Journal of Environment and Earth Science
ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online)
Vol.5, No.12, 2015
www.iiste.org

Zinc oxide nanoparticle impact on solid waste anaerobic digestion and biogas production

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Abstract

Engineered nanoparticles incorporated into consumer products can enter the environment during the manufacture, use of the product and waste disposal. The effect of zinc oxide (ZnO) nanoparticle on anaerobic digestion was assessed using 250 mL batch digesters, fed with a blend of municipal solid waste and cow dung, spiked with 15 to 60 mg kg^{-1} shock dose of ZnO nanoparticle for 60 days at 35 ± 2 °C. The mean volatile fatty acid composition was 0.9 to 2.8 times higher in relation to the control. The difference was significant ($p = 0.05$) and suggests that the microbial community were unable to effectively use the available substrate although low ZnO concentration (15 mg kg^{-1}) exerted no pronounced adverse effect on the digestion process. Based on *Archaea*-specific phospholipid etherlipid (PLEL) derived saturated and monounsaturated isoprenoids, hydrogenotrophic and methylotrophic biomarkers were dominant with 1.0 to 2.3 times lower concentration in the ZnO nanoparticle spiked digester. The biogas produced was concentration-dependent and ranged from 1.1 to 4.3 times lower in the ZnO nanoparticle spiked digestate relative to the control. The results provide evidence on the inhibitory effect of ZnO nanoparticle on the performance, microbial richness and evenness during anaerobic digestion of municipal solid waste.

Keywords: ZnO nanoparticle, municipal solid waste, anaerobic digestion, biogas, inhibitory effect

1. Introduction

Engineered nanoparticles (ENPs) are materials less than 100 nm in size widely incorporated into consumer products because of their novel physicochemical properties and biological effects. More than 1500 consumer products contain a form of nanoparticle and through the production, use of the products and disposal of nano-enabled waste, there is a tendency that aged-nanoparticles will accumulate in environmental matrices (Woodrow Wilson Database 2015). In recent years, it is common knowledge that zinc oxide (ZnO) nanoparticles and other engineered nanoparticles such as silver oxide and titanium dioxide are used in many consumer products in health and fitness, textile and food because of their novel physicochemical characteristics and biocidal effects (Suresh et al., 2010; Verma et al., 2010; Sondi and Salopek-Sondi, 2004). Most ENPs will sorb to organic materials in the environment once they are released form the consumer products. The presence of ENP-enriched organic matter is of critical concern from environmental perspective because of the plausible impact on non-target biologically sensitive microbial communities involved in anaerobic digestion.

In the absence of oxygen, microbial species can degrade and stabilize organic matter and at the same time generate biogas and biomass; a process commonly called anaerobic digestion, is one of the most efficient waste treatment biotechnologies (Appels et al., 2008). Based on temperature requirements, the anaerobic digestion process is broadly classified into mesophilic (20 to 45 ^{0}C) and thermophilic (50 – 70 ^{0}C) anaerobic digestion. Anaerobic digestion can substantially kill pathogens, reduce pollution and generate clean renewable energy for domestic and industrial use. The initial stage of the process is a rate-limiting step where complex organic polymers such as carbohydrates, protein and fat are hydrolysed by fermenting bacteria into utilizable monomeric substrate and precursors which are converted to methane (Schink, 1997; Appels et al., 2008).

AD microbial community have different optimum conditions for proper functioning and in order to ensure efficient reactor performance it is necessary to maintain a balance between the different process parameters (Demirel and Yenigun, 2002). The conditions required by the microbial community can be attributed to the sensitive nature of the methanogenic organisms which are usually affected by physicochemical composition of the substrate such as the accumulation of free ammonia, pH, alkalinity, hydrogen, sodium, potassium, heavy metals, surfactants and other exogenous agents (Appels et al., 2008; Kayhanian, 1994). In addition, upset and failure or instability of anaerobic digestion process can be as a result of imbalance in microbial community abundance and diversity (Demirel and Yenigun, 2002). Other than the intrinsic inhibitory components in the substrate, of significant concern is the release of xenobiotic compounds such as engineered nanoparticles from consumer products into the environment. Thus, the performance of anaerobic digestion in the presence of ENPs will depend on resilient and adapted syntrophic bacterial and archaeal communities in the reactor. Besides, toxic metabolic products can accumulate and may constitute a rate-limiting step when any of the successive steps of

