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Elemental copper nanoparticle toxicity to different trophic groups involved in anaerobic and anoxic wastewater treatment processes

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HIGHLIGHTS

- Cu^0 NPs are inhibitory to various anaerobic and anoxic biological processes.
- Cu^0 NPs and $CuCl_2$ strongly inhibited glucose fermentation.
- SOP was overall more tolerant to Cu^0 NP and $CuCl_2$ toxicity.
- Results suggest that release of soluble Cu(II) by Cu^0 NPs causes inhibition.

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ABSTRACT

Elemental copper nanoparticles (Cu^0 NPs) are potentially inhibitory to the different key microbial trophic groups involved in biological wastewater treatment processes. Cu-based NPs are known to be toxic to methanogens at low concentrations. However, very little is known about the toxic effect of Cu^0 NPs on other microbial groups involved in either upper trophic levels of anaerobic digestion or anoxic nitrogen removal processes. This study evaluated the toxicity of Cu^0 NPs to glucose fermentation, syntrophic propionate oxidation and denitrification in shaken batch bioassays with soluble substrates. Batch experiments were also supplemented with $CuCl_2$ to evaluate the inhibitory impact of soluble Cu(II) ions. Syntrophic propionate oxidation and glucose fermentation were the least and most inhibited processes with inhibition constant (K_i) values of 0.202 and 0.047 mM of added Cu^0 NPs, respectively. Further analyses revealed that the K_i values calculated as a function of the free soluble Cu concentration were <0.003 mM for every biological process tested and most of these K_i values were similar in order of magnitude regardless of whether the Cu source was $CuCl_2$ or Cu^0 NPs. The results taken as a whole indicate that Cu^0 NPs are toxic to all the microbial processes studied. Therefore, Cu^0 NPs can potentially be an important inhibitor of anaerobic wastewater treatment processes that rely on these trophic groups. The evidence suggests that the inhibitory impact of Cu^0 NPs was mainly due to the release of toxic Cu(II) ions originating from the corrosion and dissolution of Cu^0 NPs.

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1. Introduction

Engineered nanoparticles (NPs) are manufactured materials with at least one dimension ≤100 nm. NPs are applied widely in industrial processes and consumer products (Auffan et al., 2009). Specifically, copper-based NPs are applied in several products such as wood preservative, catalyst, antimicrobial substances or printable electronics; and are additionally formed as byproducts during semiconductor manufacturing (Wang et al., 2013). Elemental copper (Cu^0) and copper oxide nanoparticles (CuO and Cu_2O) are the most common types of Cu NPs used in technological applications (Wang et al., 2013). Cu-based NPs are also generated as a byproduct of chemical mechanical polishing in the semiconductor industry (Golden et al., 2000). Consequently, NPs after being used are very likely to be discharged to domestic wastewater treatment plants, namely activated sludge processes and other biological operations (Keller and Lazareva, 2013).

Recent studies show that NPs commonly applied to commercial products such as Ag^0, TiO_2, and ZnO accumulate in the sludge biosolids of both pilot and full scale activated sludge municipal treatment plants (Kiser et al., 2009, 2010; Ma et al., 2013). These findings raise the concern that other commonly applied NPs such as Cu-based NPs will have a similar fate. Thus, potential inhibitory NPs can be magnified in concentration during treatment of certain waste streams such as anaerobic sludge digestion or the liquors from dewatering of digested sludge.

Methanogens are key microorganisms in anaerobic stabilization of waste sludge due to their role in converting acetate and hydrogen into methane and Cu-based NPs are highly inhibitory to them (Gonzalez-Estrella et al., 2013; Otero-González et al., 2014). However, very little or no research has been performed on the toxic effect of Cu-

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based NPs to other anaerobic trophic groups of the anaerobic digestion process or those involved in N-removal. Therefore, this study evaluated the toxic effect of Cu^0 NPs and its soluble salt analog ($CuCl_2$) on three anaerobic trophic groups. These included glucose fermentation and syntrophic propionate oxidation (SPO) involved in the anaerobic digestion of soluble carbonaceous substrates as well as denitrification which is an important process for N-removal.

