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Aged-engineered nanoparticles effect on sludge anaerobic digestion performance and associated microbial communities

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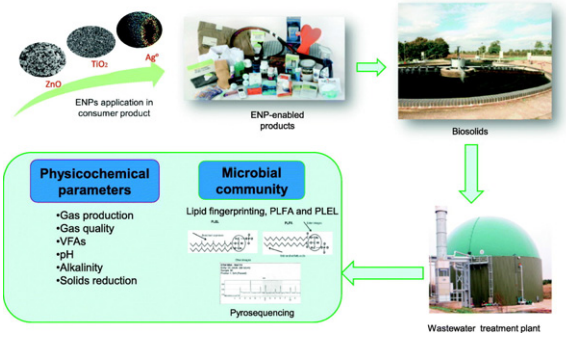
HIGHLIGHTS

- Aged engineered nanoparticles (ENP) did not affect significantly AD process and performance.
- Aged engineered nanoparticles (ENPs) lower H_2S and did not affect CH_4 production.
- *Actinobacteria* and *Fusobacteria* were competitively tolerant to ENP.
- *M. acetivorans* and *M. barkeri* are nano-tolerant methanogens in AD.

GRAPHICAL ABSTRACT



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ABSTRACT

To investigate the potential effect of aged engineered nanoparticles (a-ENPs) on sludge digestion performance, 150 L pilot anaerobic digesters (AD) were fed with a blend of primary and waste activated sludge spiked either with a mixture of silver oxide, titanium dioxide and zinc oxide or a mixture of their equivalent bulk metal salts to achieve a target concentration of 250, 2000, and 2800 mg kg^{-1} dry weight, respectively. Volatile fatty acids (VFA) were 1.2 times higher in the spiked digesters and significantly different ($p = 0.05$) from the control conditions. Specifically, isovaleric acid concentration was 2 times lower in the control digester compared to the spiked digesters, whereas hydrogen sulfide was 2 times lower in the ENPs spiked digester indicating inhibitory effect on sulfate reducing microorganisms. Based on the ether-linked isoprenoids concentration, the total abundance of methanogens was 1.4 times lower in the ENPs spiked digester than in the control and metal salt spiked digesters. Pyrosequencing indicated 80% decrease in abundance and diversity of methanogens in ENPs spiked digester compared to the control digester. *Methanosarcina acetivorans* and *Methanosarcina barkeri* were identified as nano-tolerant as their relative abundance increased by a factor of 6 and 11, respectively, compared to the other digesters. The results further provide compelling evidence on the resilience of *Fusobacteria*, *Actinobacteria* and the Trojan horse-like effect of ENPs which offered a competitive advantage to some organisms while reducing microbial abundance and diversity.

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1. Introduction

In recent years, engineered nanoparticles (ENPs) have been incorporated into many consumer products for their broad microbiostatic and biocidal properties (Suresh et al., 2010). Their extensive use and eventual release in the environment have raised concerned on the potential impacts on the environment and human health (Wiesner et al., 2009). Wastewater treatment plants have been identified as one of the main route (Gottschalk and Nowack, 2011), as most ENPs released from consumer products will sorb to biosolids and be transported to the wastewater treatment plant. Reported concentrations in biosolids vary from relatively low levels of 12–25 mg $kg^{-1}{}_{biosolids}$ and 7–39 mg/$kg_{biosolids}$ for zinc and silver respectively to higher values of 3–100 mg/$kg_{biosolids}$ for titanium (Blaser et al., 2008; Kiser et al., 2009; USEPA, 2009). In this context, ENPs has not only elicited concerns on their potential effects on humans and the environment but also on their influence on the biological processes occurring within wastewater treatment plants (Maurer-Jones et al., 2013). Microbiologically mediated processes can alter ENP core and surface functionality that will influence the fate, transport and toxicity of ENPs turning them into 'aged ENPs' (Barton et al., 2014; Eduok et al., 2015). It is important to remember that most of the published work on the biological effect of nanomaterials have been done at laboratory scale using pristine forms of ENPs and pure cultures of microorganisms; therefore caution should be taken in extrapolating the observed effects to microbial community in complex samples such as wastewater sludge (Eduok et al., 2013). The antimicrobial action of ENPs on aerobic wastewater processes has been well studied and documented (Zhang et al., 2016; Zheng et al., 2015; Li et al., 2014; Sun et al., 2013; Chen et al., 2012; Zheng et al., 2011; Brar et al., 2010; Choi and Hu, 2008) although there are still gaps in knowledge in relation to their impact on anaerobic processes, such as anaerobic digestion (AD) to generate biogas. Biogas production during AD process involves a complex multi-step sequence of substrate hydrolysis, fermentation and methanogenesis catalysed by diverse and unique microbial communities (Appels et al., 2008; Johnson et al., 2003; Schink, 1997) with different optimum requirements for growth and metabolism. To ensure efficient reactor performance it is important to maintain a subtle balance between the different process parameters and potential inhibitors, including heavy metals (Demirel and Yenigun, 2002). Heavy metals such as Zn, Cr, Cu, and Cd are able to hinder microbial activity in AD (Aquino and Stuckey, 2007). In contrast, their correspondent metal nanoparticles have not always exhibited the same physicochemical and toxicological effects. For example, EC_{50} concentration for inhibition of methane production by copper was attained at 10.7 mg L^{-1} for CuO nanoparticles and 129 mg L^{-1} for the correspondent bulk CuO (Luna-del Risco et al., 2011). Similarly, inhibition was recorded at 57.4 mg L^{-1} for ZnO nanoparticles and at 101 mg L^{-1} for the bulk correspondent ZnO (Luna-del Risco et al., 2011). Another author reported 18.3% and 75.1% methane inhibition at the concentrations of 30 and 150 mg ZnO/gTSS for the nanoparticle and the bulk respectively (Mu et al., 2011). In the case of silver (Ag) the reactors containing AgNPs treated sludge have not shown any significant difference in methane production compared to the controls at concentrations up to 40 mg L^{-1} (Yang et al., 2013). The performance of anaerobic digesters containing ENPs-enriched sludge depends on the antagonistic and/or synergistic interactions between bacteria and *Archaea*, although empirical data on the impact of aged-ENPs on Bacteria and Archaea in anaerobic process is still scarce. In this study, three parallel pilot-scale anaerobic digesters were used to assess the effect of aged-ENPs such as silver oxide (Ag°), titanium dioxide (TiO_2) and zinc oxide (ZnO) on the microbial community, their influence on volatile fatty acids (VFA) and biogas production.