the anaerobic process is adversely affected (Appels et al., 2008).
The empirical data on the effect of ENPs released from consumer products into wastewater treatment plants (Benn and Westerhoff, 2008; Blaser et al., 2008; Geranio et al., 2009) and anaerobic digestion of sludge are available (Jin et al., 2009). Information on the effect of engineered nanoparticle on anaerobic digestion of municipal solid waste is limited (Yang et al., 2012), thus, a clear understanding of how microbial community interact with ENPs is necessary from an environmental perspective. Like most xenobiotic compounds, the adverse effect of ENPs such as ZnO nanoparticle on microorganisms is emerging (Liang et al., 2010) whereas accumulation of ENPs in organic materials can adversely affect the efficiency of anaerobic digestion processes which rely on the diverse microbial communities. This study provides empirical evidence on the inhibitory effect of ZnO nanoparticle on the microbial community structure and abundance in natural enrichment of methanogenic A*rchaea* from cow dung used as seed inoculum to digest municipal solid waste components and generate biogas.

2. Materials and methods

2.1 Experimental set up

The measure of how substances are degraded to generate biogas especially methane, commonly called biochemical methane potential was done by a modified approach of Owen et al., (1979). ZnO nanoparticle spiked complex and undefined municipal solid waste instead of chemically defined medium was used as substrate. 120 g of homogenized solid waste collected from the Uyo municipal waste dump site was equilibrated to the assay temperature of 35 ± 2^0 C and inoculated with 20g of aged cow dung to naturally enrich the substrate with methanogenic Archaea. Prior to inoculation, fresh cow dung used as seed inoculum was obtained from the Uyo municipal abbattoir was incubated at 35 ± 2^0 C for 15 days until no significant biogas was detected. The sludge-inoculum mixture was homogenized and characterized by determining the total and volatile fatty acid (APHA, 2005). The pH of the blended substrate-seed inoculum was adjusted with sodium bicarbonate anhydrous (Na2CO3, 99.5% pure) to 7.2 ± 0.2 and fed into the anaerobic digesters. Zinc oxide nanoparticles (ZnO, 99.5 %, 20 nm) from NanoAmor (Nanostructured & Amorphous Materials Inc. Houston, USA) as characterized by the manufacturer was used directly for the test. No further characterization of the ZnO nanoparticles was carried out. The laboratory-scale anaerobic microcosms containing the substrate-inoculum mixtures were spiked with 15, 30, 45, and 60 mg kg^{-1} shock dose of zinc oxide nanoparticle. The inoculated media were allowed to equilibrate for one hour at 35^0 C and the gas volume normalized at ambient pressure. A control without ZnO nanoparticle spike was also set up. The experiment was carried out in triplicate reactors for the tested concentrations and incubated at mesophilic temperature of 37 ± 2^0 C in a wet digestion condition and continuously agitation at 120 rpm for 60 days.

2.2 Measurement of Volatile fatty acid (VFA)

The changes in volatile fatty acid composition was measured in duplicate anaerobic digestate samples centrifuged at 5000 rpm for 20 min and filtered through 0.45 µm and then 0.2 µm syringe filter. Samples were preserved by adding 10 µL of sulphuric acid to 9 mL of filtered sample and frozen until analysis. Prior to analysis, the samples were thawed, shaken and analysed according to standard methods (APHA, 2005). Volatile fatty acid (VFA) concentrations were determined by high performance liquid chromatography (HPLC) on a Kontron 535 detector (Bio-Tek, Vermont, USA) with a Bio-Rad (California, USA) HPLC column for fermentation monitoring. The column was maintained at 60^oC with an eluent of 1 mM H_2SO_4 at a flow rate of 0.8 mL min^{-1}. VFA concentrations were detected with ultraviolet light at 208 nm. The generated and transformed VFA profiles was obtained using EZChrom® software.

