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# Transport, fate, and long-term impacts of metal oxide nanoparticles on the stability of an anaerobic methanogenic system with anaerobic granular sludge

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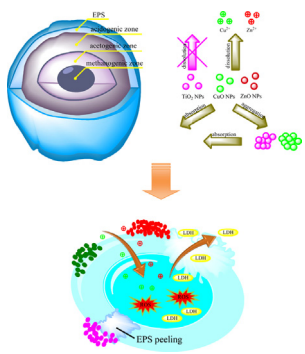
## HIGHLIGHTS

- Long-term impacts of $TiO_2$, CuO and ZnO NPs on AGS were compared.
- Methanogenesis was temporarily stimulated by low doses of ZnO NPs.
- Metabolism inhibition varied depending on both the NPs type and dose.
- Direct contacts and ions release were the main mechanisms for the NPs cytotoxicity.
- Most metal oxide NPs aggregated in the sludge and caused changes of AGS structure.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The fate and long-term effect of different metal oxide ($TiO_2$, CuO and ZnO) nanoparticles (NPs) on anaerobic granular sludge (AGS) was evaluated in an anaerobic methanogenic system. Operation stability and structural characteristics of the granules were compared, the metabolism changes in the microbial community were quantified, and NPs fate were investigated. CuO NPs had greatest toxic effect on AGS after extended exposure, whereas ZnO NPs benefited methanogenesis temporarily (no more than 5 d). The inhibition on AGS caused by NPs varied due to the unique structure of AGS and different toxic mechanism. Structural changes of AGS provided new evidence that tested NPs have different toxicity.



## 1. Introduction

The rapid development of nanotechnology has resulted in the increasing integration of engineered nanoparticles into diverse commercial products and manufacturing processes. As a class of important functional nanometer-scale materials, metal oxide nanoparticles (NPs), such as ZnO, $TiO_2$, and CuO, have attracted much attention for a variety of applications including cosmetics, sunscreen, textiles, bactericides, metal catalysts, wastewater treatment, etc. (Paull et al., 2003), because of their different or enhanced properties compared with their conventional 'bulk' (microsized) counterparts (Ivask et al., 2014). The extensive use of metal oxide NPs has raised concerns about their potential toxicity, and harmful effects of metal oxide NPs on different organisms have been documented (Farré et al., 2009), suggesting that biological processes in natural and engineered systems might be affected.

Wastewater treatment plants (WWTP) are considered as the last barriers to the release of metal oxide NPs from domestic and industrial sources to aquatic or terrestrial environments, and metal

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oxide NPs have been detected both in wastewater and sewage sludge (Ganesh et al., 2010). Although NPs can be removed via each of the principal segments of wastewater treatment, sorption by activated sludge and aggregation/settling to activated sludge are reportedly the predominant removal mechanism for NPs (Kiser et al., 2010), especially for metal oxide NPs due to their larger aggregates and fewer negative charges compared to other nanomaterials (e.g., metallic NPs and C60) (Wang et al., 2012). Studies have demonstrated a strong need to clarify the impact of metal oxide NPs on activated sludge.

Anaerobic granular sludge (AGS) consists of functionally and phylogenetically distinct microbial groups including acidogens, syntrophic acetogenic/methanogenic consortia and aceticlastic methanogens (Batstone et al., 2004). Compared with other activated sludge treatments, anaerobic biogranulation is suitable for the treatment of various wastewaters because of its high biomass concentration, dense and strong microbial aggregate structure, and excellent settling capacity (Hulshoff Pol et al., 2004); however, these characteristics might lead to different responses to metal oxide NPs from other types of activated sludge (Ma et al., 2013). Although some studies have evaluated the effect of metal oxide NPs on the anaerobic activated sludge process, emphasis has been on the short-term sudden exposure to metal oxide NPs by batch tests (Gonzalez-Estrella et al., 2013; Luna-delRisco et al., 2011; Ma et al., 2013; Mu et al., 2011), which might not be appropriate to interpretate the NPs cumulative effects on anaerobic activated sludge or the performance of anaerobic WWTP over extended SRT values. Only few studies assessed the long-term toxicity of certain types of metal oxide NPs to AGS (Mu and Chen, 2011; Otero-González et al., 2014a, b), and dynamic changes in the metabolism capability of functional microorganisms have been analyzed inadequately. Moreover, little evidence is available on similarities and differences caused by different metal oxide NPs on AGS over a long-term and persistent exposure.

