Journal of Hazardous Materials 189 (2011) 603–608

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Particle-size effect of CuO and ZnO on biogas and methane production during anaerobic digestion

Mario Luna-delRisco[a,*], Kaja Orupõld[a], Henri-Charles Dubourguier[a,b,1]

[a] Estonian University of Life Sciences, Estonia
[b] National Institute of Chemical Physics and Biophysics, Estonia

ARTICLE INFO

Article history:
Received 19 November 2010
Received in revised form 7 February 2011
Accepted 27 February 2011
Available online 4 March 2011

Keywords:
Copper oxide
Zinc oxide
Nanoparticles
Anaerobic digestion
Inhibition
Ecotoxicity

ABSTRACT

The effects of bulk- and nano-sized CuO and ZnO particles on biogas and methane production during anaerobic digestion of cattle manure were studied for a period of 14 days at 36 °C using the ISO 13641-2 guidelines. Biogas production was severely affected at concentrations of bulk and nanoparticles over 120 and 15 mg/L for CuO and 240 and 120 mg/L for ZnO, respectively. EC50 concentrations for methane inhibition were estimated to be 129 mg Cu/L for bulk CuO, 10.7 mg Cu/L for nano CuO, 101 mg Zn/L for bulk ZnO and 57.4 mg Zn/L for nano ZnO. The solubility of CuO nanoparticles in the reaction mixture was observed after 14 days of incubation and was significantly higher than the levels observed for ZnO. These results are of significant importance, as it is the first time that the effects of metal oxide particle size on biogas and methane production have been studied.

1. Introduction

Over the past several years, modern industrial research has adopted new technologies to utilise an increasing number of materials at a nanometer scale. These advances allow for improved characteristics so that more complex tasks can be achieved [1]. Currently, interest in these new technologies has resulted in increased funding from private and governmental sources. A wide range of novel applications improved by these new nano materials include anti-reflection coatings, high conductivity and mechanical resistant materials, energy-efficient batteries, antibacterial silver coatings on wound dressings, sensors for disease detection, soil decontamination agents, water filtration materials, biodegradable polymers and highly efficient clear inorganic sunscreens [2–4].

Industrial effluents containing suspensions of these particles may drastically harm the environment and this may be particularly true for aquatic habitats [5–12]. Another negative impact may occur in water and wastewater treatment plants, as no specific filtering mechanisms are typically installed to avoid the entrance of nano-sized particles into the system. In addition, dispersal of contaminated sewage sludge into the soil will spread toxic substances to living organisms, groundwater and sub-surface water systems [4].

In recent years, the ecotoxicity of engineered nanoparticles has been of great interest due to their potential harmful effects on human and other vertebrate health [13–16]. Most studies have examined the effects on aquatic environments [17,18]. However, very few studies have been carried out examining contaminated sediments or non-aquatic environments.

Different toxicity tests examining bulk and nanoparticles of copper oxide (CuO) and zinc oxide (ZnO) have been documented in the literature [19–23]. Results have shown higher toxicity from metal oxide nanoparticles than their bulk particle counterparts. Toxicity assays with these nanoparticles to the microalgae *Pseudokirchneriella subcapitata* have shown high toxicity at exceedingly low concentrations, such as 0.042 mg/L of zinc and 0.71 mg/L of copper [19]. In their study of *P. subcapitata*, toxicity was attributed to the higher solubility of the metal oxide nanoparticles. In experiments conducted by Mortimer et al. [24] examining the toxicity of CuO and ZnO nanoparticles to the protozoa *Tetrahymena thermophila*, the results showed a significant difference in toxicity between nano and bulk CuO particles. Nano CuO was 10 times more toxic than the bulk form.

There remains a lack of information regarding the adverse effect of CuO and ZnO nanoparticles on the environment when assessing different organisms. Currently, data regarding the effect of

* Corresponding author. Tel.: +372 7313255.
E-mail addresses: mario.luna@emu.ee, env.marioluna@gmail.com (M. Luna-delRisco).
[1] Deceased.

