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Diminished inhibitory impact of ZnO nanoparticles on anaerobic fermentation by the presence of TiO_2 nanoparticles: Phenomenon and mechanism

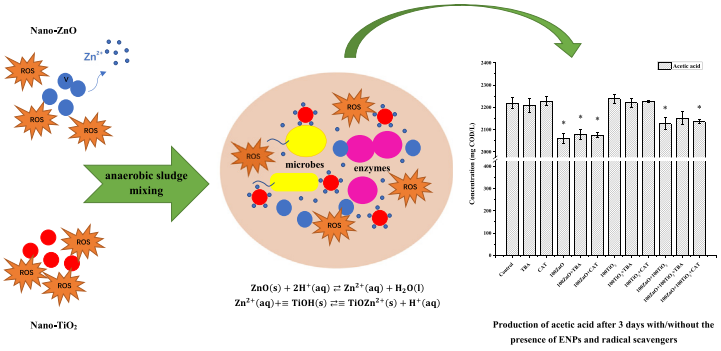
Lingling Zhang [a,b,*], Zhaoxi Zhang [a,b], Xi He [c], Lei Zheng [a,b], Shikun Cheng [a,b], Zifu Li [a,b]

[a] Beijing Key Laboratory of Resource-oriented Treatment of Industrial Pollutants, Beijing 100083, PR China
[b] School of Energy and Environmental Engineering, University of Science and Technology Beijing, Beijing 100083, PR China
[c] Beijing Drainage Group Water Engineering Design &Research Institute Co., Ltd, Beijing 100068, PR China

HIGHLIGHTS

- Combined impact of multiple ENMs on fermentation was not simply superposed.
- The presence of TiO_2 ENMs decreased the impact of ZnO ENMs on fermentation.
- Zn^{2+} decrease was the main reason for the reduced toxicity in multi-ENMs systems.
- Both •OH and H_2O_2 are proved to contribute to the toxicity mechanism.
- Enzyme activity, cell viability and bacteria abundance recovered with TiO_2 ENMs.

GRAPHICAL ABSTRACT



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ABSTRACT

Engineered nanoparticle materials (ENMs) are widely and increasingly produced and employed in many sectors. The use of diverse ENMs potentially leads to the release of multiple ENMs into the environment. These ENMs after discharge will be end in wastewater treatment plant and present in sludge. This work investigated the effect of multi-ENMs systems of ZnO and TiO_2 on sludge anaerobic fermentation and the related toxicity mechanism. Results revealed that the toxicity of ZnO ENMs on anaerobic fermentation was reduced in the presence of TiO_2 ENMs. Investigation on the change of free Zn^{2+} and reactive oxygen species (•OH and H_2O_2) suggested that both of free Zn^{2+} and ROS contributed to the toxicity mechanism. Zn^{2+} decrease was the main reason for the reduced toxicity in multi-ENMs systems. ROS mainly led to the reduction of cell viability in anaerobic fermentation systems. The presence of TiO_2 in the multi-ENMs systems promoted the recovery of enzyme activity, cell viability and bacteria abundance.

1. Introduction

Nanotechnology is considered one of the most significant advancements in science and technology today (Abdelsalam et al., 2016). Engineered nanoparticles materials (ENMs) are widely and increasingly

* Corresponding author at: University of Science and Technology Beijing, Xueyuan Road No.30, Haidian District, Beijing 100083, PR China.
E-mail address: linglingzhangll@hotmail.com (L. Zhang).

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produced and employed in many industries, such as clothing, sunscreens, cosmetics, tires, electronics and medicines due to their unique properties (Gottschalk et al., 2009a). Production and consumption of ZnO and TiO_2 nanoparticles was found to stand in the top list of various ENMs in China and all over the world (Gao et al., 2013; Klaine et al., 2008). Although the current environmental dosage of ENMs is at μg/L or mg/L level, their release into the environment may continuously increase during large-scale production (Gottschalk et al., 2013; Mueller and Nowack, 2008), and therefore pose potential risks to environment. One of the major acceptors for the released ENMs is municipal wastewater treatment plants (WWTPs), where the ENMs are adsorbed, aggregated and settled via activated sludge, and most of the ENMs will enter the sludge treatment systems (Kim et al., 2010a; Zheng et al., 2011a). Anaerobic fermentation is efficient to treat sludge, and after organic wastes are digested high-quality organic fertilizers can be obtained. Volatile fatty acids (VFAs) as important bioproduct can act as the external carbon source during the biological nitrogen and phosphorus removal in WWTPs (Duan et al., 2016; Luo et al., 2014). Therefore, it is worthy to understand the effect of ENMs on hydrolysis and acidification. Many researchers have investigated the behavior of ENMs and their systematic relevant risk in the environment. However, most of the literatures focused on the influence of the whole anaerobic digestion process such as biogas production (Luna-Delrisco et al., 2011; Wang et al., 2016), and little is known about the ENMs impact on anaerobic fermentation.

Previous studies have discussed the toxicity of ZnO ENMs on anaerobic digestion. Mu et al. studied the influence of ZnO ENMs on methane production at both short-term and long-term exposure during sludge anaerobic digestion, suggesting methane production was decreased (Mu and Chen, 2011; Mu et al., 2011). Nguyen et al. also found the inhibition of ZnO ENMs on biogas production and investigated the toxicity of ENMs on *Escherichia coli* (Nguyen et al., 2015). The impact of TiO_2 ENMs on anaerobic digestion suggested that the presence of TiO_2 could not affect anaerobic digestion (Mu et al., 2011; Sakarya et al., 2015). However, there is little work focus on the combined impact on fermentation of different type of ENMs. In reality, wastewater sludge inevitably exposed to different ENMs.

