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Toxicity assessment of inorganic nanoparticles to acetoclastic and hydrogenotrophic methanogenic activity in anaerobic granular sludge

Jorge Gonzalez-Estrella *, Reyes Sierra-Alvarez, James A. Field

Department of Chemical and Environmental Engineering, University of Arizona, P.O. Box 210011, Tucson, AZ 85721, USA

HIGHLIGHTS

- Eleven different nanoparticles were tested as inhibitors of methanogenesis.
- Methanogenic activity was inhibited the most by Cu^0 and ZnO nanoparticles.
- Corrosion of nanoparticles to toxic metal ions was main mechanism of toxicity.

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ABSTRACT

Release of engineered nanoparticles (NPs) to municipal wastewater from industrial and residential sources could impact biological systems in wastewater treatment plants. Methanogenic inhibition can cause failure of anaerobic waste(water) treatment. This study investigated the inhibitory effect of a wide array of inorganic NPs (Ag^0, Al_2O_3, CeO_2, Cu^0, CuO, Fe^0, Fe_2O_3, Mn_2O_3, SiO_2, TiO_2, and ZnO supplied up to 1500 mg L^{-1}) to acetoclastic and hydrogenotrophic methanogenic activity of anaerobic granular sludge. Of all the NPs tested, only Cu^0 and ZnO caused severe methanogenic inhibition. The 50% inhibiting concentrations determined towards acetoclastic and hydrogenotrophic methanogens were 62 and 68 mg L^{-1} for Cu^0 NP; and 87 and 250 mg L^{-1} for ZnO NP, respectively. CuO NPs also caused inhibition of acetoclastic methanogens. Cu^{2+} and Zn^{2+} salts caused similar levels of inhibition as Cu^0 and ZnO NPs based on equilibrium soluble metal concentrations measured during the assays, suggesting that the toxicity was due to the release of metal ions by NP-corrosion. A commercial dispersant, Dispex, intended to increase NP stability did not affect the inhibitory impact of the NPs. The results taken as a whole suggest that Zn- and Cu-containing NPs can release metal ions that are inhibitory for methanogenesis.

1. Introduction

The advance of nanotechnology has led to an increase in the production of engineered nanoparticles (NPs). NPs are defined as having at least one-dimension smaller than 100 nm [1]. Nanotechnology is rapidly developing and is becoming applied in several industrial sectors such as medicine and semiconductor manufacturing. NPs are already utilized in consumer products like cosmetics, personal-care products, paints and coatings [2,3]. The nanotechnology market is projected to be $1 trillion in 2015, employing 2 million people [4].

Concerns about the environmental and health impacts of NPs are increasing. However, compared to NP synthesis and applications, relatively little research has been focused on environmental and health impacts [2]. The small size of NPs may enhance processes like dissolution, redox reactions or generation of reactive oxygen species, impacting environmental and human health [5,6]. NPs disposed via wastewater streams will often end up in wastewater treatment plants with biological treatment operations [2]; where little is known about their fate [7–9]. The extent of NP removal, NP-toxicity to biological treatment, and potential sorption of NPs onto biosolids remain largely unknown [10]. Additionally, variable wastewater composition can influence the physicochemical properties of NPs differently [2,11].

NPs may impact several key unit operations at wastewater treatment plants. Some studies found that NPs remain partly retained in the sludge of the aerobic activated sludge process [7,12]. Thus, NPs are expected to enter unit operations used for treating waste activated sludge. Anaerobic digestion (involving methanogenesis) is one of the most frequently applied methods of stabilizing excess wastewater sludge [13]. With the exception of a few preliminary studies [10,14–17], little is known regarding the toxicity of NPs to methanogens. The aim of this study was to investigate the

Abbreviations: COD, chemical oxygen demand; NMA, normalized methanogenic activity; NPs, engineered nanoparticles; PSD, particle size distribution; VSS, volatile suspended solids; ZP, zeta potential.

