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Fate of antibiotic resistance bacteria and genes during enhanced anaerobic digestion of sewage sludge by microwave pretreatment

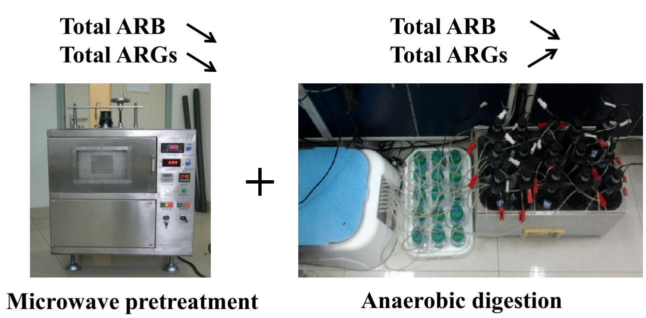
Juan Tong [a], Jibao Liu [a], Xiang Zheng [b], Junya Zhang [a], Xiaotang Ni [a], Meixue Chen [a], Yuansong Wei [a,c,*]

[a] *State Key Joint Laboratory of Environmental Simulation and Pollution Control, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China*
[b] *School of Environment and Natural Resources, Renmin University of China, Beijing 100872, China*
[c] *University of Chinese Academy of Sciences, Beijing 100049, China*

HIGHLIGHTS

- Combined microwave sludge pretreatment and AD could efficiently reduce ARB.
- Most ARGs tended to decrease in pretreatment but enrich after AD.
- *tet*X kept declination during the pretreatment and AD.
- AD with appropriate pretreatment showed slightly better ARB and ARGs reduction.

GRAPHICAL ABSTRACT



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ABSTRACT

The fate of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) were investigated during the sludge anaerobic digestion (AD) with microwave-acid (MW-H), microwave (MW) and microwave-H_2O_2-alkaline (MW-H_2O_2) pretreatments. Results showed that combined MW pretreatment especially for the MW-H pretreatment could efficiently reduce the ARB concentration, and most ARG concentrations tended to attenuate during the pretreatment. The subsequent AD showed evident removal of the ARB, but most ARGs were enriched after AD. Only the concentration of *tet*X kept continuous declination during the whole sludge treatment. The total ARGs concentration showed significant correlation with 16S rRNA during the pretreatment and AD. Compared with unpretreated sludge, the AD of MW and MW-H_2O_2 pretreated sludge presented slightly better ARB and ARGs reduction efficiency.

1. Introduction

Wastewater treatment plants (WWTPs) have been considered to be important reservoirs of antibiotic resistance in recent years (Zhang et al., 2009; LaPara et al., 2011). Many researchers have demonstrated that the activated sludge and biosolids of WWTPs contained large amounts of ARB and antibiotic resistant genes (ARGs) during the wastewater treatment process (Munir et al., 2011; Naquin et al., 2015).

And the sewage sludge contributes a greater amount of the ARGs and ARB released to the environment relative to that of the effluent wastewater (Munir et al., 2011) because the ARGs removed from influent wastewater are transferred to the sludge (Calero-Caceres et al., 2014). Significant amounts of sewage sludge are produced every year in China, for example, 6.25 million tons of dry solids sludge were produced in 2013 (Yang et al., 2015). Sewage sludge treatment and disposal are key control processes for preventing the spread of antibiotic resistant bacteria and genes

* Corresponding author at: State Key Joint Laboratory of Environmental Simulation and Pollution Control, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China. Tel.: +86 010 62849690; fax: +86 010 62849145.

E-mail address: yswei@rcees.ac.cn (Y. Wei).

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from the WWTPs to the environment, and more attention should be paid on the spread of antibiotic resistance during the sludge treatment and disposal.

Researchers have different opinions on the removal efficiencies of ARB and ARGs during AD. A number of researchers have proposed that thermophilic digestion presented an improved reduction of ARGs and *intI*1 compared with mesophilic digestion (Ghosh et al., 2009; Diehl and LaPara, 2010), whereas others believed that thermophilic digestion was only more effective at reducing certain ARGs and *intI*1 and had a similar or poorer removal performance of other ARGs compared with that of mesophilic digestion (Ma et al., 2011). Thermal hydrolysis pretreatment could significantly reduce all ARGs, but they generally rebounded during subsequent anaerobic and aerobic digestion treatments (Ma et al., 2011). It was reported that the portion of ARGs encoding resistance via target modification increased after AD (Zhang et al., 2015). In general, the mechanisms underlying ARB and ARGs removal and horizontal gene transfer (HGT) during sludge treatment are poorly understood, and satisfactory and practicable sludge treatment processes for ARGs and ARB spread control are still not available.

Sludge pretreatment such as thermal hydrolysis (Cano et al., 2014) and combined microwave (MW) process (Appels et al., 2013), can increase soluble COD (SCOD) of the sludge and then lead to better methane production during AD. Enhanced AD with pretreatment is an important tendency for sewage sludge treatment in the future. And understanding the distribution and fate of the ARGs and ARB during the enhanced AD is necessary to reduce the spread of antibiotic resistance from WWTPs to the environment. Among these pretreatment processes, the MW treatment showed better bacteria inactivation compared with the thermal treatment because of the athermal effect (Hong et al., 2004), which could effectively inactivate certain bacteria, such as coliforms, *Salmonella* spp. and *Clostridium perfringens*, as well as the total bacteria counts during sludge treatment (Hong et al., 2004; Tyagi and Lo, 2012; Kuglarz et al., 2013). The MW treatment (95 °C, 2 min) has been reported to completely eliminate the fecal coliforms in waste activated sludge (WAS) (Tyagi and Lo, 2012), and in another study, after 90 s of MW radiation, the total and fecal coliforms could not be detected in the primary sludge (PS) or WAS (Hong et al., 2004). However, the distribution and fate of ARGs and anaerobic ARB during enhanced AD of sewage sludge pretreated with the combined MW processes remain unknown.

