Journal of Hazardous Materials xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

The behavior of melamine in biological wastewater treatment system

Hongxue An[a,b], Xiaoming Li[a,b,*], Qi Yang[a,b], Dongbo Wang[a,b,*], Ting Xie[a,b], Jianwei Zhao[a,b], Qiuxiang Xu[a,b], Fei Chen[a,b], Yu Zhong[a,b], Yujie Yuan[a,b], Guangming Zeng[a,b]

[a] College of Environmental Science and Engineering, Hunan University, Changsha 410082, China
[b] Key Laboratory of Environmental Biology and Pollution Control, Hunan University, Ministry of Education, Changsha 410082, China

HIGHLIGHTS

- The removed of melamine (MA) was mainly performed by activated sludge adsorption instead of biodegradation.
- High concentration of MA could not easily removal and had adverse impacts on biological wastewater treatment.
- MA inhibited the enzyme activities of NOR, NR, NIR and PPX, which were closely relevant to nitrogen and phosphorus removal.
- High MA concentrations promoted the metabolism of glycogen, thereby providing the advantage for the growth of GAOs.

ARTICLE INFO

Article history:
Received 8 June 2016
Received in revised form 13 October 2016
Accepted 16 October 2016
Available online xxx

Keywords:
Melamine
Biological nitrogen and phosphorus removal
Activated sludge
Toxicity

ABSTRACT

Melamine (MA) is a significant raw material for industry and home furnishing, and an intermediate for pharmacy. However it is also a hazardous material when being added to food as a protein substitute due to the high nitrogen content. In this study, the behavior of MA in activated sludge was investigated. Experiments showed that MA was removed during biological wastewater treatment process, and the removal was mainly achieved by activated sludge adsorption instead of biodegradation. Low levels of MA (0.001–0.10 mg/L) in wastewater had negligible influence on the performance of activated sludge, but high levels of MA deteriorated biological nitrogen and phosphorus removal. The presence of MA (1.00 and 5.00 mg/L) decreased total nitrogen removal efficiency from 94.15% to 79.47% and 68.04%, respectively. The corresponding concentration of effluent phosphorus increased from 0.11 to 1.45 and 2.06 mg/L, respectively. It was also observed that MA inhibited the enzyme activities of nitrite oxidoreductase, nitrate reductase, nitrite reductase and exopolyphosphatase, which were closely relevant to nitrogen and phosphorus removal. Further investigation showed that the presence of high MA concentrations promoted the consumption and synthesis of glycogen, thereby providing the advantage for the growth of glycogen accumulating organisms.

1. Introduction

Melamine (MA, 1,3,5-triazine-2,4,6-triamine or 2,4,6-triamine-1,3,5-triazine, $C_3N_3(NH_2)_3$), which is a heterocyclic aromatic compound and a type of industrial material with high nitrogen content (66.67% by mass), widely exists in the environment. It is a significant raw material for the synthesis of melamine formaldehyde resin, such as manufacture of laminates, plastics, coatings, commercial filters, adhesives, dishware, and kitchenware. MA can be also used as water-reducer, formaldehyde detergent, and a chemical intermediate for pharmaceutical manufacturing. In addition, MA and MA salts (such as cyanurate, phosphate, pyrophosphate, polyphosphate, borate, phthalate and oxalate) are extensively used in production and daily life as environmental friendly flame retardants [1,2]. The extensive application of MA inevitably causes its entrance into the environment, especially the water environment. According to the survey on MA level in rivers from 1986 to 1994 in Japan, the concentration of MA ranged from ≤0.0001 to 0.0076 mg/L[3]. Ono et al. reported that wastewater steam from a MA factory had a total solids percentage of 1.80%,

* Corresponding authors at: College of Environmental Science and Engineering, Hunan University, Changsha 410082, China.
E-mail addresses: xmli@hnu.edu.cn (X. Li), w.dongbo@yahoo.com, dongbowang@hnu.edu.cn (D. Wang).

http://dx.doi.org/10.1016/j.jhazmat.2016.10.036
0304-3894/

yielded MA accounted for 1.27% of the total solids [4]. Qin et al. found that the concentration of MA from different MA factories ranged from 22 to 100 μg/L, while levels of 0.167, 1.974, 6.253 and 226.766 mg/L were detected in four other samples. They also measured MA from irrigation water samples, and the highest concentration of 0.198 mg/L was found [5].

MA was widely known by the public due to the relevant unpleasant social events occurred in recent years. It could not be distinguished with protein by Kjeldahl method, thus MA had been illegally adulterated in food to give the illusion of an increase of the apparent protein content [6]. Therefore, MA was detected in the products such as pet foods, powdered infant formula, and hydrated lime nitrogen granular fertilizer [7–9]. Histopathological alterations of tilapia were evident in the liver, gills and kidney in turn, and the severity of lesions associated with the subjected dosage of melamine (10 g/kg feed) [10]. Feed contaminated with MA (250, 500 and 1000 mg/kg) for rainbow trout could cause lipid peroxidation. Fish muscle residues of MA exhibited a dose-response relationship [11]. Mice test (MA, 30, 140 and 700 mg/(kg d)) found that MA could disrupt the blood-testis barrier and cause testicular toxicity [12], and it had ability to increase sperm abnormality rate and DNA damage [13]. The combination of MA and cyanuric acid was responsible for acute renal failure in cats [14]. Monkeys' gavage with MA (700 mg/(kg d)) caused the clinical signs including turbid and whitish urine, urine crystals, red blood cell changes, increased serum alanine aminotransferase, nephrotoxicity, pericarditis and hematopoiesis increasing [15]. According to the carcinogenic potential of MA for humans and animals, MA was classified as a category III carcinogen (the carcinogenicity to humans has not yet to be classified).