2. Materials and methods

2.1. Chemicals

Cu^0NPs (40–60 nm, 99%) were purchased from Sky-Spring Nanomaterials Inc. (Houston, TX). $CuCl_2 \cdot H_2O$ (99%), sodium acetate (99.9%) and propionic acid (>99.4%) were acquired from Sigma Aldrich (St. Louis, MO, USA). D-Glucose was bought from Fisher Scientific (Waltham, MA, USA). N_2/CO_2 (80/20, v/v) gas mix and CH_4 standard gas (99%) were acquired from Air Liquid America (Plumstedsville, PA, USA).

2.2. NP dispersions and metal solutions

Cu^0 NP stock dispersions were sonicated (DEX® 130, 130 W, 20 kHz, Newtown, CT) at 70% amplitude for 5 min. The average hydrodynamic particle size of the NPs in the biological media used in this study was 648 ± 14 nm and the zeta potential −16.3 ± 1.8 mV. Details of the stability of Cu^0 NPs in anaerobic media have been previously described (Gonzalez-Estrella et al., 2013). $CuCl_2$ solutions were prepared by dissolving the salt in 0.01 M HCl.

2.3. Anaerobic sludge

The anaerobic granular sludge used for glucose fermentation, SPO and denitrification assays was obtained from a full-scale upflow anaerobic sludge bed reactor treating brewery wastewater (Mahou, Guadalajara, Spain). The sludge was stored at 4 °C. The content of the sludge in volatile suspended solids (VSS) was 7.0% of the wet weight.

2.4. Culture media

Glucose fermentation and SPO assays were performed in an anaerobic medium at pH 7.2 containing (mg L^{-1}): NH_4Cl (280), $NaHCO_3$ (3000), K_2HPO_4 (250), $CaCl_2 \cdot 2H_2O$ (10), $MgCl_2 \cdot 6H_2O$ (183), yeast extract (100) and 1 mL L^{-1} of trace elements. Denitrification bioassays were performed in a basal medium containing (mg L^{-1}): K_2HPO_4 (250); $(NH_4)HCO_3$ (417); $NaHCO_3$ (2680), yeast extract (10) and 1 mL L^{-1} of trace elements. Trace element solutions for the glucose fermentation, SPO, and denitrification contained (mg L^{-1}): H_3BO_3 (50), $FeCl_2 \cdot 4H_2O$ (2000), $ZnCl_2$ (50), $MnCl_2 \cdot 4H_2O$ (50), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (50), $AlCl_3 \cdot 6H_2O$ (90), $CoCl_2 \cdot 6H_2O$ (2000), $NiCl_2 \cdot 6H_2O$ (50), $CuCl_2 \cdot 2H_2O$ (30), $NaSeO_3 \cdot 5H_2O$ (100), EDTA (1000), resazurin (200) and 36% HCl (1 mL L^{-1}).