0304-3894/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2011.02.085

Table 1
Reaction mixture used during the toxicity test.

Reaction mixture	Composition
Volume of inoculum	9 mL
Concentration of inoculum in test bottles	0.20 g/L
Test medium	9 mL
Dilution water	70 mL
Concentration of cattle manure in test bottles	9 g TS/L
Total liquid volume	88 mL

particle-size (nano and bulk) towards the toxicity on the production of biogas by anaerobic bacteria have not been studied. However, data concerning the inhibitory effect of different copper and zinc chemical forms on biogas production are well-documented in the literature [25–29].

The aim of this study was to investigate and compare the effect of CuO and ZnO particle size on the production of biogas and methane. The results of this study offer scientists and engineers new insights into the understanding of biological processes inhibition, including anaerobic digestion by materials with varying particle sizes.

2. Materials and methods

2.1. Inoculum and substrate

The inoculum was collected from the Paljassaare anaerobic reactor, which is a sewage treatment facility located in the northwestern part of Tallinn, Estonia. The sludge was stored for 2 days at 36 °C under a headspace with a N_2/CO_2 ratio of 80:20. Previous to the experimental setup, the sludge was gently stirred and filtered with a 1 mm^2 sieve to allow for the removal of large particles. The sludge was then diluted with distilled water to reach a fresh matter mass concentration of 2.1 g TS/L (ISO 13641-2) [30]. Cattle manure was chosen as the substrate for the analyses. Samples were collected from a cattle farm located in Jõgeva, Estonia. Collected samples were dried at 60 °C for two days. Milling and sieving of the samples were performed to ensure that a homogeneous particle size diameter of 1 mm was achieved.

2.2. Toxicity test

The toxicity experiment was carried out according to the ISO 13641-2 guidelines [30]. One variation was that cattle manure was used as the substrate instead of yeast extract. CuO and ZnO were purchased from Sigma–Aldrich. CuO and ZnO particle sizes were as follows: bulk CuO ~5 μm, nano CuO ~30 nm, bulk ZnO ~1 μm and nano ZnO 50–70 nm. Stock suspensions of 10 g/L were prepared in milliQ water on the day of the experiment. Stock suspensions were diluted to reach a series of mass concentrations ranging from 7.5 to 480 mg/L.

The test was performed in 160 mL gas-tight closed serum bottles containing 88 mL of reaction mixture (Table 1) and 5 mL of the inhibitor suspension. All experiments were conducted in triplicate. In addition, a set of three bottles containing only the reaction mixture was prepared to act as a control. For experimental validation, a batch of test bottles containing 3,5-dichlorophenol in addition to the reaction mixture was analysed. The experiment was also carried out with a series of mass concentrations ranging from 7.5 to 240 mg/L. An EC50 equal to 71 mg/L and pH between 6.9 and 7.1 at the end of the experiment validated the test. All samples were incubated at 36 °C and gently stirred twice daily during the 14 day experimental period.

Preparation of the test medium for the determination of anaerobic bacteria methane production inhibition consisted of a solution prepared with the following compounds (g/L): KH_2PO_4, 2.7; K_2HPO_4, 5.45; NH_4Cl, 5.3; $CaCl_2{\cdot}2H_2O$, 0.75; $MgCl_2{\cdot}6H_2O$, 1.0; $FeCl_2{\cdot}4H_2O$, 0.2; resazurin, 0.01; $Na_2S{\cdot}9H_2O$, 1.0. Trace element solution (g/L): $MnCl_2{\cdot}4H_2O$, 0.5; H_3BO_3, 0.05; $ZnCl_2$, 0.05; $CuCl_2{\cdot}H_2O$, 0.035; $Na_2MoO_4{\cdot}2H_2O$, 0.01; $CoCl_2{\cdot}6H_2O$, 1.0; $NiCl_2{\cdot}6H_2O$, 0.1; and Na_2SeO_3, 0.05.