Many studies have been carried out to examine the toxicities of MA to organisms, such as fish, rats, cats, and monkeys, and their toxic effects on these species have been reported. As the final defense line prior to entering into the aquatic environment, wastewater treatment plant (WWTP) with activated sludge has been reported to remove many pollutants via aggregation, settling, precipitation, biosorption, degradation, or other processes. The main function of activated sludge is to achieve nitrogen and phosphorus removal from wastewater via a series of biochemical processes, such as nitrification, denitrification, phosphorus anaerobic release and aerobic/anoxic uptake [16]. MA entered into in wastewater treatment process via drainage may also bring risks to these biochemical processes.

Up to now, the behavior of MA in biological wastewater treatment system was rarely reported. Thus, the aim of this work was to evaluate the mass balance of MA in wastewater treatment process and to investigate the potential effect of MA on biological nitrogen and phosphorus removal in a long-term exposure period in an anaerobic/aerobic/anoxic sequencing batch reactor (SBR). This study could provide a theoretical reference for stringent regulation on MA production, marketing, application and ultimate disposal, and give a reminder for water environment security management.

2. Materials and methods

2.1. Synthetic wastewater and seed sludge

The seed sludge was obtained from the secondary sedimentation tank outlet of a WWTP in Changsha, China. The synthetic wastewater consisted of CH_3COONa, NH_4Cl, KH_2PO_4, $MgSO_4$ and $CaCl_2$ to get the average initial concentrations of chemical oxygen demand (COD_{Cr}) 300–350 mg/L, NH_4^+-N 35 mg/L, PO_4^{3-}-P 10 mg/L, and pH 7.00 –7.20. The initial pH was adjusted by 1 M HCl and 1 M $NaHCO_3$, respectively.

Table 1
The dosages of MA in each SBR.

	SBR Reactors					
	SBR1 (Control)	SBR2	SBR3	SBR4	SBR5	SBR6
MA concentration (mg/L)	0	0.001	0.01	0.10	1.00	5.00

2.2. Sequencing batch reactor operation

The study was conducted in a SBR with a working volume of 18 L. The SBR operated 3 cycles daily, and each cycle consisted of a 90 min anaerobic period, a 150 min aerobic period, and a 120 min anoxic period, followed by 55 min settling, 5 min decanting and 60 min idle periods. In the decanting period, 12 L of the supernatant was discharged from the SBR, and 12 L synthetic wastewater was pumped into the reactor during the first 2 min of the anaerobic period. At the beginning of the anaerobic period, the initial pH in the SBR was adjusted to 7.00 ± 0.05 by adding either 1 M HCl or 1 M $NaHCO_3$. The sludge retention time was maintained at approximately 15 d by withdrawing 1.2 L of the sludge mixtures once per day at the end of the anoxic phase before settling.

2.3. Exposure experiments of MA in SBR system

The adaption stage of activated sludge was lasted for 120 days to achieve stable operation. Afterwards, the mixture in the SBR was divided evenly into 6 parts and transferred into 6 identical reactors, with a working volume of 3L each. The 6 reactors were operated the same as parent SBR. These 6 reactors received wastewaters containing 0, 0.001, 0.01, 0.10, 1.0 or 5.0 mg/L of MA, which were prepared by adding relevant volumes of MA stock solution to wastewater (Table 1). The exposure experiments were continuously operated for 90 d.

2.4. Analytical methods

The analysis of COD, NH_4^+-N, NO_3^--N, NO_2^--N, PO_4^{3-}-P, suspended solid (SS) and volatile suspended solid (VSS) were conducted in accordance with the Standard Methods [17,18]. Sulfuric acid-anthrone colorimetry was used for glycogen detection [19,20]. The measurements of ammonia monooxygenase (AMO), nitrite oxidoreductase (NOR), nitrate reductase (NR), nitrite reductase (NIR), exopolyphosphatase (PPX), and polyphosphate kinase (PPK) activities were referred to the methods of others (detailed in Supplementary material) [16,19,21]. The Scanning electron microscope (SEM) analysis was used to detect the surface morphology of active sludge after long-term exposed to MA by the FEI Quanta 200 SEM at 20 kV (detailed in Supplementary material) [16,19]. All experiments were performed in triplicate.

2.5. PHA analysis by GC

Poly-3-hydroxybutyrate (PHB), poly-3-hydroxyvalerate (PHV), and poly-3-hydroxy-2- methylvalerate (PH2MV) were measured by gas chromatography (GC, Shimadzu 2010C) with a HP-5 column (30 m length × 0.32 mm id × 0.25 μm film) (Supplementary material) [19,21,22]. The total polyhydroxyalkanoates (PHA) was calculated as the sum of measured PHB, PHV, and PH2MV.

2.6. Melamine extraction and HPLC analysis

MA (HPLC, ≥98%) was purchased from Shanghai Yuanye biological technology Co.Ltd. (Shanghai, China). Extraction of MA in sample was based upon solid phase extraction (SPE) by poly-Sery

Please cite this article in press as: H. An, et al., The behavior of melamine in biological wastewater treatment system, J. Hazard. Mater. (2016), http://dx.doi.org/10.1016/j.jhazmat.2016.10.036