Science of the Total Environment 550 (2016) 184–191

Contents lists available at ScienceDirect

Science of the Total Environment

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

Reduction of antibiotic resistance genes in municipal wastewater effluent by advanced oxidation processes

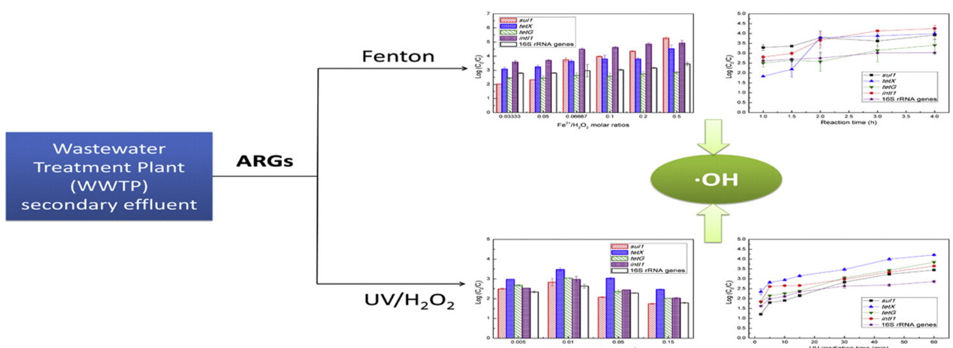
Yingying Zhang, Yao Zhuang, Jinju Geng *, Hongqiang Ren, Ke Xu, Lili Ding

State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, Jiangsu 210023, People's Republic of China

HIGHLIGHTS

- AOPs including Fenton oxidation and UV/H_2O_2 process could reduce ARGs effectively.
- Fenton oxidation is slightly more effective than UV/H_2O_2 process in ARG reduction.
- Removal of ARGs by AOPs follows the first-order reaction kinetic model.
- Selected ARGs and 16S rRNA genes exhibit similar change trends during AOPs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 August 2015
Received in revised form 12 January 2016
Accepted 14 January 2016
Available online xxxx

Editor: Kevin V. Thomas

Keywords:
Antibiotic resistance genes
Advanced oxidation processes
Fenton oxidation
UV/H_2O_2
Municipal wastewater effluent

ABSTRACT

This study investigated the reduction of antibiotic resistance genes (ARGs), *intI1* and 16S rRNA genes, by advanced oxidation processes (AOPs), namely Fenton oxidation (Fe^{2+}/H_2O_2) and UV/H_2O_2 process. The ARGs include *sul1*, *tetX*, and *tetG* from municipal wastewater effluent. The results indicated that the Fenton oxidation and UV/H_2O_2 process could reduce selected ARGs effectively. Oxidation by the Fenton process was slightly better than that of the UV/H_2O_2 method. Particularly, for the Fenton oxidation, under the optimal condition wherein Fe^{2+}/H_2O_2 had a molar ratio of 0.1 and a H_2O_2 concentration of 0.01 mol L^{-1} with a pH of 3.0 and reaction time of 2 h, 2.58–3.79 logs of target genes were removed. Under the initial effluent pH condition (pH = 7.0), the removal was 2.26–3.35 logs. For the UV/H_2O_2 process, when the pH was 3.5 with a H_2O_2 concentration of 0.01 mol L^{-1} accompanied by 30 min of UV irradiation, all ARGs could achieve a reduction of 2.8–3.5 logs, and 1.55–2.32 logs at a pH of 7.0. The Fenton oxidation and UV/H_2O_2 process followed the first-order reaction kinetic model. The removal of target genes was affected by many parameters, including initial Fe^{2+}/H_2O_2 molar ratios, H_2O_2 concentration, solution pH, and reaction time. Among these factors, reagent concentrations and pH values are the most important factors during AOPs.