2.3. Phospholipid etherlipid (PLEL) analysis

The bacterial community structure was determined by phospholipid fatty acid (PLFA) and phospholipid etherlipid (PLEL), the chloroform-methanol-citrate buffer approach as described by Frostegard et al., (1991) was adopted. Briefly, 10 g of freeze-dried digestate sample was extracted using citrate buffer- chloroform-methanol at the ratio of 0.8:1:2 (v/v/v), subjected to solid-phase fractionation and transesterified by mild alkaline methanolysis (Dowling et al., 1986) to form fatty acid methyl esters (FAMEs). Aliquots of the phospholipid fraction were subjected to PLEL analysis. Acidic hydrolysis and methylation cleavage of the polar head group to obtain ether core lipids was performed according to the method of Bai et al., (2000). After solvent removal, the aliquot of the phospholipid fraction was redissolved in 2 ml of methanol: chloroform: 37% hydrochloric acid (10:1:1, v/v/v) and kept at 60°C over night. 4 mL water was added after cooling and the etherlipids was hexane-

extracted. The organic phase was combined and dried with sodium sulfate. Hydriodic acid (HI) was used to release the etherlinked hydrocarbons (as alkyliodides) from etherlipids and dried under nitrogen stream.
The samples were incubated at 100°C for 18 h and after cooling, 4 mL water was added to stop the reaction. The samples were hexane-extracted and the hexane phases washed with 4 ml water (15 sec shaking), 10 mL sodium carbonate 10 % (30 sec shaking) and 10 mL sodium thiosulfate 50% (30 sec shaking). The lower phase was discarded after 15 min and the hexane phase dried with sodium sulfate. Reductive dehalogenisation was done by adding 300 mg zinc to the dried sample of alkyliodides in a centrifugation tube. 3 mL of acetic acid (100%) was added, shaken for 20 sec and incubated at 100°C for 18 h. The neutralisation was done after cooling with 5 mL 0.1 M sodium carbonate. Further to this, the samples were hexane-extracted and the hexane phases washed with 10 mL 0.1 M sodium carbonate and water (30 sec shaking). The aqueous phase was discarded after 15 min and the hexane phase dried with sodium sulfate.
To the dried samples, 200 µL nonadecanoic acid methyl ester (Sigma-Aldrich, UK) was added as internal standard to each sample, transferred to GC vials and analysed by gas chromatography (GC) (Agilent Technologies 6890N) coupled to a flame ionization detector. The GC was fitted with a split/splitless injector and a HP-5 (Agilent Technologies) fused silica capillary column (30 m length, 0.32 mm id, 0.25 µm film). Helium at 1 mL per min was the carrier gas and the FAMEs separated using a temperature program, starting at 50°C for 1 min (splitless hold time), increasing at 25°C per min to 160°C followed by 2°C per min to 240°C and 25°C per min until reaching 310°C. Samples (1 µL) were injected using an auto-sampler (injector temperature of 310°C) and FAMEs detected using a FID operating at 320°C. ChemStation software (Agilent G2070) for GC was used to quantify and identify the peaks retention times from the compound of interest relative to the internal standard expressed as expressed as µg n-FAME g^{-1} samples and mass spectral data compared with Supelco peak standard reference data (Sigma-Aldrich Ltd., Dorset, UK).

2.4 Biogas measurements

The cumulative gas volume from the different treatments were measured at 48 hour interval for 30 days and thereafter every 72 hours. Gas volume was determined by volumetric method (Valcke and Verstraete, 1983) by connecting the reactor to a graduated reverse cylinder device filled with water as a barrier solution. The liquid displacement was measured and converted to biogas volume using the formula:

$$\text{Biogas [mL]} = \pi r^2 h^*(k + H - h) / k$$

Where,

r = internal radius of the column (cm);

h = production of biogas as water level decreased in the column (cm);

H = working length of gas collection column (cm);

k = standard atmospheric pressure (1033 cm water gauge).

Results are expressed as the mean of triplicate determinations from the experimental reactors.

2.5 Statistical Analysis

Kruskal-Wallis test was performed using Statistica software® version 12 (Statsoft, Tulsa, OK, USA). The values are presented as mean ± standard deviation with levels of significance maintained at 95% for each test. PLEL data were log-transformed to reduce skewness in distribution, subjected to species-dependent hierarchical cluster analysis and non-metric multidimensional scaling (MDS) ordination based on Bray-Curtis similarities using PRIMER version 6 (Clarke and Warwick, 2001).

