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Effects of individual and complex ciprofloxacin, fullerene C_{60}, and ZnO nanoparticles on sludge digestion: Methane production, metabolism, and microbial community

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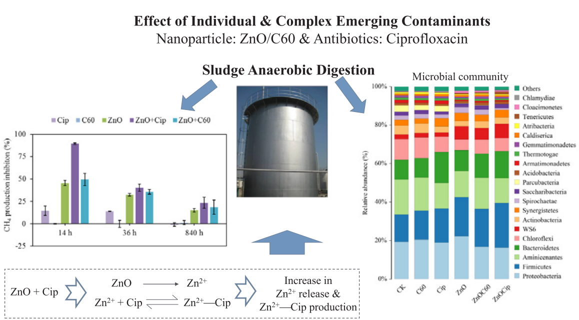
GRAPHICAL ABSTRACT



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ABSTRACT

Antibiotics and nanoparticles, emerging contaminants, present great environmental risks and human health concerns. Sludge adsorption, a biological wastewater treatment removal mechanism, targets ciprofloxacin (Cip) antibiotics, C_{60}, and ZnO, leaving complex pollution in sludge anaerobic digestion. This study investigated the mechanisms through which individual and combined ZnO, Cip, and C_{60} affect sludge anaerobic digestion by studying their effects on CH_4 production, metabolism, and microbial community. ZnO was generally more toxic to CH_4 production than Cip. The ZnO + Cip complex was more influential (> 29%) than ZnO or ZnO + C_{60}, with short-lasting acute and additive toxicity effects on methanogenesis and degradation of protein and carbohydrate. ZnO + C_{60} and ZnO + Cip exerted apparent additional complex effects on *Firmicutes*, *Aminicenantes*, *Chloroflexi*, and *Parcubacteria*. These results would potentially aid toxicity control related to complex pollution, and improve energy production and reduce pollution risks when used in land applications.

1. Introduction

Antibiotics and nanoparticles (NPs), two types of contaminants that have emerged in recent years, are causing great environmental risk and human health concerns (Ivanová et al., 2018). Fluoroquinolones are a class of broad-spectrum antibiotics used in human and veterinary medicine and farming, and ciprofloxacin (Cip) is a second-generation fluoroquinolone with very strong and long-term antimicrobial activity.

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Increased antibiotic usage may create an abundance of antibiotic-resistant genes in the environment, increasing bacterial resistance (Luo et al., 2010). Calero-Cáceres et al. detected quinolone (*qnr*A, *qnr*S) resistance genes in the water and sediment of the Mediterranean river (Calero-Cáceres et al., 2017).

Unlike antibiotics, NPs toxicity in microorganisms is primarily caused by the release of metal ions, production of reactive oxygen species (ROS), and direct cell damage. One widely used carbon-based nanoparticle, C_{60}, could cause an increase in superoxide dismutase (SOD) activity in *Bacillus* and subsequently inhibit growth and reduce the respiration rates of *Bacillus subtilis* and *Escherichia coli* (Huang et al., 2014). The potential elution of Zn^{2+} from ZnO, a metal oxide NP, may induce the production of ROS in microorganisms. Beyond Zn^{2+} elution and ROS production, ZnO NPs can directly damage cell walls, increasing cell membrane permeability and resulting in the inhibition of bacteria growth (Kumar et al., 2011).

Such emerging antibiotic and NP contaminants have been widely detected in wastewater and sludge (Demirel, 2016; Yang et al., 2016). Sludge adsorption is a major mechanism for removing antibiotics and nanoparticles during commonplace biological wastewater treatments (Kunhikrishnan et al., 2015). Pollutants demonstrate concentration levels of up to 31.0 mg/L, up to 20 μg/L, and 0.1–8.6 mg/L in terms of Cip (Guo et al., 2018; Rodriguez-Mozaz et al., 2015), C_{60} (Emke et al., 2015; Farré et al., 2010), and ZnO (Choi et al., 2017; USEPA 2009) within municipal and industrial wastewaters. In municipal sludge, Cip concentrations could reach up to ~13.8 mg/kg with a > 95% removal primarily due to sludge adsorption (Ivanová et al., 2017). Furthermore, at sludge concentrations of 2000 mg/L, 74% of C_{60} nanoparticles can be removed via adsorption (Yang et al., 2013). Choi et al. found ~80% of ZnO NPs were removed via primary and secondary sludge adsorption in conventional municipal biological treatment processes (Choi et al., 2017).

Once these pollutants have accumulated in the sludge at wastewater treatment plants (WWTP), excess sludge treatment becomes highly important. Anaerobic sludge digestion is a widely used, harmless sludge pretreatment process used in land applications to control the discharge of related pollutants into the environment and energy production (Feng et al., 2015). With regards to anaerobic digestion sludge treatment, a treatment capacity of 120–600 ton dry weight (DW) sludge/day corresponds to an operational cost of approximately $ 80–120 per ton of DW sludge. At the same time, the treatment could produce a benefit of $ 52–139 per ton of DW sludge from the electricity produced from methane and save transport costs of the order of $ 40–93 per ton of DW sludge owing to sludge-volume reduction (Yang et al. 2015a).