1. Introduction

With the frequent consumption of antibiotics for human domestic or hospital use and veterinary and agriculture purposes, these compounds have been continuously released into the environment, resulting in a widespread invasion of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) into wastewater, groundwater, and streams (Kummerer, 2009; Yang et al., 2014). Antibiotic resistance provides organisms with the ability to potentially transmit resistance to other organisms by horizontal gene transfer, which is mediated by mobile genetic elements (MGEs), including plasmids, transposons, bacteriophages, integrons, and combinations of them (Philippe and Douady, 2003; Rizzo et al., 2013). The World Health Organization (WHO)

* Corresponding author.
E-mail address: jjgeng@nju.edu.cn (J. Geng).

http://dx.doi.org/10.1016/j.scitotenv.2016.01.078

identified the development of antibiotic resistance as one of the major global threats for human society (WHO, 2013). ARGs have been recognized as the emerging environmental contaminants (Kemper, 2008; Pruden et al., 2006; Zhang et al., 2009).

Municipal wastewater treatment plants (WWTPs) are considered as the main anthropogenic reservoirs for ARG spread into the environment (LaPara et al., 2011; McKinney and Pruden, 2012; Michael et al., 2013; Rizzo et al., 2013). Conventional WWTPs are typically based on biological processes, which only partially remove ARGs; consequently, they are released into the effluent (Behera et al., 2011; Lupo et al., 2012). In addition, biological processes are suspected to contribute to ARG selection as well as resistance transfer among bacteria (da Silva et al., 2006). Particularly, activated sludge in WWTPs was reported to significantly promote the frequency of conjugative transfer of ARGs, because of a high density of bacterial community (Kim et al., 2014; Yang et al., 2013). ARGs detected in effluents from WWTPs are at levels far above those in typical aquatic environments (McKinney and Pruden, 2012). In recent years, at least 38 types of tetracycline resistance genes (*tets*) have been identified. Particularly, *tetX* and *tetG* showed high concentrations in different environmental compartments in China (Cheng et al., 2013; Shi et al., 2013), and *tetG* encodes that an efflux pump was found frequently in integrons or plasmids (Auerbach et al., 2007). *Sul1* is the most detected sulfonamide resistance gene (*sul*) in the environment (Munir et al., 2011). Integrons are always investigated, particularly Class 1 integrons, which contain the *intI1* gene because of its ability to capture and spread gene cassettes containing ARGs (Chen and Zhang, 2013). As the expression of background bacteria has become more prevalent, 16S rRNA genes have been identified as taxonomic markers of bacteria (Ju et al., 2014).

In general, ARGs in WWTP effluent can only be removed partly by the conventional disinfection process, including chlorination, UV irradiation, and ozonation, when doses are higher than those commonly reported in WWTPs (Zhang et al., 2015b; Zhuang et al., 2015), which may generate disinfection by-products. Advanced oxidation processes (AOPs) (such as Fenton oxidation, photo-Fenton process, TiO_2 photocatalysis, and UV/H_2O_2 process) are among the most applied and studied advanced treatment technologies, which aim at improving the quality of the secondary effluent before disposal or reuse (Rizzo et al., 2013). They are characterized by different ways, because they can generate the highly reactive and nonselective hydroxyl radical (•OH) and other reactive oxygen species (Gogate and Pandit, 2004b), which can oxidize a broad range of organic pollutants significantly (Alfano et al., 2001; Bin and Sobera-Madej, 2012; Pereira et al., 2014; Zuorro et al., 2014). Recent studies have indicated that AOPs have the potential to damage DNA so as to inactivate some bacteria and genes in wastewater. For example, Karaolia et al. (2014) revealed that solar-driven Fenton oxidation using 50 mg L^{-1} of hydrogen peroxide and 5 mg L^{-1} of Fe^{3+} with a pH around 4.0 could eliminate ARB in different aqueous matrices including distilled water and simulated and real wastewater effluents. UV–TiO_2 photocatalysis has been reported to inactivate a wide range of bacteria, viruses, and protozoa commonly found in water and wastewater (McCullagh et al., 2007). Despite the availability of numerous studies on the effect of AOPs on inactivation of a wide range of organisms in water (De Luca et al., 2013; Gerrity et al., 2011; Malato et al., 2009) and a few reports of the ability of AOPs to inactivate ARB (McCullagh et al., 2007; Oncu et al., 2011; Rizzo et al., 2014; Tsai et al., 2010), to the best of our knowledge, research information on the reduction of ARGs by AOPs is limited, even at the laboratory scale.