3. Results and discussion

3.1 Effect on volatile fatty acid (VFA) composition

Anaerobic digestion of organic matter generates volatile fatty acids that can be converted to biogas. The accumulation of volatile fatty acid is a measure of the inhibitory effect of the ZnO nanoparticle on the methanogenic activities of the microbial community (Appels et al., 2011). The effect of ZnO nanoparticles on the volatile fatty acid composition of the digestate is shown in Figure 1. There was accumulation of volatile fatty acid in the ZnO spiked digestate and the difference was significant ($p = 0.05$) relative to the control. The difference in total volatile fatty acid composition in relation to the control was 1.0 in 15 mg kg^{-1}), 1.3 in the 30 and 45 mg kg^{-1} and 1.6 in the 60 mg kg^{-1} spiked digestate. The volatile fatty acid accumulated in the 15 mg kg^{-1} spiked digestate compared with the control was low but the difference was significant ($p = 0.05$) and indicates that ZnO nanoparticle at low concentrations can inhibit the activities of methanogenic microbial community. Although acetic, propionic and n-butyric acids accumulated with a difference of 1.1 times higher relative to the

control, the concentration of i-butyric, isovaleric and n-valeric acids ranged from 0.8 to 0.9 times lower than in the control. Acetic acid accumulation in the different treatments and can be attributed to reduced metabolic activity of aceticlastic methanogens. The nutrients in the spiked digestate were poorly utilized by the organisms because of reduced population of microbes which resulted in fatty acid accumulation. Other reasons for volatile fatty acid accumulation in the digestate includes temperature fluctuations and the short residence time. However, organisms in the control were actively metabolizing as indicated by the reduced volatile fatty acid concentration in the digestate.

As the concentration of ZnO spiked into the digesters increased, there was a corresponding reduction in biogas production. For instance, at 15 mg kg^{-1}, a slightly enhanced ability to use the volatile fatty acids was conferred on the methanogenic microbial community. In contrast, the level of volatile fatty acid accumulated in the 30, 45 and 60 mg kg^{-1} spiked digestate was consistent with inhibition of methanogenic activities as evidenced in the reduced biogas produced from the ZnO spiked reactors. This result is consistent with the study of Wagner et al., (2010) in which fatty acid accumulation was implicated in the reduced efficiency of anaerobic digestion process. Despite the fluctuations in the concentration of individual VFA in the spiked digestate, the sum of volatile fatty acid accumulated was barely affected and corroborates with the studies of Yang et al., (2012) that VFA can accumulate in response to increased concentration of engineered nanoparticles in a reactor.

