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Exposure to polystyrene nanoplastic leads to inhibition of anaerobic digestion system

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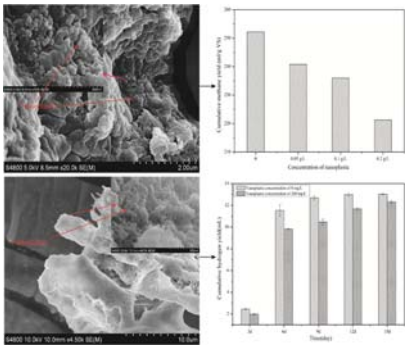
HIGHLIGHTS

- Impacts of nanoplastic on anaerobic digestion system was investigated
- Exposure to nanoplastic leads to inhibition of anaerobic digestion system
- The inhibitory effect was positive correlation with nonaplastic's concentration
- Different microorganism showed unequal response to nanoplastic

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, impacts of nanoplastic on the pure and mixed anaerobic digestion systems were investigated. Results showed the growth and metabolism of *Acetobacteroides hydrogenigenes* were partly inhibited by nanoplastic existed in the pure anaerobic digestion system. The anaerobic digestion of sewage sludge was also obviously inhibited by nanoplastic existed in the mixed anaerobic digestion system. Both the methane yield and methane production rate of the mixed anaerobic digestion system showed negative correlation with the nanoplastic concentration. Compared with anaerobic digestion system without nanoplastic, methane yield and maximum daily methane yield at the nanoplastic concentration of 0.2 g/L decreased for 14.4% and 40.7%, respectively. In addition, the start-up of mixed anaerobic digestion system was prolonged by addition of nanoplastic. Microbial community structure analysis indicated the microbial community structures were also affected by nanoplastic existed in the system. At the nanoplastic concentration of 0.2 g/L, the relative abundances of family *Cloacamonaceae, Porphyromonadaceae, Anaerolinaceae* and *Gracilibacteraceae* decreased partly. Conversely, the relative abundances of family *Anaerolinaceae*, *Clostridiaceae*, *Geobacteraceae*, *Dethiosulfovibrionaceae* and *Desulfobulbaceae* improved partly.

1. Introduction

Plastic is an artificial chemical, which is widely used in daily life (e.g. packaging, films, covers, bags, and containers) (Huerta Lwanga et al. 2016). The numerous application of plastic prompts the plastic industry

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expanded yearly by 8.7% from 1950 to 2012 (Bouwmeester et al. 2015). The production of plastic in 2013 was 299 million metric tons and it was estimated to be 33 billion tons for 2050 (Sussarellu et al. 2016). When the disused plastic emits into environment, it will fragmentized into micro/nano-plastic (<5 mm) by the biological-, photo-, and/or mechanical degradation (Cole et al. 2016). The produced micro/nano-plastic has a great chance to enter and enrich in municipal wastewater treatment plants (WWTPs). In addition, the disused plastic existed in WWTPs can also fragmentize to micro/nano-plastic. WWTPs were also demonstrated as potential sources of microplastics in aquatic systems (Carr et al. 2016; Murphy et al. 2016).

Due to the increased surface area, micro/nano-plastic holds a greater potential to absorbing and desorbing toxic chemicals (Song et al. 2015). In addition, due to the poor biodegradation micro/nano-plastic could interact with organisms by ingestion of consumers, which could facilitate the accumulation of persistent organic pollutants (POPs) into food webs (McCormick et al. 2014). Recently, the toxic impacts of micro/nano-plastic have received increasing attention. Many literatures have demonstrated the toxic impacts of micro/nano-plastic on marine ecosystems (Besseling et al. 2013; Cole et al. 2013; Davarpanah and Guilhermino 2015; Lönnstedt and Eklöv 2016), fresh water ecosystems (Besseling et al. 2014; Eerkes-Medrano et al. 2015; McCormick et al. 2014) and terrestrial ecosystems (Gaylor et al. 2013; Huerta Lwanga et al. 2016).

Anaerobic digestion has been widely applied for stabilization of sewage sludge (Gonzalez-Gil et al. 2016; Liu et al. 2016). The impacts of nano/micro-plastic exist in the sewage sludge on the anaerobic digestion system were less reported. In this study, the impacts of nanoplastic on the pure and mixed anaerobic digestion culture were investigated and analyzed. Scanning electron microscopy (SEM) was used to analyze the interaction among nanoplastic, sewage sludge and microorganisms. In addition, the impacts of nanoplastic on the microbial community structure of anaerobic digestion system were also studied.

2. Experimental section

2.1. Preparation of nanoplastic

Polystyrene nanoparticle with average particle size of 54.8 nm (the size distribution of nanoplastic was shown in Fig. S1) was used to study the effect of nanoplastic on the anaerobic digestion system. Polystyrene nanoparticle was prepared through nitrogen-protected emulsion polymerization with styrene as a monomer and APS as initiator (Wang et al., 2016). The average particle size of nanoplastic was tested by Malvern Nano-s90 Laser Particle Size Analyzer (Malvern, UK).

