Available online at www.sciencedirect.com

ScienceDirect

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

Fish larval deformity caused by aldehydes and unknown byproducts in ozonated effluents from municipal wastewater treatment systems

Zhiming Yan, Yu Zhang, Hongying Yuan, Zhe Tian, Min Yang**

State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China

ARTICLE INFO

Article history:
Received 8 February 2014
Received in revised form
30 June 2014
Accepted 18 August 2014
Available online 27 August 2014

Keywords:
Secondary effluent
Ozonated by-products
Larval deformity
Aldehydes
Reclaimed water

ABSTRACT

Ozonated secondary effluents (SEs) from municipal wastewater treatment plants (MWTPs) have been found to cause developmental retardation of fish embryos. This study explored the potential cause of the embryo toxicity formed in ozonated SEs by exposing Japanese medaka (*Oryzias latipes*) (d-rR) embryos to ozonated SE from a MWTP in Tianjin, China. The increase of ozone dose from 0.26 to 0.96 mg O_3/mg DOC_0 (consumed ozone per initial DOC), which produced total aldehyde (mixture of formaldehyde, acetaldehyde, propionaldehyde, and glyoxal) from 41.5 to 114.7 μg/L, resulted in an increase in the percentage of deformed larvae from 2.2% to 24.1%. Increases in larval deformity and embryo mortality were also observed in ozonated SEs from other MWTPs. The exposure experiment using the mixture aldehyde solution showed that the production of aldehydes could explain approximately 13.6% of larval deformity caused by ozonation of SEs. Pilot experimental results in Tianjin and Beijing, China showed that biofiltration as a post-treatment technology was effective in removing the aldehydes as well as reducing embryo toxicity caused by ozonation.

1. Introduction

Because of the increasing shortage of water resources, greater attention has been paid to the reuse of secondary effluents (SEs) from municipal wastewater treatment plants (MWTPs) (Gardner et al., 2001; Liberti and Notarnicola, 1999). Since municipal wastewater often contains micropollutants (Schwarzenbach et al., 2006), further treatment of SEs is usually required before reuse to prevent potentially adverse aqua-ecological effects (Aguayo et al., 2004; Dizer et al., 2002; Zha and Wang, 2005). Among existing wastewater reclamation technologies, ozone has been shown to effectively remove various hazardous pollutants such as endocrine disrupting chemicals (EDCs) and genotoxic chemicals (Cao et al., 2009), a wide range of pharmaceuticals (Huber et al., 2005) as well as pathogenic microbes (Tyrrell et al., 1995) from SEs. Ozonation is also effective for removing color- and odor-causing compounds (Rice et al., 1981). Thus, ozone has been considered a promising technology for wastewater reclamation (Paraskeva and Graham, 2002; Xu et al., 2002; Petala et al., 2006; Petal et al., 2008), and has been increasingly applied in many countries.

However, some adverse effects, such as developmental retardation of Japanese medaka (Cao et al., 2009) and rainbow trout embryos (Stalter et al., 2010), and decrease in reproduction and biomass of lumbriculus variegatus (Magdeburg et al.,

* Corresponding authors.
E-mail address: yangmin@rcees.ac.cn (M. Yang).
http://dx.doi.org/10.1016/j.watres.2014.08.019
0043-1354/

2012), have been observed in ozonated SEs, which might be attributed to the formation of ozonation by-products (OBPs). Aldehydes, particularly formaldehyde, acetaldehyde, and glyoxal, are well known OBPs (Schechter and Singer, 1995; Wert et al., 2007). Some aldehydic compounds have shown carcinogenicity to humans (Zhang et al., 2009) and teratogenicity to rat embryos (Campbell and Fantel, 1983; Thrasher and Kilburn, 2001). Merk et al. reported that formaldehyde can induce DNA–protein crosslinks, which are primary DNA lesions (Merk and Speit, 1998). At the same time, the formation of bromate and brominated organic compounds has also been reported at relatively high ozone doses (Huang et al., 2005; Kim et al., 2007). The sum of aldehydes (e.g. formaldehyde, glyoxal, acetaldehyde), carboxylic acids (e.g. formate), ketones and brominated organic compounds possibly formed due to ozonation have been speculated as the potential cause for the adverse ecotoxicological effects to fish (Stalter et al., 2010). However, direct evidence regarding the effects of the ozonation byproducts remains unavailable.