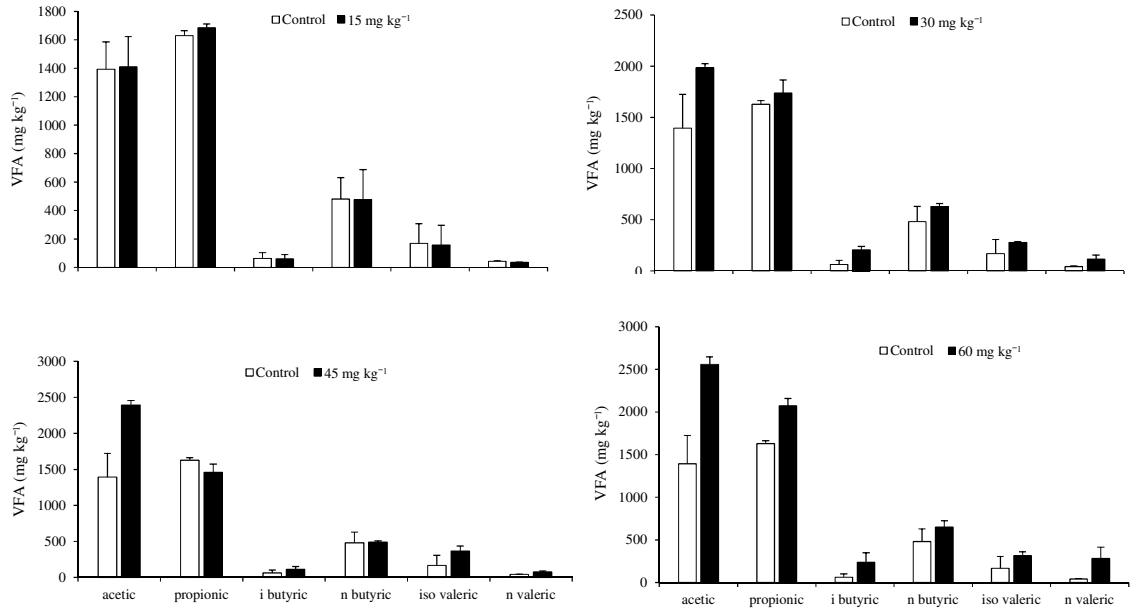

Figure 1 Levels of volatile fatty acid in digestate spiked with different concentrations of zinc oxide nanoparticle after 60 days incubation. Error bars denote standard deviation

In different cultural conditions, however, isovaleric acid is a major end product of amino acid metabolism generated in large amount by multi-enzyme complex of resting microbial cells especially during the stationary growth phase (Thierry et al., 2002; Kaneda, 1991). Moreover, post synthetic modification of isovaleric acid can enhance the ability of structural molecules to maintain membrane fluidity for growth under stress (Kaneda, 1991). The concentration of isovaleric acid in the 15 mg kg^{-1} spiked digestate was 0.9 times lower than in the control. The evidence suggests that the microbial community were actively metabolising whereas, a range of 1.7 to 2.2 times higher concentration observed in the 30 to 60 mg kg^{-1} spiked digestate indicated that most of the cells were in state of rest. Also, it would appear that the cells produced isovaleric acid to maintain the wall and membrane integrity against the harmful impact of the ZnO nanoparticles.

3.2 Community abundance and structural change of Archaea in the reactors

The characteristic Archaea-specific phospholipid etherlipid (PLEL) identified by GC-MS after hydrolysis, cleavage and reductive halogenation of the polar lipids from microorganism in the digestate are presented in Figure 2. The side chains were composed of saturated isoprenoids i15:0, i20:0, i25:0, i40:0, i40:0-1cy and the monounsaturated isoprenoid i20:1. The i40:0-1cy indicates an isoprenoid with 1 cyclo-pentane ring (Gattinger et al., 2003). The results suggest the abundance of hydrogenotrophic and methylotrophic methanogens influenced by their presence in the dung from intestinal tract of ruminants used as the inoculum. Most of the aceticlastic

methanogens probably were derived from the solid waste component and although their presence in the cow dung has been reported (Hungate et al., 1970). Moreover, production of methane from acetate is usually not significant in the ruminant intestine relative to other environmental compartments (Sharp et al., 1998). Apart from the ruminant intestine, other methanogens probably were from the soil and human fecal material which were an integral part of the solid waste. It is worthy of mention that solid waste including human fecal material are dumped at the municipal waste site where the waste management procedures are unclear. Thus, some of the methanogens recovered were those from human intestinal tract. In relation to the animal intestinal tract, notable genera of methanogenic organisms implicated in biogas production include *Methanobrevibacter* (Miller et al., 1986; Lin and Miller, 1998), *Methanosphaera* (Miller and Wolin, 1986) and *Methanogenium* (Miller et al., 1986; Konig, 1986). However, ZnO nanoparticles exerted inhibitory effect on the methanogenic *Archaea* which resulted in their reduced abundance compared with the control.

