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Research article

The fate of cyanuric acid in biological wastewater treatment system and its impact on biological nutrient removal

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ABSTRACT

Cyanuric acid (CA) is widely used in living and production. It is a kind of environmental priority pollutants which exists chronically in soil and water, but is difficult to be chemically hydrolyzed or oxidized. The behavior of CA at different levels of 0, 0.01, 0.10 and 1.00 mg L^{-1} in biological wastewater treatment process was investigated in this paper. Experimental results showed that CA (0.01 and 0.10 mg L^{-1}) was removed in biological wastewater treatment process, which was mainly achieved by biodegradation of particular species (*Acidovorax* and *Pseudomonas*) in the anaerobic condition. However, 1.00 mg L^{-1} CA was reluctant to be degraded in biological wastewater treatment system. With the CA level increase from 0 to 1.00 mg L^{-1}, total nitrogen removal efficiency decreased from 97.23 to 74.72%. The presence of CA promoted both the synthesis and decomposition metabolisms of poly-hydroxyalkanoates and glycogen, thereby providing the advantage for phosphorus removal. CA could inhibit nitrification process because of inhibition to nitrite oxidizing bacteria (NOB). Moreover, the microbial community of activated sludge was changed by the exposure of CA. Polyphosphate accumulating organisms, such as *Bacteroidetes*, *Chloroflexi* and *Saccharibacteria* increased, but the abundance of *Nitrospirae* was decreased.

1. Introduction

Cyanuric acid (CA), $C_3H_3N_3O_3$, 1,3,5 - triazine - 2,4,6 - triol, which is an odorless white hygroscopic crystalline powder and important commercial compound, was first synthesized by Wöhler in 1829 (Dodge et al., 2013). As a kind of raw material, CA is used to synthesize hundreds of fine chemicals, such as cross-linked polymers on fire retardant, antioxidant, decolorizer, paint, adhesive, herbicide, drug, and polymer modifier.

Besides, it is widely added to outdoor pools and recreational waters to stabilize chlorine (Zeng et al., 2003). The maximum stabilization of CA occurs in outdoor pools and recreational waters between 50 ppm and 100 ppm (Zeng et al., 2003). It is also utilized to disinfect for swimming pool, drinking water and breeding environment, especially in case of emergency (Magnuson et al., 2001). Besides, CA could be added to fodder as a kind of non-protein nitrogen source for ruminant which is approved by Food and Drug Administration (FDA). CA is an important intermediate metabolite of triazine herbicides (e.g. atrazine, simazine and prometryn) which are anthropogenic chemicals frequently found in water bodies, particularly following floods and periods of heavy rain and runoff from agricultural lands (Xiao et al., 2015). Hence the degradation of these pesticides by microbial degradation or chemical methods leads to CA accumulation (Benzaquén et al., 2016).

CA is included in the EC environmental priority pollutants list (Galindez-Najera et al., 2009). It has shown chemical toxicity to aquatic organisms such as carps, larval lampreys and zooplankton (Galindez-Najera et al., 2009). Furthermore, CA in admixture with melamine adding to pet food, infant milk and dairy product is toxic. It is suspected to be potential toxicity to gastrointestinal or liver in

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humans and fatal to animals owing to kidney failure. CA could slow the oxidation of organic compounds and inhibit the growth rates of bacteria, viruses, and algae in disinfection, but high CA levels impaired the disinfection process, leaving people vulnerable to the attacks and infections of viruses, bacteria, and protozoa (Yeom et al., 2015).

CA is generally difficult to be chemically hydrolyzed or oxidized (Benzaquén et al., 2016). In previous studies, researchers tried to use chemical oxidization (Rodriguez et al., 2004), photocatalysis (García-López et al., 2007) or combined methods (Watanabe et al., 2005) to mineralize s-triazine herbicides and dyes containing the triazine ring, but all the attempts were almost terminated at CA as the end product. The extensive application of CA inevitably causes it entering into the environment, especially the water environment. Wastewater treatment plant (WWTP) is the final defense line before the contaminants entering the aquatic environment. On the one side, the biological processes in WWTP may absorb and decompose CA to avoid the contact of CA and water environment. On the other side, these biological processes in WWTPs aer executed by a series of microbes, thus the presence of CA or its metabolic intermediate could cause adverse impacts on these microbes. In previous articles, most of contaminants entering into WWTPs were verified to affect wastewater and sludge treatment (Xu et al., 2017; Yang et al., 2017; Yi et al., 2017). However, up until now, the fate of CA in biological wastewater treatment system and its impact on biological nutrient removal have been barely reported.

The purpose of this work was therefore to investigate the behavior of CA in wastewater treatment process. First, the removal and degradation potential of CA in the wastewater treatment system were evaluated. Then, the effect of CA ranging from 0, 0.01, 0.10 and 1.00 mg L^{-1} on biological nitrogen and phosphorus removal were investigated. Finally, the mechanisms of CA affecting biological nitrogen and phosphorus removal were explored from the aspects of the transformations of metabolic intermediates, the variation of extracellular polymeric substance, and the change of microbial community. The findings obtained in this study can serve as a model to better understand the fate of a man-made chemical in WWTPs.