Since Cip and NPs are primarily removed by sludge adsorption during wastewater treatment, complex pollution from them surely exists in the sludge anaerobic digestion system. Some previous studies have attempted to illuminate the complex effects of antibiotics and metal ions on the environment. Their findings can be summarized into three core discoveries: (1) coordination between pollutants. Bagheri et al. found that Pd^{2+} can form a 2:1 complex with tetracycline (Bagheri, 2015), (2) interactive effects on biodegradation due to the toxicity towards microorganisms. Lu et al. found the root number of *Eichhornia crassipes* was reduced by 21% with tetracycline single stress; however, the root number decreased by 39% under complex stresses from Cu^{2+} and tetracycline, showing significant enhancements in toxic effect (Lu et al., 2014). During the wastewater treatment process, complex pollution from chlortetracycline and metal ions (Ca^{2+}, Mg^{2+}, Cu^{2+}) could increase the toxicity of gram-positive bacteria in the sludge, but failed to significantly affect gram-negative bacteria (Pulicharla et al., 2015), and (3) interactive influencing of adsorption on environmental media via hydrophobic and electrostatic forces. Cd^{2+}, Cu^{2+}, and Pb^{2+} can significantly enhance the adsorption of tetracycline in soil, correlating with their coordination (Zhao et al., 2013)

Since NPs are characterized by nanoparticle sizes, large surface areas, metal ions releasing, and antimicrobial effects, they have great potential to create strong complex effects with antibiotics through coordination, adsorption, degradation and other means. However, the method by which these individual and complex processes affect sludge digestion remains unclear. This study, considering concentrations occurring in activated sludge, took ZnO, C_{60}, and Cip as model metal NPs, carbon NPs, and antibiotics. The aim was to investigate the mechanisms through which individual and combined ZnO, Cip, and C_{60} affected sludge anaerobic digestion by studying effects on the CH_4 production, metabolism, and microbial community. To our best understanding, this represents the first attempt to study the complex effects of these nanoparticles and Cip antibiotics on sludge digestion. These results could thus aid toxicity control for complex pollution created by emerging contaminants in sludge digestion, improving energy production and reducing pollution risks once used in land applications.

2. Materials and methods

2.1. Anaerobic sludge

Anaerobic sludge was collected from mesophilic anaerobic digestion reactors running at a treatment capacity of 800 ton/day, SRT ~ 20 day, and 35 ± 2 °C located in a full-scale sludge treatment plant in Tianjin city, China. The main sludge characteristics during the sampling period were: pH 7.58, total solid 80.57 ± 8.85 g/L, total suspended solid 75.69 ± 5.56 g/L, volatile solids 39.94 ± 8.51 g/L, and volatile suspended solid 31.62 ± 5.46 g/L.

2.2. Preparation of ZnO NPs, C_{60} NPs, and Cip solution

Commercial antibiotic Cip (> 98% purity), ZnO NPs (> 99.8% purity) and C_{60} (> 99.9% purity) were purchased from Macklin biochemical Co., Ltd, China. Stock suspensions of ZnO and C_{60} were prepared via ultra-sonication (97.5 w) using an ultrasonic homogenizer JY92-IIN (Scientz, China) for 0.5 h and 20 h, respectively. The average particle sizes of ZnO and C_{60} in the stock suspensions were measured as 119.7 and 129.5 nm, respectively. Their zeta potentials were 20.7 and −16.7 mV using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). All solutions were prepared using Milli-Q quality pure water (18 MΩ resistance, Elga Ultrapure, UK).

2.3. Digestion reactor and exposure experiments

The digestion process was conducted using an automated methane potential testing system (RTK-BMP, RTKINS, China) with 18 channel reactors, each of which consisted primarily of a reaction flask (500 ml), mechanical mixture rotor, CO_2 absorption bottle, and CH_4 measuring system. The host computer automatically controlled the digestion temperature, mixture rate, and measurement and recording of CH_4 production amounts with 0.1 ml accuracy.

The digestion experiment was conducted according to ISO 13641–1, 2003 (E) with minor modifications (ISO, 2013). The substrate contained nutrient broth, yeast extract, and glucose, each at 2.0 g/L concentration. A 1.0 g/L $NaHCO_3$ buffer was added to the reactors, preventing abrupt pH changes during the sludge process. The total solids concentration in the reaction flask was adjusted to 30.0 g/L. Nitrogen was introduced into the reaction flask for 4 min before the reaction began to create an anaerobic environment. The digestion temperature was maintained at 35 °C using the above system. The amount of spiked model pollutants varied across a wide range, covering their concentrations common in municipal and industry sludges. All experiments were performed in duplicate in this study.

2.4. Analytical methods

pH was measured with a pH meter (Mettler-Toledo, Switzerland).