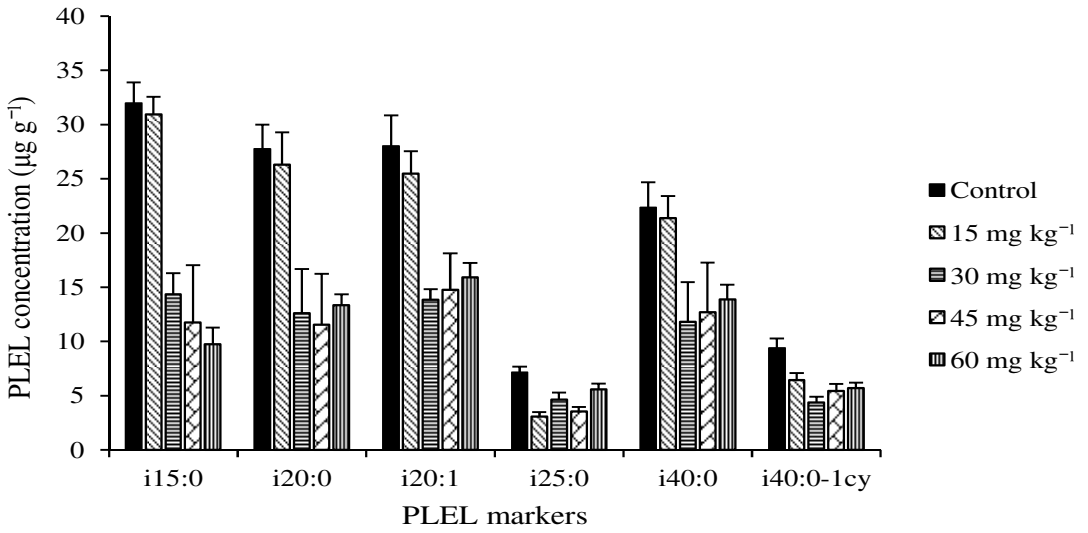

Figure 2 Effect of zinc oxide nanoparticle on the abundance of *Archaea* based on phospholipid etherlipid biomarkers. Error bars represent standard deviation.

The non-metric 2-dimensional MDS of archaeal biomarkers in the different treatments by Bray-Curtis similarity matrix is presented in Figure 3. The result indicates that the triplicate samples of the PLEL markers (n =15) share a similarity index at 20% and 40%. At 60% similarity, the sample markers are grouped into 3 discrete clusters whereas 7 clusters are formed at 80% similarity level. The result indicate that the inter-sample relationships are relatively simple with the gradient of change indicated by the clustering of the PLEL markers. The tight clustering of the sample markers indicate similar taxonomic relatedness of the methanogens. In Figure 3, the stress is low at 0.05 and gives confidence that the 2-dimensional plot of the group-averaged clustering is an accurate representation of the sample relationships. Although in 2-dimesional ordinations, stress increases with reducing dimensionality and increasing quantity of data; the stress value of 0.05 gives an excellent representation without a prospect of misinterpretation (Clark and Warwick, 2001).

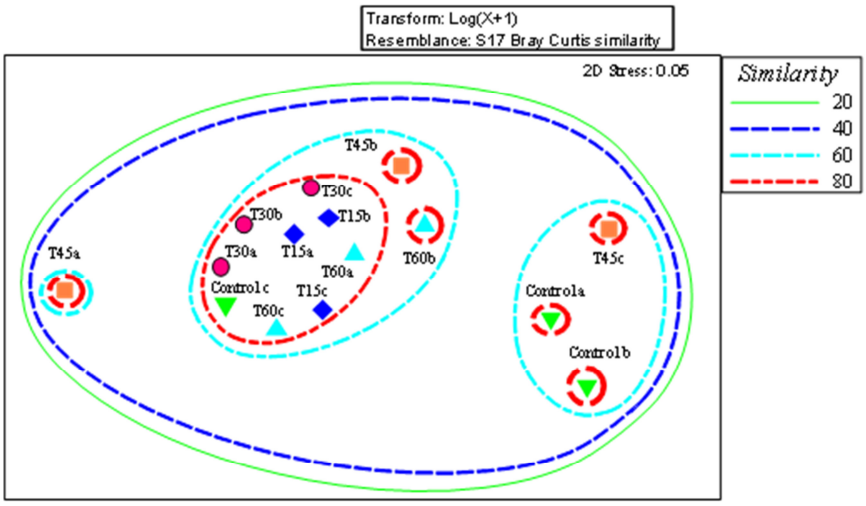

Figure 3. Non-metric 2-dimensional MDS of archaeal biomarkers in the different treatments by Bray-Curtis similarity matrix. The 7 groups at 80 % similarity level forming 3 cluster at similarity level of 60 % are indicated