In our previous studies, the factors of the MW combination process were studied, and the microwave-H_2O_2 process was optimized (Wang et al., 2009, 2015). According to our previous studies, three kinds of combined MW pretreatments with subsequent AD were thus investigated in this study for the distribution and fate of ARGs and anaerobic ARB of tetracyclines and β-lactam antibiotics, which is the most popular antibiotics used as veterinary drug and therapy drug for human disease, respectively. To the best of our knowledge, this is the first study on the antibiotic resistance during the AD of sewage sludge with combined MW pretreatment. This study provides a new awareness about the fate of ARGs and ARB during the sludge pretreatment and the subsequent AD.

2. Methods

2.1. Source of sewage sludge

Sludge samples were collected from the inlet sludge of mesophilic egg-shaped digesters in a municipal WWTP in Beijing, China, which was designed to treat 6×10^5 m^3/d of wastewater. The feed sludge of the egg-shaped digesters included only primary sludge (PS), which had a water content of 96.5–97.5%. The mesophilic egg-shaped digesters were operated at a temperature of 38 °C and an SRT of 24–28 d, and they treat 2200–2600 m^3 of primary sludge per day. Sludge samples were transferred to an ice box, immediately transported to the laboratory and stored at 4 °C. Prior to the analysis and further experiments, the sludge samples were filtered through a 1 mm mesh to remove large particles, and they were then dried to measure the total solids (TS) and volatile solids (VS) according to the APHA (Rice et al., 2012).

2.2. Sludge pretreatment methods

Based on our previous research (Wang et al., 2009), a lab-scale microwave reactor with a 25 L of cavity volume was constructed by the Julong Co. Ltd. (Baoding, China), equipped with a lift mixer and a thermocouple temperature sensor operating at 2450 MHz. The power of the MW reactor could be adjusted within the range of 0–1000 W. In this study, the MW reactor was operated at 600 W and had an agitation rate of 50 rpm. Four sampling events were assessed in independent experiments using the combined MW pretreatment, and 500 mL samples of the egg-shaped digester inlet sludge were pretreated under each one of the following conditions: MW (sludge at pH = 7.2 and heated by MW irradiation from 20 °C to 100 °C); MW-H (sludge adjusted by 5 mol/L HCl to pH = 2.5 and heated by MW irradiation from 20 °C to 100 °C); and MW-H_2O_2 (sludge adjusted by 5 mol/L NaOH to pH = 10, heated by MW irradiation from 20 °C to 80 °C, dosed with H_2O_2 (30%) at an H_2O_2:TS ratio of 0.06 (*w*/*w*), and continuously heated with MW irradiation to 100 °C). The reaction time was set at 5 min with a heating rate of 16 °C/min for each test. The pretreated sludge was cooled to room temperature for the TS, VS, pH and alkalinity analyses, and it was neutralized to pH 7.2 before the ARB and ARGs analyses and subsequent AD.

2.3. Enhanced mesophilic anaerobic digestion

The methane production performance of the different pretreated sludge was determined by biochemical methane potential (BMP) assays at 38 °C and an SRT of 30 d using an AMPTS-I instrument (Bioprocess Control, Lund, SWE). The total volume and effective volume of each serum bottle was 650 mL and 400 mL, respectively. A mixture of MW/MW-H/MW-H_2O_2 pretreated sludge and inlet sludge from the egg-shaped digesters at a 3:7 *v*/*v* ratio was added to the serum bottles to compare the influence of different sludge pretreatment methods on the fate of ARB and ARGs, whereas 400 mL of the egg-shaped digester inlet sludge was used as the control group. Three independent experiments were conducted for each test group. The characteristics of each sludge sample group included a pH of 7.2 and an alkalinity of 5081–6868 mg/L.

2.4. Detection and quantification of anaerobic ARB

The egg-shaped digester inlet sludge, pretreated sludge and digested sludge were collected to analyze the distribution and fate of the anaerobic ARB and ARGs. The anaerobic ARB were enumerated by sample dilution and plating on Wilkins-Chalgren anaerobe agars (Oxoid, UK) (Macfarlane et al., 2004) containing an antibiotic at the following concentrations according to the previous studies (Macauley et al., 2006; Brooks et al., 2007): 32 mg/L tetracycline (Sigma, US); 32 mg/L chlortetracycline (Sigma, US); 32 mg/L oxytetracycline (LKT Labs, US); 32 mg/L ampicillin (Goldbio, US); 32 mg/L cephalothin (Sigma, US); 32 mg/L cefotaxime (Goldbio, US). To break up the sludge flocs, each sludge sample was first subjected to a ten-fold dilution using phosphate-buffered saline (PBS, pH = 7.4), and the samples were then mixed with several sterile 3 mm glass beads in a shaker incubator at 180 rpm for 1 h.