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Effects of metal oxide nanoparticles (TiO_2, Al_2O_3, SiO_2 and ZnO) on waste activated sludge anaerobic digestion

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ABSTRACT

The effect of metal oxide nanoparticles (nano-TiO_2, nano-Al_2O_3, nano-SiO_2 and nano-ZnO) on anaerobic digestion was investigated by fermentation experiments using waste activated sludge as the substrates. Nano-TiO_2, nano-Al_2O_3 and nano-SiO_2 in doses up to 150 milligram per gram total suspended solids (mg/g-TSS) showed no inhibitory effect, whereas nano-ZnO showed inhibitory effect with its dosages increased. The methane generation was the same as that in the control when in the presence of 6 mg/g-TSS of nano-ZnO, however, which decreased respectively to 77.2% and 18.9% of the control at 30 and 150 mg/g-TSS. The released Zn^{2+} from nano-ZnO was an important reason for its inhibitory effect on methane generation. It was found that higher dosages of nano-ZnO inhibited the steps of sludge hydrolysis, acidification and methanation. Also, the activities of protease, acetate kinase (AK) and coenzyme F_{420} were inhibited by higher dosages of nano-ZnO during WAS anaerobic digestion.

1. Introduction

Researches about nanotechnology have drawn much attention due to the unique physicochemical properties of nanoparticles, such as enhanced magnetism, electricity, and optics (Maynard et al., 2006; Roco, 2005). Metal oxide nanoparticles, such as titanium dioxide (TiO_2), aluminum oxide (Al_2O_3), silicon dioxide (SiO_2) and zinc oxide (ZnO), have received increasing interests due to their widespread industrial, medical and military applications (Ellsworth et al., 2000; Miziolek, 2002; Serda et al., 2009). With the world wide utilization of these metal oxide nanoparticles, their potential effects on environment have been investigated, but most studies focused on the toxicity to human health, and soil and aquatic organisms (Franklin et al., 2007; Ge et al., 2011; Limbach et al., 2007).

The increasing use of nanoparticles introduces them intentionally or unintentionally into wastewater treatment plants (WWTPs), which are the last barriers prior to nanoparticles waste (caused mainly by both daily activities and industrial use) environmental release. The existence of nanomaterials in WWTPs has been reported (Gottschalk et al., 2009; Kiser et al., 2009), and the adsorption of activated sludge was reported to be the main mechanism for nanoparticles removal in WWTPs (Ganesh et al., 2010; Kiser et al., 2009, 2010; Limbach et al., 2008). Therefore, nanoparticles would eventually end up in sludge. Large amounts of WAS are produced in municipal WWTPs, which need to be treated before being discharged to the environment. WAS anaerobic digestion for methane generation is a sustainable sludge treatment practice in which both pollution control and energy (methane) recovery can be achieved. Nevertheless the influences of metal oxide nanoparticles on sludge anaerobic digestion have seldom been investigated.

There are some publications discussing the toxicity of metal oxide nanoparticles to pure microbes. For example, nano-Al_2O_3, nano-SiO_2 and nano-ZnO were observed to be harmful to *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas fluorescens* (Jiang et al., 2009). Adams et al. (2006) found that the antibacterial effects of nanoparticles on *B. subtilis* and *E. coli* increased from SiO_2 to TiO_2 to ZnO. Nano-ZnO was observed to cause significant toxicity to the viability of gram negative bacterial cells (Sinha et al., 2011). It is well known that large numbers of different microorganisms participate in sludge anaerobic digestion due to several stages (solubilization, hydrolysis, acidification and methanation) involved. Thus, it is impossible to deduce the negative effect of these metal oxide nanoparticles on sludge anaerobic digestion microorganisms according to the current knowledge of pure microbial species.

The purpose of this study was to investigate the influences of four metal oxide nanoparticles (TiO_2, Al_2O_3, SiO_2 and ZnO) on methane generation during sludge anaerobic digestion and to dig out the mechanisms. Firstly, the effects of three dosages (6, 30 and 150 mg/g-TSS) of these four nanoparticles on methane generation were studied when WAS was anaerobically digested in batch tests. Then, the mechanisms for nanoparticles affecting methane generation were investigated from the role of dissolved metal ions and the changes of products and key enzymes involved each stage (solubilization, hydrolysis, acidification and methanation) of sludge anaerobic digestion.