Before sample incubation, the pH of the test medium was measured to validate that the experiment was correctly set up. The pH measured from the test bottles was in the range of 6.9 ± 0.3.

2.3. Analytical methods

Gas production kinetics were determined using a calibrated pressure transmitter (SIEMENS). Gas samples were collected using a glass syringe. Methane concentrations from the biogas samples were analysed chromatographically using a gas chromatograph (Varian Inc., Model CP-4900) equipped with two columns as follows: a Molsieve 5A Backflush heated column (20 m × 0.53 mm) and a PoraPLOT U heated column (10 m × 0.53 mm). Helium and argon were used as carrier gases in columns 1 and 2, respectively. Copper and zinc concentrations in the supernatant were measured using a flame atomic absorption spectrometer (Shimadzu Co., Model AAS-6800) after a 20-min centrifugation at 11,000 rpm. Furthermore, acidification (1% HNO_3) and glass microfiber filtration (type: GF/C; Whatman Co.) were performed. Operational configuration of the instrument was set according to the manufacturer's recommendations as follows: wave length (nm) of 324.8 and 213.9; lamp current (mA) of 10/500 and 10/300; acetylene (C_2H_2) flow rate (L/min) of 1.8 and 2.0; slit width (nm) of 0.5 for copper and zinc, respectively.

2.4. Calculations

Biogas production was estimated by measuring the increase in test bottle pressure. The inhibition of methane production was calculated by comparing the volume of methane produced in bottles containing the inhibitor with the controls. Calculation of common toxicity parameters (i.e., EC10, EC20, and EC50) was carried out using the Log-Normal model application within REGTOX software. The half effective concentration, EC50, corresponds to the concentration of inhibitor required to cause a 50% reduction of methane production when compared with the control tests. Analyses on statistical differences between the effects of CuO and ZnO bulk and nanoparticles were performed using STATISTICA software. One-way analysis of variance (ANOVA) followed by *t*-test was used to determine statistical significance ($p < 0.05$).

3. Results and discussion

3.1. Effect of CuO and ZnO bulk and nanoparticles on biogas production

CuO and ZnO nanoparticles and microparticles were inoculated in a batch mode with anaerobically digested sludge at 36 °C for 14 days. Biogas production was used as an indicator of anaerobic digestion imbalance [31–33]. Production of biogas during the incubation period from the control and test samples with different ranges of CuO and ZnO bulk and nanoparticle mass concentrations is illustrated in Figs. 1 and 2, respectively. In the experiment, nanoparticles of CuO (Fig. 1) showed higher toxicity to anaerobic bacteria than bulk CuO, bulk ZnO and nano ZnO. A CuO nanoparticle concentration of 15 mg/L provoked a 30% reduction in biogas production when compared with the total biogas produced in the control sample at day 14. Biogas production in the presence of CuO microparticles was less inhibited, whereas concentrations of 120 and 240 mg/L of bulk CuO caused reductions of 19% and 60%, respectively. Statistical analyses validated the differences between the two groups of CuO particles tested (i.e., bulk and nanoparticles)