* Corresponding author. Tel.: +1 520 621 6162.
E-mail address: jorgegonzaleze@arizona.edu (J. Gonzalez-Estrella).

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inhibitory effect of inorganic NPs on the methanogenic activity of acetoclastic and hydrogenotrophic methanogens in anaerobic granular sludge. Likewise, this study evaluated the aggregation properties of NPs in an anaerobic basal medium. Lastly, the impact of a surfactant intended to stabilize NP dispersions on NP toxicity was evaluated.

2. Materials and methods

2.1. Chemicals

The commercial ammonium polyacrylate dispersant (Dispex A40, average MW ~4000) was obtained from BASF (Freeport, TX, USA). Sodium acetate (99.9%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). H_2/CO_2 (80/20, v/v) gas mix was delivered by Airweld (Phoenix, AZ, USA). N_2/CO_2 (80/20, v/v) gas mix and CH_4 standard gas (99%) were acquired from Air Liquid America (Plumsteedsville, PA, USA). Sodium bicarbonate was purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.2. Nanoparticles and stability of NP dispersions

Ag^0, Al_2O_3, Cu^0, CuO, CeO_2, Fe^0, Fe_2O_3, Mn_2O_3, SiO_2, TiO_2, and ZnO NPs were tested as inhibitors of methanogenic activity with and without the use of Dispex. The source of the NPs and the manufacturer reported size and purity are as follows: Ag^0 (size of <100 nm, purity of 99.5%), Al_2O_3 (<50 nm, 99%), CeO_2 (50 nm, 99.95%), Cu^0 (40–60 nm, 99%), CuO (40 nm, >99%), and SiO_2 (10–20 nm, 99.5%) were purchased from Sigma–Aldrich (St. Louis, MO); Fe^0 (46–60 nm, 99.9%), Fe_2O_3 (40 nm, 99%), Mn_2O_3 (98%), and ZnO (10–30 nm, 99.8%) were acquired from SkySpring Nanomaterials Inc. (Houston, TX); and TiO_2 (~25 nm, 99.5%) was a gift from Aerosil (Parsippany, NJ). All NPs were obtained as dry powders.

The stability of the NPs was evaluated by determining the particle size distribution (PSD) and zeta potential (ZP) according to a previous study [18]. All stock solutions were also prepared as previously described [18]. Aliquots (3 mL) of the stock solutions (2500 mg L^{-1}) were amended into the 160 mL serum bottles containing 27 mL of 1.1× concentrated anaerobic basal medium, 1.1× concentrated basal medium with Dispex, DI water, or acidified DI water (pH 2). Afterwards, all bottles were flushed with a N_2/CO_2 (80:20, v/v) gas mixture. Subsequently, pH, PSD and ZP were measured. In order to imitate the conditions at which the toxicity assays were performed, all bottles were shaken for 24 h at 120 rpm at 30 °C. Next, the samples were allowed to settle for 30–45 min under static conditions, and samples of the supernatant were collected carefully to avoid carryover of any settled material. Samples were analyzed immediately for PSD and ZP. The PSD and ZP is shown in Table 1S (Supplementary Information). The soluble concentration of metals in the samples was only determined for those NPs that showed toxicity by filtering the samples through 25 nm membranes.

2.3. Sludge source

The methanogenic anaerobic granular sludge was obtained from a full-scale upward anaerobic sludge bed reactor treating brewery wastewater (Mahou, Guadalajara, Spain). The sludge was sieved to remove fine particles and excess water. The content of volatile suspended solids (VSS) was 7.92% of the wet weight. The sludge was stored at 4 °C. The maximum methanogenic activity of the sludge in assays utilizing acetate and hydrogen as substrate was 317.6 ± 29.5 and 566.7 ± 34.8 mg CH_4-chemical oxygen demand (COD) per gram volatile suspended solids (VSS) per day, respectively.

