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Comparative mammalian cell cytotoxicity of wastewater with elevated bromide and iodide after chlorination, chloramination, or ozonation

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A B S T R A C T

Recycling wastewater is becoming more common as communities around the world try to better control their water resources against an increased frequency of either prolonged droughts or intense flooding. For communities in coastal areas, wastewaters may contain elevated levels of bromide (Br^-) and iodide (I^-) from seawater intrusion or high mineral content of source waters. Disinfection of such wastewater is mandatory to prevent the spread of pathogens, however little is known about the toxicity of wastewater after disinfection in the presence of Br^- and I^-. In this study we compared the induction of chronic cytotoxicity in mammalian cells in samples of municipal secondary wastewater effluent amended with elevated levels of Br^-/I^- after disinfection by chlorine, chloramines or ozone to identify which disinfection process generated wastewater with the lowest level of adverse biological response. Chlorination increased mammalian cell cytotoxicity by 5 times as compared to non-disinfected controls. Chloramination produced disinfected wastewater that expressed 6.3 times more cytotoxicity than the non-disinfected controls and was 1.3 times more cytotoxic than the chlorinated samples. Ozonation produced wastewater with cytotoxicity comparable to the non-disinfected controls and was at least 4 times less cytotoxic than the chlorine disinfected wastewaters. These results indicate that compared to chlorination and chloramination, ozonation of wastewater with high Br^-/I^- levels yielded the lowest mammalian cell cytotoxicity, suggesting its potential as a more favorable method to disinfect wastewater with minimizing the biological toxicity in mind.

Introduction

Wastewater reuse was identified as an important means to alleviate pressure on freshwater resources and is attracting attention in the United States (National Research Council Committee, 2012). Reuse requires that the recycled wastewater is disinfected to prevent the spread of pathogens. Due to its efficacy and affordability, chlorine-based disinfection is the most widely adopted technology for wastewater disinfection (Metcalf & Eddy Inc., 2013). Chlorine-based disinfection includes free chlorine (referred to as chlorination hereinafter) or chloramines (referred to as chloramination hereinafter). The latter occurs

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when the ammonia concentration of the wastewater is sufficiently high. However, chlorine is not only ineffective at low concentrations against certain pathogens, such as *Cryptosporidium parvum* (Korich et al., 1990), but also generates disinfection byproducts (DBPs) (Crittenden et al., 2012; Reckhow et al., 1990). Compared to chlorination, chloramination produces lower concentrations of regulated DBPs, such as trihalomethanes (THMs) and haloacetic acids (HAAs), and therefore many drinking water utilities are considering switching from chlorination to chloramination (Seidel et al., 2005). In coastal areas and regions with high halogen content in source waters, Br^- and I^-, two common halide anions that are present at seaside water reclamation facilities, are elevated (Crittenden et al., 2012; Ludzack and Noran, 1965). Chlorination and chloramination can oxidize Br^- to hypobromous acid and I^- to hypoiodous acid (Qi et al., 2004; Sun et al., 2009). In the presence of wastewater organic matter (WOM) the slow decay kinetics of the hypobromous and hypoiodous acid may encourage the formation of brominated DBPs (Br-DBPs) and iodinated DBPs (I-DBPs) from reactions between hypobromous (Qi et al., 2004; Sun et al., 2009) and hypoiodous acids with organic precursors (Bichsel and Von Gunten, 2000). Thus, the formation of Br- and I-DBPs during wastewater chlorination and chloramination could be a potential public health concern when the disinfected wastewater is recycled.

In addition to chlorine-based disinfection, ozonation is being increasingly used for wastewater disinfection (Gottschalk et al., 2010). However, more studies of pathogen disinfection, DBP formation, and cytotoxicity related to ozonation were conducted in drinking water rather than in wastewater. Under drinking water conditions, ozone has been shown to inactivate a number of pathogens such as the Norwalk virus (Shin and Sobsey, 2003), poliovirus (Majumdar et al., 1973), and even chlorine-resistant pathogens such as *C. parvum* (Cho and Yoon, 2007; Corona-Vasquez et al., 2002; Kim et al., 2007, 2004; Tang et al., 2005). However, enhanced Br^-/I^- levels found in source waters in coastal areas could pose a potential problem because ozonation has been shown to generate Br- and I-DBPs (Bichsel and Von Gunten, 1999, 2000; Zeng et al., 2016). Compared to the chlorinated DBPs (Cl-DBPs), Br-DBPs and I-DBPs are more cytotoxic and genotoxic to mammalian cells (Plewa and Wagner, 2009; Richardson et al., 2008). Because ozonation of wastewater for reuse has been shown to form a broad range of DBPs due to the higher concentration of organic and inorganic constituents (Wert et al., 2007), safe practice of wastewater reuse requires systematic studies focusing on toxicity of ozonated wastewater.