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2. Methods

2.1. Waste activated sludge: origin and chemical properties

The WAS used in this study was withdrawn from the secondary sedimentation tank of a municipal WWTP in Shanghai, China. The sludge was concentrated by settling at 4 °C for 24 h, and its main characteristics (average data and standard deviations of three tests) are as follows: pH 6.7 ± 0.2, total suspended solids (TSS) 10,070 ± 780 mg/L, volatile suspended solids (VSS) 7700 ± 450 mg/L, soluble chemical oxygen demand (SCOD) 90 ± 14 mg/L, total chemical oxygen demand (TCOD) 10,700 ± 200 mg/L, total carbohydrate 900 ± 530 mg-COD/L, and total protein 5685 ± 150 mg-COD/L. The natural concentrations of titanium, aluminum, silicon and zinc in the WAS used in this study were 3.4 ± 0.2, 14.7 ± 0.9, 52.5 ± 3.5 and 0.8 ± 0.2 mg/g-TSS, respectively.

2.2. Metal oxide nanoparticles and their dissolved metal ions

Nano-TiO_2 (<25 nm, anatase), nano-Al_2O_3 (<50 nm), nano-SiO_2 (10–20 nm, amorphous) and nano-ZnO (<100 nm) were purchased from Sigma Aldrich (St. Louis, MO). The suspensions of nanoparticles stock (2000 mg/L) were prepared by adding 2000 mg nanoparticles to 1.0 L distilled water (pH 7.0) containing 0.1 mM sodium dodecylbenzene sulfonate (SDBS) to enhance the stability of nano-suspension, followed by 1 h of ultrasonication (40 kHz, 250 W). The analyses of these suspensions by dynamic light scattering (DLS) using a Malvern Autosizer 4700 (Malvern Instruments, UK) (Franklin et al., 2007) indicated that the average particle sizes of nano-TiO_2, nano-Al_2O_3, nano-SiO_2 and nano-ZnO were 185 ± 40, 130 ± 30, 110 ± 40 and 140 ± 20 nm, respectively. The specific surface area (SSA) of nano-TiO_2, nano-Al_2O_3, nano-SiO_2 and nano-ZnO analyzed by Micromeritics Tristar 3000 analyzer at 77 K using the Brunauer–Emmett–Teller (BET) method was 110.0 ± 8.5, 138.0 ± 7.0, 52.5 ± 4.0 and 42.5 ± 2.5 m^2/g, respectively. The X-ray diffraction (XRD) analysis using a Rigaku D/Max-RB diffractometer equipped with a rotating anode and a Cu Kα radiation source is shown in Fig. S1 (Supplementary material).

It has been reported that sometimes the toxicity of nanoparticles comes from the dissolution of nanoparticles (Brunner et al., 2006; Franklin et al., 2007; Wong et al., 2010; Xia et al., 2008). Thus, in this study, three concentrations of nanoparticles in 0.1 mM SDBS water solution were prepared with the stock dispersion, and the mixtures were maintained in an air-batch shaker (150 rpm) at 35 ± 1 °C for 48 h. At different times, the samples were withdrawn and centrifuged at 12,000 rpm for 30 min. The supernatant was collected, filtered through 0.22 μm mixed cellulose ester membrane, and determined by inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer Optima 2100 DV, USA) after acidified with 4% ultrahigh purity HNO_3 (Franklin et al., 2007; Jiang et al., 2009). In this study among four nanoparticles only nano-ZnO showed significant amount of metal ion (Zn^{2+}) release, and the released metal ions were negligible in the suspensions of nano-TiO_2, nano-Al_2O_3 and nano-SiO_2. The released Zn^{2+} was respectively 4.4, 11.6 and 17.6 mg/L at nano-ZnO dosage of 6, 30 and 150 mg/g-TSS.