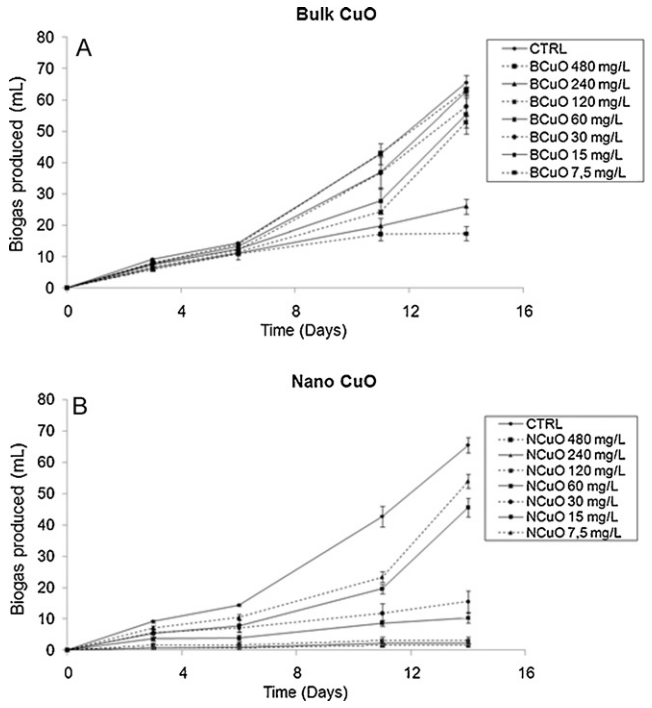

Fig. 1. Biogas inhibition from CuO bulk (A) and nano-sized particles (B).

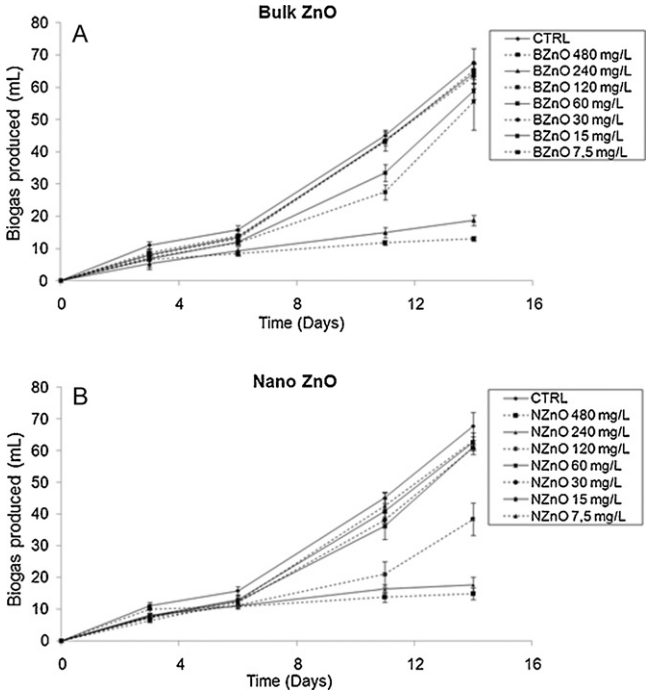

Fig. 2. Biogas inhibition from ZnO bulk (A) and nano-sized particles (B).

($p < 0.05$). As reported by Heinlaan et al. [21], Kasemets et al. [22] and Neal [36], nanoparticles are toxic to bacteria due to the release of cell membrane damaging bioavailable metal ions, and therefore, the inhibition of biogas production can occur.

Biogas production in test samples containing ZnO nanoparticles compared to bulk ZnO is illustrated in Fig. 2. ZnO nanoparticle concentrations of 120 and 240 mg/L presented an inhibition of 43% and 74% of the biogas yield at day 14, respectively. In comparison, test bottles containing bulk ZnO presented a total biogas reduction of 18% and 72% at day 14, respectively. However, no significant difference of biogas inhibition from ZnO bulk and nanoparticles was observed.

A further evaluation of the results presented in Figs. 1 and 2 indicates that the inhibition of biogas production also depends on exposure time. During the first six days of incubation, test samples with bulk CuO, bulk ZnO and nano ZnO were not statistically different from the control sample. However, inhibition of biogas production in test bottles containing CuO nanoparticles occurred at the beginning of the experiment. In addition, results from Figs. 1 and 2 highlight a significant increase in biogas production from day 11 to day 14 for test bottles with CuO concentrations less than 120 mg/L bulk particles and 15 mg/L for nanoparticles. This was also the case for ZnO at concentrations less than 120 mg/L for both bulk and nanoparticles. We suggest that anaerobic bacteria can adapt to medium containing inhibitors, possibly by enzymatic induction, tolerance development or to changes in the microbial metabolism [34], all of which result in an increase of biogas production over time.