The cytotoxicity and genotoxicity of disinfected drinking water were found to highly correlate with total organic bromine (TOBr) and total organic iodine (TOI) and weakly and inversely correlate with total organic chlorine (TOCl) (Yang et al., 2014). Thus, the generated Br- and I-DBPs rather than the Cl-DBPs were proposed to be the forcing agents for cytotoxicity and genotoxicity in drinking water containing high level of Br^-/I^- (Yang et al., 2014). However, lowered genotoxicity in the presence of Br^- after chlorination of a municipal secondary effluent was also reported (Wu et al., 2010). These partially contradictory results may be attributed to the complex chemical composition of the wastewater, suggesting the need to comparatively quantify the cytotoxicity of wastewater for reuse after different disinfection technologies. The objective of this study was, therefore, to identify which disinfection technology would generate disinfected wastewater effluents with the lowest mammalian cell cytotoxicity when enhanced Br^-/I^- levels were present in a secondary effluent wastewater. The use of a single wastewater source in this study allowed the comparison to be conducted without the complication of different WOM. The findings will shed light on selecting the disinfection technology that minimizes the potential biological toxicity.

1. Materials and methods

1.1. *Water sampling, processing, and characterization*

Samples were collected from the Northeast Wastewater Treatment Plant (NEP) in Urbana, Illinois. At the NEP, the raw sewage flows through a series of preliminary, primary, secondary, and tertiary treatments to remove the majority of the solids, organic matter, and ammonia, before it is disinfected and discharged. The samples were taken from a secondary clarifier after the activated sludge treatment but before the nitrification tower. To eliminate the interference of suspended solids, the samples were filtered through 1.6 μm glass fiber filters and stored in the dark at 4°C until used (within a week of collection). The total organic carbon (TOC) was measured by a Shimadzu TOC analyzer (Shimadzu Scientific Instruments, Columbia, MD) to be 7.3 mg C/L. The absorbance at 254 nm was measured by a Beckman UV–vis spectrophotometer (Beckman Coulter Life Sciences, Indianapolis, IN) to be 0.144 cm^{-1}. Specific UV Absorbance at 254 nm ($SUVA_{254}$) was therefore calculated to be 1.97 m/(mg·L). Background total bromine and iodine was determined by ICP-MS previously to be both below 0 μg/L (Dong, 2016). Ammonium nitrogen (7.5 mg/L), free and total chlorine were measured using Hach kits (Loveland, CO). Combined chlorine was calculated as the difference between the total and free chlorine. The pH of the filtered wastewater was determined to be 7.5 at room temperature.

1.2. *Disinfection experiments*

Thirteen liters of the secondary effluent samples was treated with free chlorine (75 mg/L as Cl_2, Cl_2 to NH_3-N mass ratio of 10), combined chlorine (17.3 mg/L as total Cl_2, Cl_2 to NH_3-N mass ratio of 2.3), or ozone (3 mg/L) in the presence of Br^- (500 μg/L) and I^- (100 μg/L). All disinfectant concentrations were of engineering relevance. The concentrations of Br^- and I^- were used in previous studies to represent waters that were impacted by high levels of Br^- and I^-, such as desalinated seawater (Wu et al., 2010; Yang et al., 2014). For chlorine-based disinfection experiments, all reactions were carried out in amber glass bottles with Teflon-lined caps wrapped in aluminum foil. Ozonation took place in clear round bottom flasks sealed with caps. To achieve breakpoint chlorination, previous studies reported Cl_2 to NH_3-N mass ratio of between 7.6 to 10 (Yang et al., 2014) and 15 (Agus et al., 2009). We conducted preliminary experiments to ensure that when operated at a Cl_2 to NH_3-N mass ratio of 10, more than 97% of available chlorine was free chlorine after rapid mixing. Similarly, for chloramination, a

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