2. Materials and methods

2.1. Pilot plant set up

Three parallel pilot-scale plants, each consisting of primary clarifier (180 L), secondary clarifier (~150 L), activated sludge tank (~300 L) and anaerobic digester (150 L) were used in the study (Fig. S1-S1 in supporting information) as previously described by Eduok et al. (2015). Identical conditions were maintained in the three plants with exception that treatment lines 1 and 2 were spiked with ENPs and bulk metal salts respectively, whereas treatment line 3 served as control (unspiked). The three ENPs used were chosen based on their wide application in many consumer products with particle size of 20 nm for Ag^0 and ZnO, and 21 nm for TiO_2. The ENPs are most likely to be transformed and accumulate in biosolids from wastewater treatment processes and ultimately in the soil. Silver was proprietary solution of Ag nanoparticles coated with polyvinylpyrrolidone (PVP). Zinc was a high purity and high quality zinc oxide nano-powder commercially known as Nanosun™. Titanium was high purity titanium oxide nano-powder commercially known as Aeroxide P25 (Degussa, Germany). The solution of mixed ENPs was made up of 0.01 mg L^{-1} Ag^0, 0.08 mg L^{-1} TiO_2, and 0.12 mg L^{-1} ZnO and the activated sludge was spiked at the rate of 0.14 mL min^{-1} (equivalent to 0.67 mL^{-1} L day^{-1}) for 315 days (details in supporting information). An equivalent concentration of bulk metal salts consisting of silver nitrate ($AgNO_3$), TiO_2, and anhydrous zinc nitrate ($Zn(NO_3)_2 \cdot 6H_2O$) and unspiked sludge (control) was used for comparison. The mixed ENPs and metal salt suspensions were maintained in dispersed state by continuous stirring at 200 rpm.

2.2. Anaerobic digester plants set up

The anaerobic digesters (AD) were batch-fed daily with freshly blended primary sludge (8 L) and thickened WAS (2 L) of 0.405 kg VS m^3 d^{-1} equivalent organic loading rate (OLR) without pre-treatment. The digester temperature was maintained with the aid of heating jacket at 35 ± 2 °C in a wet digestion condition. Prior to spiking with either ENPs or metal salt blended sludge, all digesters were operated at a fixed sludge retention time (SRT) of 15 days with a working volume of 121.5 L and subsequently operated over 3 SRT (45 days) to stabilize. ENPs-enriched waste activated sludge (WAS), thickened using Polygold® C420 coagulant (Goldcrest, Barnsley, UK) to 2 L and blended with 8 L settled primary sludge was spiked with 5 mg L^{-1} Ag^0, 40 mg L^{-1} TiO_2, and 56 mg L^{-1} ZnO and batch-fed into the digesters. To minimize the fluctuation in pH as a result of the high organic loading rate, the pH of blended sludge was adjusted with sodium bicarbonate anhydrous (Na_2CO_3, 99.5% pure) buffer to 7.2 ± 0.2. The targeted final concentration of the nanoparticles in the blended sludge (WAS + primary sludge) was 250, 2000, and 2800 mg kg^{-1} for Ag^0, TiO_2 and ZnO, respectively. Equivalent concentration of bulk metal salts comprising of titanium dioxide (TiO_2), silver nitrate ($AgNO_3$) and anhydrous zinc nitrate ($Zn(NO_3)_2.6H_2O$) and unspiked sludge (control) was used for comparison. The spiked concentrations of ENPs and metal salts represent the worst case scenario of the maximum allowable concentrations in sludge spread to agricultural land with reference to the Transatlantic Initiative on Nanoparticle and the Environment (TINE) project (NE/H01375X/1). Digestate circulation at a rate of 240 L h^{-1} was applied using a Mono pump (620S, Watson Marlow, UK) to ensure complete mixing of the digester content. 10 L of digestate was removed from each digester after HRT of 15 days and stored in holding tanks at 4 °C. The digestate produced from the 3 CE plants over 295 days of operation were dewatered in 200 L batches to 25% dry solid using the laboratory filter press. Prior to dewatering, 1.25 g L^{-1} solution of Polygold® C540 coagulant (Goldcrest, Barnsley, UK) was added to thicken the digestate and enhance solid-liquid separation. 10 g subsamples of dewatered digestate and filtrate were then stored at 4 °C until analysis.