This study aimed to reveal the potential compounds in ozonated MWTP SEs causing increased fish embryo toxicity. Firstly, we evaluated the adverse effects of ozonation on the development of Japanese medaka (d-rR strain medaka) embryos by exposing them to ozonated SEs from several MWTPs in Tianjin and Beijing, China, and analyzed potential OBPs including four aldehydic compounds (formaldehyde, acetaldehyde, propionaldehyde, and glyoxal), seven frequently observed brominated organic compounds, and bromate in ozonated SEs. Since the aldehydic compounds were the only detectable OBPs in the ozonated SEs, their adverse effects were further confirmed in aldehyde exposure experiments. Finally, we investigated the effectiveness of biofiltration in removing the adverse effects of ozonation in two pilot systems. The results of this study will help better understand and control adverse effects caused by ozonation of SEs.

2. Materials and methods

2.1. Water samples and chemicals

Water samples were collected from effluents of three MWTPs (MWTP-JZZ , MWTP-GBD, and MWTP-KG) and different treatment steps of two pilot plants (Pilot-GBD and Pilot-KG) located in Beijing and Tianjin, China, respectively. Samples were transferred to the laboratory within 4 h after sampling and analyzed within one day. The characteristics of the water samples are summarized in Table S1. The SE samples contained 6.7–9.4 mg/L DOC and 0.6–2.3 mg/L NH_3–N.

Analytical standards for the four aldehydes were obtained from Accustandard® Inc (New Haven, USA). The O-2,3,4,5,6-pentafluorobenzylhydroxyl-amine (PFBHA) was purchased from Floka Co. (Switzerland) for derivatization of aldehydes. All other chemical reagents were of analytical grade if not noted specially. Tap water dechlorinated with an activated carbon column was used as the control water in the exposure experiments and the solvent of aldehyde solutions. No aldehydic compounds were detected in the tap water.

2.2. Pilot experiment

Pilot-GBD system consisting of an ozonation column and a biological aeration filter (BAF) was located in MWTP-GBD in Beijing. Ceramic pellets with an average diameter of 5 mm were used as the biocarrier for the BAF. The empty bed contact time (EBCT) for the BAF was 15 min. The SE from the MWTP-GBD (anaerobic-anoxic-aerobic process with a 1,000,000 m^3/d capacity) was fed into the system at a flow rate of 500 m^3 per day, and the ozone dose applied was approximately 0.7–0.9 mg O_3/mg DOC_0 (consumed ozone per initial DOC).

Pilot-KG system consisting of ozonation and biological activated carbon (BAC) columns was located in MWTP-KG in Tianjin. The EBCT for the BAC was 15 min. The SE from the MWTP-KG (membrane bio-reactor with a 20,000 m^3/d capacity; PVDF membrane with a mean pore size of 0.02 μm was used) was fed into the system at a flow rate of 100 m^3 per day, and the ozone dose applied was approximately 0.4–0.6 mg O_3/mg DOC_0. The two systems were operated for more than one year before the study.

2.3. Bench experiments

The SE from MWTP-JZZ was ozonated in a glass column with an effective volume of 2.5 L. Ozone gas generated from an ozone generator (OS–1N, Mitsubishi Inc. Corp.) connected with an oxygen producer (CP-20, Mitsubishi Inc. Corp.) was introduced into the reactor from the bottom at a flow rate of 0.3 L/min. Feed gas and off-gas ozone concentrations were measured according to Standard Method 422 (APHA, 1985). The contact time in the reactor was 5 min, and different ozone doses were obtained by adjusting the voltage of the ozone generator. The ozonated water samples were supplied for aldehydic compound analysis and exposure experiments using Japanese medaka (*Oryzias latipes*) (d-rR) embryos 2 h after ozonation to ensure residual ozone was depleted.

2.4. Analytical methods

The pH, dissolved oxygen (DO), and conductivity were determined using a portable multi-parameter meter (HACH, USA). The DOC was analyzed on a Total Organic Carbon Analyzer (Phoenix, 8000, Tekmar-Dohrmann Co. USA), and UV absorbance at 254 nm (UV254) by UV3100 (Hitachi Ltd. Japan). Ammonia was determined by colorimetry using nesslerization, and chroma was measured by colorimetry according to the Standard Method of Water and Wastewater Monitoring (State Environmental Protection Administration of China, 2002). The four aldehydes were analyzed by gas chromatography with mass spectrograph (6890GC/5973MSD, Angilent, USA) after derivatization of filtered water samples using O-2,3,4,5,6-pentafluorobenzylhydroxylamine (PFBHA), with analytical method details given in the Supporting Information. The four brominated acetic acids, including dibromoacetic acid (DBAA), bromodichloroacetic acid (BDCAA), bromochloroacetic acid (BCAA), and monobromoacetic acid (MBAA), were detected on a UPLC-MS/MS (ACQUITY UPLC system, Waters, USA) with an Acquity UPLC BEH C8 column (2.1 mm × 100 mm, 1.7 μm particles, Waters USA) according to previous methods (Meng et al., 2010). The three brominated