2.5. Microbial inhibition bioassays

2.5.1. Glucose fermentation and SPO bioassays

Glucose fermentation experiments were performed in 160-mL serum flasks supplemented with inoculum (1.5 g VSS L^{-1}) and basal medium to 50 mL. All bottles were flushed with N_2/CO_2 (80:20, v/v) and, subsequently, they were spiked with glucose or propionate (0.5 g chemical oxygen demand (COD) L^{-1}). Next, the bottles were pre-incubated overnight (10–12 h) at 30 ± 2 °C in an orbital shaker at 115 rpm to adapt the inoculum to the added substrate. In the morning, the flasks were spiked with additional electron donor (0.5 g COD L^{-1}), and a known volume of the Cu^0 NPs stock dispersion, Cu(II) stock solution, or deionized water, depending on the experiment. The concentrations of Cu supplied to the glucose fermentation experiments ranged from 0 to 0.315 mM of Cu^0 NPs and from 0 to 0.629 mM of $CuCl_2$; whereas the added concentration of Cu in the SPO bioassays ranged from 0 to 0.629 mM of Cu^0 NPs and from 0 to 0.315 mM of $CuCl_2$. Assays were again pre-incubated overnight at 30 ± 2 °C in an orbital shaker at 115 rpm. A triplicate control was included in all the experiments, whereas all assays containing Cu^0 NP and $CuCl_2$ were carried out by duplicate. Liquid samples (1 or 1.5 mL) were taken several times in the first hours of incubation to measure glucose consumption, once or twice a day for propionate consumption, and twice to measure the evolution of volatile fatty acids (VFA). Gas samples (100 μL) were taken twice or three times a day to track CH_4. Finally a 1.5 mL liquid sample was taken to analyze the free metal concentration. The free metal concentration is the soluble free metal concentration measured at the end of the incubation.

2.5.2. Denitrification bioassays

Denitrification bioassays were inoculated with the same anaerobic sludge and incubated under the same conditions of the glucose fermentation and SOP bioassays. The bottles were supplied with acetate (0.3 g COD L^{-1}) and 0.39 g L^{-1} of nitrate (NO_3^-) and pre-incubated for 72 h. After the pre-incubation, another amendment of 0.3 g COD L^{-1} of acetate, 0.390 g L^{-1} of nitrate, the Cu^0 NP stock dispersion, metal stock solution, or deionized water was added to the corresponding experiments. The concentration of Cu for the SPO bioassays ranged from 0 to 0.629 mM of Cu^0 and $CuCl_2$. A triplicate control was included in all the experiments, whereas all assays containing Cu^0 NP and $CuCl_2$ were carried out by duplicate. Gas samples (100 μL) were taken twice or three times a day to track CH_4. Finally a 1.5 mL liquid sample was taken to analyze the free metal concentration.

2.6. Particle size distribution and zeta potential measurements

The zeta potential of NPs dispersions was measured with a ZetaSizer Nano ZS (Malvern, Inc., Sirouthborough, MA). Particle size distribution measurements were performed by dynamic light scattering (DLS) using the same instrument. Details of the methodology are described elsewhere (Garcia-Saucedo et al., 2011).

2.7. Analytical methods

Methane was quantified by gas chromatography with flame ionization detection (Hewlett Packard 5890 Series II). VFAs were measured by gas chromatography (7890A GC System, Agilent Technologies, Santa Clara, CA, USA) using a fused silica Stabilwax-DA column (30 m length, 0.53 mm ID, 0.25 μm particle size; Restek, State College, PA, USA) and a flame ionization detector. Details of the analysis are described in Otero-González et al. (2014). N_2 was analyzed using a Hewlett Packard 5890 Series II gas chromatograph fitted with a Carboxen 1010 Plot column (30 m × 0.32 mm, Sigma-Aldrich Corp. St. Louis, MO USA) and a thermal conductivity detector as described elsewhere (Carvajal-Arroyo et al., 2014).

Culture samples for the determination of glucose and soluble copper were centrifuged for 10 min at 13,000 rpm prior to analysis. Glucose was determined colorimetrically as described elsewhere (DuBois et al., 1956). Briefly, a sample of the supernatant (0.5 mL) was transferred into a test tube containing 0.5 mL of 5% (v/v) phenol and 2.5 mL of concentrated sulfuric acid was added. The samples were incubated at room temperature for 20 min and, subsequently, the concentration of glucose was determined by measuring the color intensity of the sample at 490 nm. The concentration of soluble Cu in the supernatant was measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES Optima 2100 DV, Perkin–Elmer TM, Shelton, CT). Samples were filtered through 25 nm membranes (VSWP, Millipore, Billerica, MA, USA) and then diluted with dilute nitric acid (3%) as required prior ICP analysis. The wavelength used for ICP-OES analysis of Cu was 324.754 nm.

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