2.2. Impacts of nanoplastic on the anaerobic digestion of sewage sludge

2.2.1. Substrate and inoculum

Sewage sludge with TS (total solid) of 10.0% and VS (volatile solid) of 76.1% (based on TS) was collected from local WWTPs in Qingdao city and used as substrate for anaerobic digestion (AD) (The TS and VS were measured by standard method ((APHA), 2006)). The collected sewage sludge was stored in a refrigerator at 4 °C until use. In order to accelerate the AD star-up of sewage sludge, biogas slurry collected from a 500 m^3 size of biogas plant (Qingdao, China) was used as inoculum. The biogas plant is running at 35 °C and used corn straw as the substrate. The TS and VS of biogas slurry were 6.26% and 73.71% (based on TS), respectively. The collected biogas slurry was also stored in a refrigerator at 4 °C. Before anaerobic digestion, biogas slurry was pre-activated at 37 °C for 3 days.

2.2.2. Anaerobic digestion of sewage sludge

Triplicate mesophilic (37 °C) anaerobic digestion of sewage sludge was conducted in 300 mL bottles with a working volume of 200 mL. During AD of sewage sludge, the total solid content of fermentation system was 4%, the ratio of $TS_{substrate}$ and $TS_{inoculum}$ was 2:1. Before anaerobic digestion, pH of the system was adjusted to 7.5 using 2 M NaOH and 2 M HCl. To explore the impact of nanoplastic on anaerobic digestion system, polystyrene nanoparticle described as Section 2.1 was added to the fermentation system. The concentrations of nanoplastic in the fermentation system were set as 0, 0.05, 0.1 and 0.2 g/L (marked as N0, N1, N2 and N3). Batch AD of sewage sludge was performed in an air bath shaker at 37 °C with 120 rpm. Biogas yield was tested by water replacement method. The concentration of methane in biogas was tested by gas chromatograph (SP 6890, Shandong Lunan, Inc., China), which equipped with a Porapak Q stainless-steel column (180 cm long and 3 mm outer diameter) and a thermal conductivity detector. The temperatures of the injector, detector, and oven were 50, 100, and 100 °C, respectively. The carrier gas was argon.

2.3. Impacts of nanoplastic on the pure anaerobic digestion culture (using an acetate-type fermentation bacterium - Acetobacteroides hydrogenigenes)

In this study, an anaerobic acetate-type fermentation bacterium-*Acetobacteroides hydrogenigenes* was selected to investigate the impact of nanoplastic on the pure anaerobic digestion culture. The impact of nanoplastic on the *Acetobacteroides hydrogenigenes* was tested in triplicate, which proceeded in 300 mL bottles with a working volume of 150 mL. During the experiment, glucose (0.3 mM) and yeast extraction (0.01%) were used to cultivate *Acetobacteroides hydrogenigenes*. The concentrations of nanoplastic in the pure anaerobic system were set as 0 and 0.2 g/L. The growth and metabolism of *Acetobacteroides hydrogenigenes* were represented by the production of hydrogen. The hydrogen production was also tested by gas chromatograph (SP 6890, Shandong Lunan, Inc., China), which equipped with a Porapak Q stainless-steel column (180 cm long and 3 mm outer diameter) and a thermal conductivity detector. The temperatures of the injector, detector, and oven were 50, 100, and 100 °C, respectively. The carrier gas was nitrogen.

2.4. Scanning electron microscopy (SEM) analysis of the interaction between nanoplastic and sewage sludge/Acetobacteroides hydrogenigenes

The *Acetobacteroides hydrogenigenes* was collected at its logarithmic phase and then dried by vacuum freeze-drying. The sewage sludge was collected after anaerobic digestion, and also dried by vacuum freeze-drying. SEM pictures were taken using Hitachi S-4800 scanning microscope at 3 kV. Samples were mounted onto aluminium pin stubs and sputter-coated with a thin layer of gold.

2.5. Microbial community analysis

During the anaerobic digestion process, the biogas slurries of N0 and N3 were collected with syringe to analyze the microbial structure variations during the anaerobic digestion process. The collected biogas slurries were stored in a − 80 °C refrigerator until next microbial structure analysis step.

During the microbial structure analysis, E.Z.N.A. stool DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) was used to extract the microbial DNA according to manufacturer's protocols. The V4-V5 region of the bacteria 16S rRNA gene were amplified by PCR (95 °C for 5 min, followed by 27 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s and a final extension at 72 °C for 5 min) using primers 515F 5'-GTGCCAGC MGCCGCGG-3'and 907R 5'-CCGTCAATTCMTTTRAGTTT-3′. PCR reactions were performed in triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA.

Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions and quantified