3.2. *Effect of CuO and ZnO bulk and nanoparticles on methane production*

The effective concentration values causing a 50% (EC50) reduction of methane production were calculated. Results were used to compare toxicities of different particle sizes (bulk and nano) for varying CuO and ZnO concentrations.

Figs. 3 and 4 illustrate the inhibition of methane production for varying copper and zinc (in their respective oxides form) concentrations during a 14-day incubation period. EC50 values for CuO bulk and nanoparticles were calculated to be 129 and 10.7 mg Cu/L, respectively. For ZnO, the EC50 levels for bulk and nanoparticles were calculated to be 101 and 57.3 mg Zn/L, respectively. Data for EC10, EC20 and EC50 values with confidence intervals are presented in Table 2.

The results presented in Fig. 3 show that CuO nanoparticles (~30 nm) inhibit the production of methane at least 10 times more effectively than the bulk counterpart. The difference between CuO bulk and nanoparticles was statistically significant ($p < 0.0001$). Complete inhibition of methane production in the presence of CuO occurred at concentrations of 330 and 30.2 mg Cu/L for bulk and nanoparticles, respectively.

Although a significant difference for biogas inhibition from ZnO bulk and nanoparticles was not found, methane inhibition was different. It can be seen in Fig. 4 that ZnO nanoparticles had higher methane inhibition than bulk ZnO. The ZnO nanoparticles (50–70 nm) were approximately twice as toxic when compared

Table 2
Toxicity of bulk and nano CuO and ZnO particles to methane-forming bacteria.

Inhibitor	EC10 (mg/L of metal)			EC20 (mg/L of metal)			EC50 (mg/L of metal)		
	Average	95% C.I.		Average	95% C.I.		Average	95% C.I.	
Bulk CuO	54.8	43.0	67.3	73.4	61.7	85.3	129	117	141
Nano CuO	3.94	3.62	4.17	5.56	5.20	5.82	10.7	10.3	11.1
Bulk ZnO	39.8	30.6	52.2	53.6	44.1	66.1	101	84	108
Nano ZnO	19.5	15.7	24.1	28.2	23.9	33.4	57.3	52.1	63.2

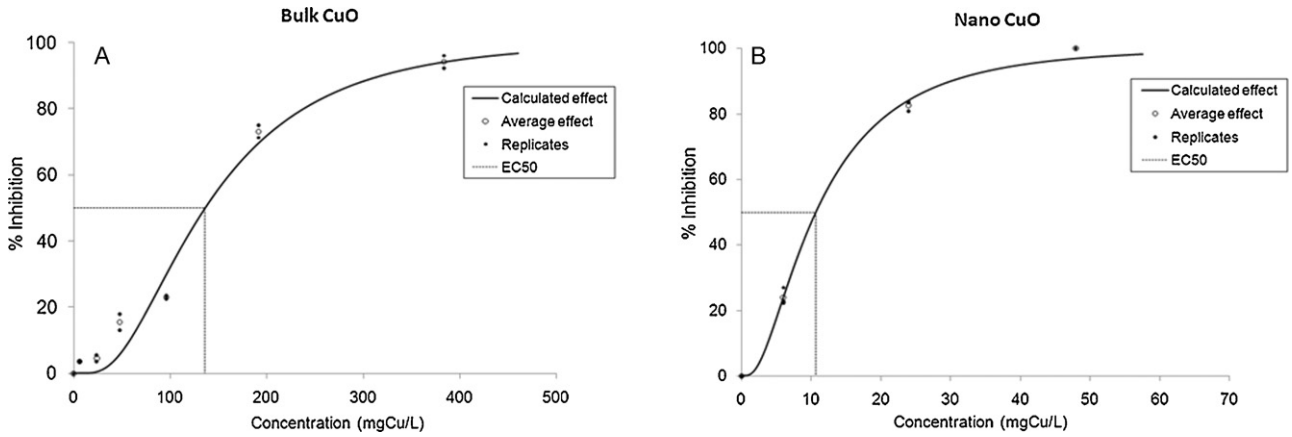

Fig. 3. Methane production dose–response curves during exposure to bulk CuO (A) and nano CuO (B) particles.

to bulk ZnO particles. The statistical difference between the two groups was calculated, with a resulting value of $p < 0.005$. Complete inhibition of methane production for ZnO bulk and nanoparticles occurred at concentrations of 246 and 181 mg Zn/L, respectively.