2.4. Batch acetoclastic and hydrogenotrophic methanogenic activity bioassays

All bioassays were carried out using an anaerobic medium pH (7.2) containing (in mg L^{-1}): NH_4Cl (280), $NaHCO_3$ (5000), K_2HPO_4 (250), $CaCl_2{\cdot}2H_2O$ (10), $MgCl_2{\cdot}6H_2O$ (100), $MgSO_4{\cdot}7H_2O$ (100); yeast extract (100) with 1 mL L^{-1} of trace elements [19]. The medium of the control without NPs, assays with Dispex without NPs, assays with NPs, and assays with NPs and Dispex was composed by combining 27 mL of a 1.1× concentrated basal medium with 3 mL of DI water, 3 mL of Dispex solution (1500 mg L^{-1}), 3 mL of NP stock (15 g L^{-1}), and 3 mL of the NPs stock (15 g L^{-1}) with Dispex (1500 mg L^{-1}), respectively.

Firstly, inoculum (1.5 g of VSS L^{-1}) and 1.1× concentrated medium (27 mL) were added to 160 mL bottles. Subsequently, all bottles were flushed with the gas mixture N_2/CO_2 (80:20, v/v). Either sodium acetate (1 g COD L^{-1}) or hydrogen gas were used as electron donors. H_2 was supplied to a final headspace concentration of 0.5 atm of H_2/CO_2 applied as an overpressure after first flushing the assay bottles with the N_2/CO_2 gas mixture. Subsequently, all the assays were pre-incubated overnight at 30 ± 2 °C in an orbital shaker at 120 rpm.

Following pre-incubation, 3 mL of DI water, 3 mL of Dispex stock solution (1500 mg L^{-1}), 3 mL of NP stock (15 g L^{-1}), and 3 mL of the NPs stock with Dispex (NPs/Dispex, 10:1, w/w) were added to the control without NPs, assays with Dispex without NPs, assays with NPs, and assays with NPs and Dispex, respectively. The controls without NPs were performed in triplicate; whereas, all the treatment assays were performed in duplicate. Once NPs were added and the experimental control was prepared, all bottles were flushed with the N_2/CO_2 gas mixture and H_2/CO_2 was added when H_2 was the intended electron donor. A second substrate feeding was supplied to assays where methanogenic inhibition was observed (i.e., assays with Fe^0, Cu^0, CuO, Mn_2O_3, and ZnO NPs). Acetate or H_2 were respiked as described above for the first feeding. All assays were incubated at 30 ± 2 °C in an orbital shaker at 120 rpm.

Gas samples (100 μL) were withdrawn from the assays two or three times a day during the experiment to measure methane production until the theoretical maximum methane production was reached. The normalized methanogenic activity (NMA) was then calculated as the percentage of the ratio of maximum methane production rates in the treatment (test concentration of NP) and the control (without NPs) as shown below:

$$\text{NMA}(\%) = \left(\frac{\text{Maximum } CH_4 \text{ production rate at tested NP concentration}}{\text{Maximum } CH_4 \text{ production rate of the control}}\right) 100$$

In the assays where methanogenic inhibition was observed, a second feeding of the electron donor was provided to explore the changes of toxicity as a function of the NP exposure time. NPs which caused enhanced methanogenic inhibition in the second feeding (Cu^0, CuO, and ZnO) were further evaluated in acetoclastic and hydrogenotrophic methanogenic assays exposed to different NP concentrations. These assays also included a first and second feeding of the respective substrates. The NMA was calculated for the different concentrations of NPs applied along with the inhibition concentration at which a 50% decrease in the specific methanogenic activity (IC_{50}, relative to the non-inhibited control) was observed. The IC_{50} concentration was calculated as described elsewhere [18].

2.5. Methanogenic inhibition by soluble Cu and Zn ions

To study the effect of soluble Cu^{2+} and Zn^{2+} on methanogens, $CuCl_2$ and $ZnCl_2$ salts were used, with the experimental conditions