2.3. Effects of nanoparticles on methane generation during WAS anaerobic digestion

In this study the environmental relevant concentration of nanoparticles was chosen to be 6 mg/g-TSS as it was reported that the titanium, aluminum and zinc content in WWTPs (84 in total) biosolids ranged from 0.02 to 7.02, 1.4 to 57.3 and 0.22 to 8.55 mg/g-TSS in USA, respectively (USEPA, 2009). Also, some researchers suggested that a much higher nanomaterial dosage should be investigated if one wants to get the final conclusion regarding the toxicity of nanomaterial (Nyberg et al., 2008). Thus, the impacts of 30 and 150 mg/g-TSS of nanoparticles were also investigated. The experiments of nanoparticles exposure affecting sludge anaerobic digestion for methane generation were carried out in series of serum bottles (500 mL), with a sludge volume of 300 mL each. The nanoparticles (TiO_2, Al_2O_3, SiO_2 and ZnO) were added to the serum bottles with the dosage of 0.006, 0.03 and 0.15 g/g-TSS, respectively. Also, two controls, one with only sludge, and another one with sludge plus 4 mg/g-TSS of SDBS (dispersing reagent), were used to investigate whether the SDBS addition induced a negative effect on methane generation during WAS anaerobic digestion. After being flushed with nitrogen gas for 5 min to remove oxygen, all bottles were capped with rubber stoppers, sealed and placed in an air-bath shaker (150 rpm) at 35 ± 1 °C. The total gas volume was measured by releasing the pressure in the bottles using a syringe (100 mL) to equilibrate with the room pressure according to the Owen method (1979), and the syringe was empty with the excess gas at ambient pressure when the gas component was analyzed. The cumulative methane gas volume was calculated by the following equation modified according to Oh et al. (2003):

$$V_{H,i} = V_{H,i-1} + C_{H,i}(V_{G,i}) - C_{H,i-1}(V_{G,i-1}) \tag{1}$$

where $V_{H,i}$ and $V_{H,i-1}$ are respectively the cumulative methane gas volumes in the current (i) and previous ($i-1$) time intervals, $V_{G,i}$ is the total biogas volumes (including the total volume of headspace in the reactor and the syringe) in the current time, $V_{G,i-1}$ is the total volume of biogas after the gas component analysis in the previous time, $C_{H,i}$ and $C_{H,i-1}$ are respectively the fractions of methane gas in the syringe or the headspace of the bottle measured using gas chromatography in the current and previous time intervals. By analyzing the changes of methane generation, the effect of nanoparticles on WAS fermentative methane generation was obtained. The influences of released metal ions from nanoparticles on WAS anaerobic digestion were conducted with the same method described above except that the corresponding dissolved ions were used to replace the nanoparticles.

2.4. Effects of nanoparticles on each stage involved in WAS anaerobic digestion

The methane generation during WAS anaerobic digestion usually includes the solubilization of sludge particular organic compounds, hydrolysis, acidification and methanation (Fig. S2 (Supplementary material). The batch experiments of influences of four nanoparticles on the solubilization of sludge particulate organic maters were the same as that described in the section of "Effects of nanoparticles on methane generation during WAS anaerobic digestion" except that the fermentation time was 2 d. Based on the analyses of soluble protein and carbohydrate in fermentation liquor, the impact of nanoparticles on sludge solubilization was obtained. The influences of nanoparticles on the other three stages (hydrolysis, acidification and methanation) were conducted in synthetic wastewater consisting of (mg/L of distilled water) 1000 KH_2PO_4, 400 $CaCl_2$, 600 $MgCl_2 \cdot 6H_2O$, 100 $FeCl_3$, 0.5 $ZnSO_4 \cdot 7H_2O$, 0.5 $CuSO_4 \cdot 5H_2O$, 0.5 $CoCl_2 \cdot 6H_2O$, 0.5 $MnCl_2 \cdot 4H_2O$, 1 $NiCl_2 \cdot 6H_2O$ and 34.8 SDBS. WAS of 30 mL, which was heat-pretreated at 102 °C for 30 min to kill methanogens (Oh et al., 2003), was used as the inocula of each bottle unless otherwise stated.

The impacts of nanoparticles on sludge hydrolysis were conducted in the following batch tests with 3510 mL synthetic wastewater (described above) containing 15.6 g bovine serum albumin (BSA, average molecular weight M_w 67,000, model protein compound used in this study) and 3.9 g dextran (model polysaccharide compound) (the mass ratio of protein to carbohydrate