2. Materials and methods

2.1. Synthetic wastewater

The synthetic wastewater consisted of CH_3COONa, NH_4Cl, KH_2PO_4, $MgSO_4$ and $CaCl_2$, and the characteristics were (average): 300–350 mg L^{-1} chemical oxygen demand (COD_{Cr}), 35 mg L^{-1} NH_4^+-N, 10 mg L^{-1} soluble orthophosphate (SOP). The synthetic wastewater also contained trace elements (each litre): 0.03 mg $CuSO_4 \cdot 5\ H_2O$, 0.06 mg $Na_2MoO_4 \cdot 2\ H_2O$, 0.12 mg $ZnSO_4 \cdot 7\ H_2O$, 0.12 mg $MnCl_2 \cdot 4\ H_2O$, 0.15 mg H_3BO_3, 0.15 mg $CoCl_2 \cdot 6\ H_2O$, 0.18 mg KI, 1.5 mg $FeCl_3 \cdot 6\ H_2O$, 10 mg EDTA. The initial pH was controlled at 7.0 ± 0.1 which was adjusted by 1 M $NaHCO_3$ and 1 M HCl, respectively.

2.2. The operation of parent sequencing batch reactor

The inoculated sludge was obtained from the secondary sedimentation tank outlet of a WWTP in Changsha, China. A parent sequencing batch reactor (SBR) with a working volume of 24 L was operated to achieve biological nitrogen and phosphorus removal. It was operated at 22 ± 1 °C with 3 cycles daily. Each cycle consists of anaerobic (90 min) - aerobic (150 min) - anoxic (120 min) - settling (55 min) - decanting (5 min) - idle periods (60 min) (Supplementary Fig. S1). 16 L of the supernatant was discharged from the reactor after the settling period, and was replaced with 16 L synthetic wastewater during the first 5 min of the anaerobic phase. At the end of the anoxic phase of the 2nd cycle 1.6 L of the mixtures was withdrawn before settling to maintain the sludge retention time (SRT) about 15 days. The experimental rationale is explained in Supplementary material.

2.3. CA exposure to activated sludge

Four replicate SBRs were operated in the test, with a working volume of 3 L each. 12 L of activated sludge mixture was withdrawn from the parent SBR and equally divided into the four reactors after the stable operation for 100 days. All SBRs were conducted the same as the parent SBR. To obtain more information about CA's impact on nitrogen and phosphorus removal, four CA concentrations of 0, 0.01, 0.10 and 1.00 mg L^{-1} were selected and performed with 4 SBRs, named SBR1, SBR2, SBR3 and SBR4, respectively. The batch experiments of exposure test were continuously operated for 90 days.

2.4. The biodegradation experiment of CA

Three wide mouth conical flasks with the volume of 500 mL each were used to investigate the biodegradation of CA. 450 mL sludge was taken from the parent SBR at idle stage, then divided equally into 3 parts and added to 3 wide mouth conical flasks (500 mL) numbered as CA-S, CA-AN and CA-AO. CA-S was set up as the control test after high-temperature sterilization. CA-AN and CA-AO were cultured under aerobic condition (aeration) and anaerobic condition (stirring), respectively. Dissolved Oxygen was approximately controlled at 2.0 mg L^{-1} in aerobic and below 0.5 mg L^{-1} in anaerobic condition. The experimental apparatus diagram was shown in Fig. S2 (in Supplementary material). 350 mL wastewater with CA was added to each flask and kept the initial concentration of CA at 5.00 mg L^{-1}. The concentration of CA in three conical flasks was detected every day.

2.5. Analytical methods

Mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), COD_{Cr}, SOP (PO_4^{3-}-P), ammonia (NH_4^+-N), nitrite (NO_2^--N), and nitrate (NO_3^--N), were analyzed in accordance with the Standard Methods (APHA, 1998). Glycogen was measured by anthrone-sulfuric acid method (Wang et al., 2017b). The total of poly-hydroxyalkanoates (PHA), including Poly-3-hydroxybutyrate (PHB), poly-3-hydroxyvalerate (PHV), and poly-3-hydroxy-2-methylvalerate (PH2MV) were detected by gas chromatography (Shimadzu, GC, 2010C), the method was reported in our previous publication (An et al., 2017).

CA and the possible intermediate products (biuret, allophanate) was analyzed by Agilent 1100 series HPLC system (with an 1100 series automatic injector) according to the previous studies (Bensalah et al., 2016). The detection method of urea was displayed in Supplementary material.

2.6. Microbial diversity analysis

The biological community diversity analysis was conducted with a SBR with the working volume of 3 L. The activated sludge was taken from the parent SBR. The operations were consistent with the 4 SBRs in Section 2.3. The experiment was composed of 3 stages with 3 CA concentration gradients (0, 0.10 and 1.00 mg L^{-1}). Each stage was lasted for 30 days. The samples of activated sludge were taken at the 30th day (ASCA0), the 60th day (ASCA1) and the