Among AOPs, Fenton oxidation and UV/H_2O_2 process have been investigated in advanced wastewater treatment, which showed promising results in the removal of contaminants of emerging concerns (Bin and Sobera-Madej, 2012; Li et al., 2012). They both provide a homogeneous treatment system with no mass transfer limitation (Will et al., 2004). The purpose of this study is to evaluate the efficiency of Fenton process and UV/H_2O_2 treatment to reduce frequently detected ARGs (*sul1*, *tetX*, *tetG*, and *intI1*) and 16S rRNA genes in actual municipal wastewater effluent. The effects of operating parameters such as initial Fe^{2+}/H_2O_2 molar ratios, H_2O_2 concentration, solution pH, and reaction time were studied. The degradation kinetics by Fenton and UV/H_2O_2 treatment was investigated as well. ARG removals under the optimal pH conditions and initial effluent pH condition (pH = 7.0) were compared. The results provide a better understanding of how ARGs can be effectively removed from WWTP effluent.

2. Materials and methods

2.1. Chemicals and reagents

The reagents used in the Fenton process are $FeSO_4 \cdot 7H_2O$ (standard purity ≥99%) and H_2O_2 (30% *w*/*w*). The pH was adjusted by adding 4 M H_2SO_4 and 2 M NaOH. All chemicals used in this study are analytically pure, obtained from Nanjing Chemical Reagent Co. Ltd., China. Milli-Q water, with a resistivity of at least 18.2 MΩ·cm, was produced from a Millipore purification system (Billerica, CA, USA). The mixed cellulose ester filter membrane (0.22 μm) was purchased from Xinya Electronics Co. Ltd., Shanghai, China.

2.2. Wastewater samples

Wastewater samples were collected from the secondary effluent in a WWTP in Nanjing, China, which uses anoxic–aerobic cyclic activated sludge technology (CAST). All samples were collected in roughly equal volumes per hour with composite samples and kept in sterile containers, which were placed in ice and transported to the laboratory for immediate analysis. The pH range of the secondary effluents was 7.0–7.2. The concentrations of chemical oxygen demand (COD), total nitrogen (TN), ammonia nitrogen (NH_3–N), and suspended solids (SS) were found to be 13–39, 9.73–19, 9–12, and 9 mg L^{-1}, respectively. The absorbance at 254 nm (UV_{254}) of the secondary effluents was 0.093–0.150. The original gene copies of the wastewater samples for *sul1*, *text*, *tetG*, *intI1*, and 16S rRNA genes ranged from 1.91×10^5 to 2.19×10^6, 1.26×10^7 to 6.31×10^7, 1.66×10^5 to 1.51×10^6, 5.25×10^5 to 3.31×10^6, and 4.37×10^7 to 3.72×10^9 copies mL^{-1}, respectively.

2.3. Fenton process

Fenton treatment was carried out at ambient temperature according to the following sequential steps. First, 500 mL of wastewater sample was taken in a beaker and magnetically stirred to ensure complete homogeneity and its pH was adjusted to test values by adding H_2SO_4 or NaOH solution. A wide pH range (2.5, 3.0, 3.5, 4.0, 5.0, and 7.0) was selected to obtain the optimal pH value to remove ARGs. The neutral pH value was also set to obtain the removal rate under real wastewater effluent condition. Second, the predetermined Fe^{2+} dose was achieved by adding the necessary amount of solid $FeSO_4 \cdot 7H_2O$. After that, a known volume of 30% H_2O_2 solution was added in a single step to initiate the oxidation process. After a fixed reaction time, stirring was ceased and Fenton oxidation was stopped by adjusting the pH value to 7 by adding NaOH solution. Finally, after the reaction was complete, 200 mL of supernatant was collected and filtered through a 0.22-μm filter for the following analysis.

2.4. UV/H_2O_2 process

Experiments to investigate the UV/H_2O_2 treatment were carried out in a cylinder Plexiglas reactor (height 310 mm and radius 450 mm). The reactor was equipped with a low-pressure 254-nm mercury vapor lamp (Model TUV 16 W T5 4P-SE, Philips) in a quartz sleeve, occupying the central position of the installation setup, which was described in a previous study (Zhang et al., 2015b). The temperature inside the reactor was kept constant (20 ± 2 °C). A 1800 mL of wastewater effluent