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Electro-peroxone pretreatment for enhanced simulated hospital wastewater treatment and antibiotic resistance genes reduction

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ABSTRACT

Hospital wastewater is one of the possible sources responsible for antibiotic resistant bacteria spread into the environment. This study proposed a promising strategy, electro-peroxone (E-peroxone) pretreatment followed by a sequencing batch reactor (SBR) for simulated hospital wastewater treatment, aiming to enhance the wastewater treatment performance and to reduce antibiotic resistance genes production simultaneously. The highest chemical oxygen demand (COD) and total organic carbon (TOC) removal efficiency of 94.3% and 92.8% were obtained using the E-peroxone-SBR process. The microbial community analysis through high-throughput sequencing showed that E-peroxone pretreatment could guarantee microbial richness and diversity in SBR, as well as reduce the microbial inhibitions caused by antibiotic and raise the amount of nitrification and denitrification genera. Specially, quantitative real-time PCRs revealed that E-peroxone pretreatment could largely reduce the numbers and contents of antibiotic resistance genes (ARGs) production in the following biological treatment unit. It was indicated that E-peroxone-SBR process may provide an effective way for hospital wastewater treatment and possible ARGs reduction.

1. Introduction

Antibiotics are widely used for bacterial diseases treatment in human and veterinary medicine, and also used as feed additives for livestock growth. An attendant increase in the prevalence of antimicrobial resistance genes (ARGs) in the environment has become a serious world-wide public health concern of the twenty-first century (Bouki et al., 2013; Ju et al., 2016; Ma et al., 2017). This global problem is particularly urgent in developing countries where high infectious disease burden and high usage of antimicrobial agents in hospitals were existed (Okeke et al., 2005; Qiao et al., 2017; Zhu et al., 2013). Hospital wastewaters contain significant amounts of antibiotic residues which are sufficient to exert selective pressure for resistance development. Therefore, as a hotspot, hospital wastewater plays a major role in the emergence and spread of antimicrobial-resistant bacteria (ARB) (Tello et al., 2012). Especially pathogens spread in hospitals, the possible transmission path of ARGs to both non-pathogenic and pathogenic strains, can easily lead to antimicrobial-resistant pathogens generation, and spread out into environment from hospitals via wastewater systems (Hocquet et al., 2016). Although antibiotic resistance has been considered a worldwide threat, this serious biosecurity risk is largely neglected in current existing environmental pollution treatment settings because the biocompatibility safety assessment of ARB is not the requisite discharge standard in wastewater treatment (Guo et al., 2016; Marti et al., 2014).

Biological approach (activated sludge processes) is the primary method currently applied for wastewater treatment. Certain concentrations of antibiotics in wastewater, in one hand, can seriously inhibit bacterial growth and lead to deterioration on bacterial functions. On the other hand, it can make it easy for bacteria to acquire resistance (Rodriguez-Mozaz et al., 2015a; Su et al., 2015; Zhang et al., 2013), and lead to more serious hurt to human beings. A number of studies assessed the presence of ARGs in hospital effluent, and revealed that the hospital wastewater is the important source of multi-resistant bacteria (Chang et al., 2010; Fuentefria et al., 2011; Hocquet et al., 2016; Korzeniewska et al., 2013; Rodriguez-Mozaz et al., 2015b; Varela et al., 2013). Therefore, further studies are required to design effective processes capable with both cost-effective pollutants removal and the

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ARGs control in hospital wastewater. Advanced oxidation processes (AOPs) are recognized as highly effective and competitive wastewater treatment technology for most organic pollutants, especially for those of high chemical stability or low biodegradability (Elmolla and Chaudhuri, 2012). However, chemical oxidation for complete mineralization of pollutant is usually expensive and thus is hindered in scaled-up wastewater treatment plants (WWTPs). To overcome the respective drawbacks of conventional biological treatment and AOPs, we proposed a promising alternative for hospital wastewaters treatment that combined AOPs with biological treatment, to enhance the pollutant degradation and ARGs removal simultaneously.

In this study, electro-peroxone (E-peroxone) technology was chosen as pretreatment AOPs method. The main mechanism of E-peroxone is that H_2O_2, generated by the electrical process in situ, reacts with O_3 to form hydroxyl radical (·OH) to degrade organic pollutants (Guo et al., 2015). This process added none chemicals and catalysts without secondary pollutants production, offering several advantages over other AOPs and providing a convenient and environmentally-friendly method for wastewater treatment (Li et al., 2013). Simulated hospital wastewater was prepared by mixing municipal wastewater with high strength antibiotic representative ciprofloxacin (CIP). CIP is one of the most important classes of antibiotics based on its therapeutic versatility, and was world-widely used in hospital for antimicrobial therapy (Dodd et al., 2005). Via residues from hospital wastewater treatment effluent and illegally direct run-off, CIP has become one of the most frequently detected antibiotics in the influent of wastewater treatment plants, untreated hospital sewage and surface waters, and therefore may cause serious environmental and biosafety problem (Ferrando-Climent et al., 2014; Mater et al., 2014; Yan et al., 2014).

In the E-peroxone pretreatment process, effects of operational conditions such as O_3 concentration, current and solution pH were firstly discussed and optimized to improve the wastewater biodegradability. Then performance of the combined process was evaluated for efficient treatment. Thirdly, effects of CIP antibiotic and E-peroxone pretreatment on the microbial community in SBR were investigated at phylum and genus level by high-throughput sequencing. Specifically, quantitative real-time PCR was performed to reveal the occurrence and abundance of CIP resistance genes, and effects of E-peroxone pretreatment on ARGs reduction in the following SBR were also discussed in detail.