The purpose of this study was to reveal the long-term impacts of three metal oxide ($TiO_2$, CuO and ZnO) NPs on the performance of anaerobic methanogenic system and the possible toxicity mechanisms; specifically, focusing on the AGS structure, with particular attention to the metabolism changes of microbial community and transformation/biophysicochemical interactions occurring between metal oxide NPs and granules. Likewise, this study elucidated the biosorption by AGS of different types of NPs and the consequent changes of granule structure, and the ion release and NPs remaining in the effluent were examined to explain the fate and transport of metal oxide NPs and the toxicity data obtained.

## 2. Materials and methods

### 2.1. Metal oxide NPs and suspension preparation

Commercial $TiO_2$, ZnO, and CuO NPs (>99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA) as dry powders.

The stock suspensions (2 g/L) were prepared by adding 2 g NPs to 1 L ultrapure water containing 0.1 g ammonium polyacrylate, which was found to be a non-toxic dispersant to methanogens (Gonzalez-Estrella et al., 2013), to avoid NPs aggregation, and sonicating for 2 h (KUDOS SK3300H, 180 W, 53 kHz, Shanghai, China). Stock solutions were stored at 4 °C and sonicated for 30 min before use.

### 2.2. Stability of metal oxide NPs dispersions

Since the composition of water could remarkably affect the behavior and fate of NPs (Zhang et al., 2008), the variation of NPs stability in both deionized (DI) water and synthetic wastewater (see section 2.3) was evaluated by particle size distribution (PSD) and zeta potential (ζ-potential). The $TiO_2$, ZnO, and CuO NPs stock solutions were diluted to 50 mg/L, and then added to serum bottles and shaken on an air-batch shaker (80 rpm) at 35 ± 1 °C for 24 h. To imitate the conditions at which the bioreactors were operated (section 2.3), the samples were allowed to settle for 15 min under static conditions. Samples for PSD and ζ-potential determinations were taken at different incubation stages (0 h, 24 h and settlement), and measurements were carried immediately after sampling.

More information about the PSD and ζ-potential measurements can be found in the Supplementary Information.

### 2.3. Seed sludge and anaerobic bioreactor operation

The seed sludge was collected from a full-scale expanded granular sludge bed (EGSB) anaerobic reactor treating soybean wastewater in Harbin, China, with total suspended solids (TSS) and volatile suspended solids (VSS) contents of 35.04 and 30.59 g/L, respectively. The average particle size of the granular sludge was 2.3 ± 0.4 mm, and the sludge was sieved though a 70-mesh sieve to remove fine particles before pumped into the reactors.

Four identical sets of laboratory-scale anaerobic sequencing batch reactors (ASBRs) were operated independently with synthetic wastewater at 35 ± 1 °C. The size parameters and operation mode of the ASBRs are provided in the Supplementary Information.

$TiO_2$, CuO and ZnO NPs were supplemented into ASBRs with the influent; in addition, one control ASBR was operated without any NPs. The experiments were conducted for over 90 days.

Based on the content of elements in WWTPs biosolids reported by USEPA (2009), and the fact that some researchers have recommended using higher nanomaterial dosages than environmentally relevant concentration to draw final conclusions regarding toxicity (Nyberg et al., 2008), a dosage of 5 mg/L NPs was used to assess the their potential effects.

The performance of the reactors with oxide NPs exposure was monitored by measuring the biogas generation, the pH, substrate degradation, concentrations and chemical composition of the fermentation product; and the NPs and soluble metal ions in the influent and effluent. Additionally, the Ti, Zn and Cu concentrations in the AGS were determined before and after experiment.

### 2.4. Electron microscopy

The surface morphology of the AGS exposed to metal oxide NPs was characterized by environmental scanning electron microscopy (ESEM) with energy-dispersive X-ray (EDX) elemental analysis (FEI Quanta 200 FEG, USA). The SEM samples were obtained randomly at the end of the study (90 d) from the bottom sampling ports of the ASBRs (approximately 10 samples from each reactor).

The surface morphology of NPs were examined by transmission electron microscopy (TEM; JEOL JEM 1400, Japan) and the particle sizes from TEM were estimated using graphics software (Photoshop 9.0).

The pretreatment of the AGS samples for SEM observation and the NPs samples for TEM observation is described in the Supplementary Information.

### 2.5. Analytical methods

Details of the procedures used for sample digestion and metal analysis are provided in the Supplementary Information.

The biogas composition and the concentrations of individual short-chain fatty acids (SCFA) were determined using a gas chromatograph (GC; Agilent 7890N, USA), and the parameters were the same as those described in a previous publication (Cheng