3.3 ZnO nanoparticle effect on biogas generation

The effect of ZnO on the cumulative biogas production by anaerobic microbial community is shown in Figure 4. The rate of biogas production indirectly measures the rate of microbial metabolic activity in the digestate. There was a concentration-dependent reduction in the biogas generated in the ZnO nanoparticle spiked digestate compared with the control. A maximum of 90% (control), 84 % (15 mg kg^{-1}), 75 % (30 mg kg^{-1}), 61% and 21% biogas production in the 45 and 60 mg kg^{-1} treatments respectively were obtained. The results indicate a range of 1.1 to 4.3 times higher biogas was produced in the control compared with the ZnO spiked digestate and the difference was significant ($p = 0.05$). The trend of biogas produced was dependent on the concentration of ZnO with time and suggests that the metabolic activities of most methanogenic *Archaea* in the ZnO-spiked digestate were reduced in relation to the control. The inhibitory effect of the ZnO nanoparticles resulted in a limited number of the organisms able to use the substrate compared with those in the control.

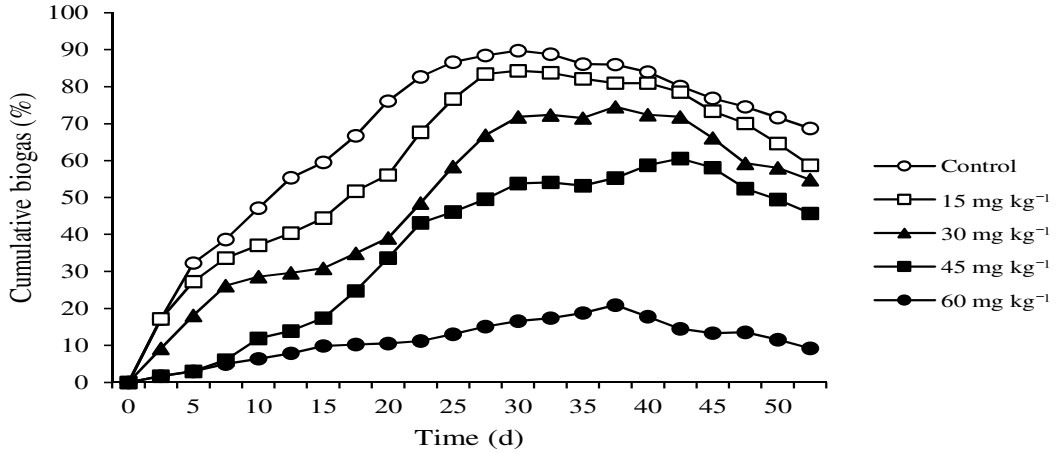

Figure 4 Cumulative biogas profiles of microbial community exposed to different concentration of ZnO nanoparticle the during 60 days incubation

Zinc oxide nanoparticles exerted inhibitory effect on microbes in the digestate evidenced by the reduced microbial density and low production of biogas. However, the specific mechanism in which the nanoparticles inhibited microbial growth is unclear although the generation of reactive oxygen species causing lipid peroxidation and death through oxidative stress are the most probable reasons for the ZnO effect (Choi and Hu, 2008). In addition, ions generated by ZnO nanoparticles can interact with key biotic receptors such as cell membrane/wall, protein and DNA (Bottero et al., 2011) to cause microbial death. The result is consistent with

the findings of Luna-delRisco et al., (2011) in which a similar effect from zinc oxide nanoparticles has been reported.

4. Conclusion

The data suggest that ZnO nanoparticles can inhibit microbial activities during anaerobic digestion of municipal solid waste and as a result, volatile fatty acids accumulated in the digestate. The presence of natural organic matter in the municipal solid waste was unable to mitigate the adverse effect of ZnO nanoparticle which resulted in reduced abundance and a shift in microbial community structure with the dominance of hydrogenotrophic and methylotrophic methanogens. Furthermore, there was no pronounced negative effect by the low concentration of ZnO nanoparticle on biogas production although adverse impact was directly correlated with increase in ZnO nanoparticle concentrations. Therefore, increased use of ZnO nanoparticle in consumer products disposed into the environment can have detrimental effect on the microbial community structure and activities resulting in reduced performance during anaerobic digestion of the municipal solid waste stream.

Acknowledgements

Christiana Udosen is gratefully acknowledged for assistance in the volatile fatty acid analysis.

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