supplied with 200 mL of aerated activated sludge (mixed liquor volatile suspended solids (MLVSS) of 2870 mg L^{-1} in Set A and MLVSS of 2420 mg L^{-1} in Set B), ammonium (and/or strain KCY1), and triclosan (2 mg L^{-1}). The initial concentration of ammonium and nitrate in Set A were 23.3 mg NH_4–N L^{-1} and 10.7 mg NO_3–N L^{-1}, respectively. For Set B, the initial concentration of ammonium and nitrate were 21.2 mg

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