Tong et al. investigated combined toxicity of ZnO and TiO_2 ENMs against *E. coli* in natural aqueous medium. Their work suggested the toxicology of ZnO ENMs is reduced in the presence of TiO_2 due to the absorption of Zn^{2+} (Tong et al., 2014; Tong et al., 2015). According to current research, the impact of anaerobic digestion caused by ZnO ENMs was mainly due to Zn^{2+} release, while TiO_2 ENMs had very little effect because of insolubility (Luna-Delrisco et al., 2011; Mu et al., 2011). It is worthwhile to understand the combined impact of multiple ENMs on anaerobic fermentation, especially the change of Zn^{2+} concentrations in supernatant and sediment. Moreover, apart from Zn^{2+}, reactive oxygen species (ROS) were reported to be another reason responsible for the toxicity of ENMs, which led to the loss of cell viability (Xia et al., 2008). Usually, ROS are produced in the presence of oxygen. However, it has been reported that •OH and H_2O_2 can also be produced under anaerobic conditions (Esposti and Mclennan, 1998; Yang et al., 2013), so the increase of ROS in the sludge was a likely reason for their adverse effect on sludge anaerobic fermentation. The role of ROS in anaerobic digested was estimated by obtaining consistent trend between ROS production and biomass viability assay in previous work (Mu and Chen, 2011). However, there is no direct evidence which can show the impact of specific ROS in the anaerobic digestion system.

Therefore, the objective of this work is to investigate the response of sludge anaerobic fermentation in the presence of multi-ENMs systems, which were with both ZnO and TiO_2 ENMs. The production of protein, polysaccharide and volatile fatty acids (VFAs) were examined by monitoring the degradation of organic matters. The influence mechanism of the multi-ENMs systems was also proposed by monitoring the changes of dissolved metal ions and ROS. Moreover, the biological changes in the fermentation systems, includes cell membrane integrity, key enzymes activity and the structure of bacterial community, were also analyzed. To our knowledge, this is the first time to investigate the impact of multiple ENMs on sludge anaerobic fermentation, and our results indicate the importance of assessing environmental risk of multiple ENMs in anaerobic fermentation systems.

2. Material and methods

2.1. Sludge and nanoparticles

Waste active sludge (WAS) used in this study was withdrawn from the secondary sedimentation tank of a municipal WWTP in Beijing, China. Seed sludge was taken from anaerobic digester at the same place. The sludge was concentrated by settling at 4 °C. Main characteristics of WAS are as Table 1.

Dry zinc oxide nanoparticles from Nano-Structured & Amorphous Materials (USA) were used in this work. ZnO ENMs sample were prepared as described in previous paper (Zhang et al., 2013). The particle size of ZnO aggregates in nanofluids was 200 nm, measured by Nano-Sizer (Malvern instrument). TiO_2 ENMs used in this study was Degussa P25, purchased from Degussa AG. TiO_2 stock solutions were prepared in distilled water and sonicated for 60 min in ultrasonic bath (Health-Sonics, 110 W, 42 kHz). Both ZnO and TiO_2 stock solutions were prepared to the desired concentrations for further study.

2.2. Fermentation experiments of hydrolysis-acidification

In order to understand the impact of multi-ENMs on the hydrolysis-acidification phase in fermentation, a 3-day of anaerobic fermentation was studied, suggested by Feng's study (Feng et al., 2014). The digested sludge was pretreated as follows: original digested sludge was heated at 102 °C for 30 min. After cooling down, 50 mM 2-bromoethanesulfonic acid (purchased from Shanghai Aladdin Bio-Chem Technology Co., LTD, China) was added to remove methanogens.

In this study, four dosages (0, 5, 30, 100 mg/g-TSS) of ZnO and TiO_2 ENMs were set to explore the impact of multiple nanoparticle on sludge anaerobic fermentation. The dosage of 5 mg/g-TSS of ZnO ENMs was chosen to be the environmentally relevant concentration according to the literature (Gottschalk et al., 2009b). The high dose was chosen to provide a sufficient Zn concentration for toxicology and mechanism study. The anaerobic digestion system that added with 5 mg/g-TSS ZnO and 5 mg/g TiO_2 ENMs was named as $5ZnO + 5TiO_2$. A series of bottles of 250 mL capacity were used for batch anaerobic digestion tests. Seed sludge and raw sludge were mixed equally (1:9, v/v), for the final active volume of 150 mL. The anaerobic digestion experiment was carried out under mesophilic (37 ± 1 °C) condition and the sludge bottles agitated manually at least twice a day to avoid stratification.

Table 1
Main characteristics of raw sludge and seed sludge.

Parameters	Raw WAS	Seed WAS
pH	7.205 ± 0.025	7.495 ± 0.015
Total solids (TS)	22.6 ± 0.3 g/L	27.1 ± 0.1 g/L
Volatile solids (VS)	12.2 ± 0.3 g/L	14.6 ± 0 g/L
Total suspended solids (TSS)	19.9 ± 0.5 g/L	24.5 ± 0.1 g/L
Volatile suspended solids (VSS)	11.7 ± 0.4 g/L	12.9 ± 0.3 g/L
Total chemical oxygen demand (TCOD)	10,837.56 mg/L	12,677.92 mg/L
Soluble chemical oxygen demand(SCOD)	654.37 mg/L	728.34 mg/L
Total protein	6681.18 ± 213.11 mg COD/L	8812.23 ± 106.56 mg COD/L
Total polysaccharide	1413.28 ± 35.64 mg COD/L	1339.00 ± 49.00 mg COD/L
Soluble protein	323.99 ± 31.97 mg COD/L	547.74 ± 63.93 mg COD/L
Soluble polysaccharide	58.15 ± 4.69 mg COD/L	55.02 ± 0.78 mg COD/L