2. Materials and methods

2.1. Chemicals and simulated hospital wastewater

Analytical grade of CIP ($C_{17}H_{18}FN_3O_3{\cdot}HCl{\cdot}H_2O$, 99.0%) was purchased from Tokyo chemical industry Co., Japan. Other chemicals (e.g. Na_2SO_4, NaOH, and K_2HPO_4, H_3PO_4 and KH_2PO_4,) used were reagent grade and purchased from Tianjin chemical Works Co., China. All solutions were prepared with deionized water.

Simulated hospital wastewater used in this study was prepared by mixing antibiotic agent CIP with domestic wastewater, initial CIP concentration of 200 mg L^{-1}, total organic carbon (TOC) 103.9 mg L^{-1}, chemical oxygen demand (COD) 510 mg L^{-1} and TN 20.96 mg L^{-1}.

2.2. Experimental setup and procedure

2.2.1. Electro-peroxone-SBR system

A schematic of the combined electro-peroxone-SBR system is shown in Fig. 1. E-peroxone part was set up in an acrylic cube reactor with effective working volume of 2 L. Ozone was produced from pure O_2 gas (99.9%) using an ozone generator with gaseous flow meter (DHX-SS-1G, Jiujiu ozone, Harbin, China), and the mixture of O_2 and O_3 from the ozone generator was bubbled into the reactor using a fine bubble diffuser placed at the bottom of the reactor. The electrolysis process was initiated by turning on the direct current (DC) power supply (DJS-292, Leici Co., Shanghai, China).

For SBR part, the effective operating liquid volume was 2.0 L. The reactor was equipped with an air pump and an air diffuser to keep dissolved oxygen (DO) above 3 mg L^{-1}, as well as a stirring plate and a stirrer bar (200 rpm) for mixing. The detailed operation parameters are described in Section 2.2.2.

2.2.2. Electro-peroxone pretreatment

Electro-peroxone pretreatments of simulated hospital wastewater were carried out in a 2.0 L reactor with a continuous supply of O_3 in Electro-peroxone reactor described as above. The O_2 and O_3 mixture was supplied into the reactor with a constant flow rate of 1 L min^{-1}. The O_3 concentration was adjusted within the range of 2 g h^{-1} to 5 g h^{-1}, by varying O_2 inlet valves and operational power of ozone generator. The supporting electrolyte was Na_2SO_4 solution 0.05 M, and the galvanostatic DC power supply was turned on after the ozone generator was switched on for 15 min to reach an almost steady ozone concentration. While the ozone generator was off, using a 6 cm^2 plate (2 cm * 3 cm) anode made of Pt, and a 48 cm^2 (6 cm * 8 cm) cathode made of activated carbon fiber.

The optimal experiment of operational parameters such as O_3 concentration, current and solution pH on the E-peroxone performance were evaluated by controlling variables method respectively: the runs were carried out at ozone dose 2–5 g h^{-1}, at pH 7 and applied current density was 400 mA; at pH 3–11, at ozone dose was 3 g h^{-1} and applied current density was 400 mA; at current density 100–400 mA, at pH 7 and ozone dose was 3 g h^{-1}. Each E-peroxone process was run for 60 min in the batch experiments.

The E-peroxone pretreatment wastewater experiments which was used for the following SBR operation were conducted under the optimized parameters of ozone dose 5 g h^{-1}, current density 400 mA and pH 9, were run for 30, 45, 60 min and 75 min respectively. E-peroxone effluents from the different reaction conditions were subjected to diverse biological tests as COD and TOC. As a result, four effluent samples were chosen at different E-peroxone reaction time to assess the combination effects on the SBR unit, whereas four different pretreatment time were selected at 30, 45, 60 and 75 min.

2.2.3. Operation of SBR

For start-up stage, the SBR was inoculated with 500 mL sludge from the aeration tank of a municipal wastewater treatment plant. Mixed liquor suspended solids (MLSS) in the reactor after inoculation was 3000 mg L^{-1}. The reactor was working under this load for 15 days until MLSS were stable. After the start-up stage, five SBRs with different E-peroxone pretreatment time effluents were simultaneously carried out and continuously operated for 30 days, which were named as S0 (wastewater without E-peroxone pretreatment), S1 (30 min E-peroxone pretreatment), S2 (45 min E-peroxone pretreatment), S3 (60 min E-peroxone pretreatment) and S4 (75 min E-peroxone pretreatment). The detailed operation conditions of the SBR system were described as follows: effective reactor volume 2.0 L, stirring velocity 200 r min^{-1}, DO 3 mg L^{-1}, hydraulic retention time 24 h, MLSS concentration 3000 mg L^{-1} and ambient temperature 25 ± 2 °C. The SBR cycle period was divided into five phases: filling (0.25 h), aeration-reaction (variable), settling (1 h), decanting (0.25 h) and idle (0.25 h). The cycle was repeated 4–6 times to allow cell acclimation and to obtain repetitive results. Daily analysis of COD and TOC of influent and effluent was carried out. MLSS concentration was monitored throughout the operation.

2.3. Analytical procedures

During the treatment, the gaseous products and consumed O_3 from the reactor was led to a terminator after measured by a gas flowmeter, where the remaining ozone was absorbed and measured by KI solution.

TOC was measured using a TOC-VCPH analyzer (Shimadzu Co.