In our experiment, CuO and ZnO nanoparticles (Figs. 3 and 4) showed higher toxicity to anaerobic bacteria than their bulk counterparts, with other research groups reporting similar results. In studies of the microalgae *P. subcapitata*, Aruoja et al. [19] found a higher toxicity for CuO nanoparticles (~30 nm; EC50 = 0.71 mg Cu/L) compared to bulk CuO (EC50 = 11.55 mg Cu/L). However, although they found high toxicity using ZnO, no statistical difference between the toxicity of bulk ZnO (EC50 = 0.037 mg Zn/L) and nano ZnO particles (50–70 nm; EC50 = 0.042 mg Zn/L) could be determined. In a study conducted by Heinlaan et al. [21], the toxicity of CuO bulk and nanoparticles (30 nm) to the bacterial species *Vibrio fischeri* presented an EC50 of 3049 ± 819 and 63 ± 22 mg Cu/L, while ZnO particles (50–70 nm) showed an inhibition with EC50 values of 1.4 ± 0.08 and 1.5 ± 0.16 mg Zn/L, respectively. However, results from studies of the crustaceans *Daphnia magna* showed EC50 values of 131.8 ± 19.7 and 2.6 ± 1.3 mg Cu/L for bulk and nano CuO, and 7.1 ± 1.1 and 2.6 ± 1.04 mg Zn/L for bulk and nano ZnO, respectively.

CuO nanoparticles were identified as the most toxic particle to anaerobic bacteria from all tested metal oxides in our study (Table 2). Our results appear to be consistent with findings from other groups studying the toxicity of metal oxide nanoparticles to several species of microorganisms. Kasemets et al. [22] studied CuO and ZnO bulk and nanoparticle toxicity at 8 h of *S. cerevisiae* growth. The results from their study show that CuO nanoparticles presented higher toxicity when compared to ZnO nanoparticles. They found an EC50 of 16.6 mg Cu/L for CuO nanoparticles, whereas ZnO nanoparticles presented an EC50 of 97.4 mg Zn/L. Comparable results were also reported by Ivask et al. [37], where bacterial toxicity tests performed with several *E. coli* strains showed higher toxicity levels for CuO nanoparticles when compared to ZnO nanoparticles.

Zayed and Winter [35] studied the influence of Cu and Zn on methane production. In their study, they tested $CuCl_2$ and $ZnCl_2$ toxicity during anaerobic digestion of whey. EC50 values of 4.7 mg Cu/L and 19.2 mg Zn/L were reported. These results are comparable with our data, where it was found that copper oxide nanoparticles had higher toxicity during methane production than zinc oxide nanoparticles. This was the case even though CuO and ZnO nanoparticles have been reported to have very low solubility in water unlike the higher solubility observed for $CuCl_2$ and $ZnCl_2$. In addition, CuO nanoparticles inhibited methane production at similar concentrations as Cu ions in the case of soluble copper salt ($CuCl_2$). However, methane inhibition from $ZnCl_2$ is approximately twice as toxic when compared to our data obtained from studies of ZnO nanoparticles.

3.3. Influence of metal ions on methane production

The presence of heavy metal ions (i.e., Cu, Zn, Fe, Ni, Co, Mo) during anaerobic biodegradation of organic matter is known to be fundamental for numerous reactions. However, high concentrations of these elements can inhibit the biological degradation

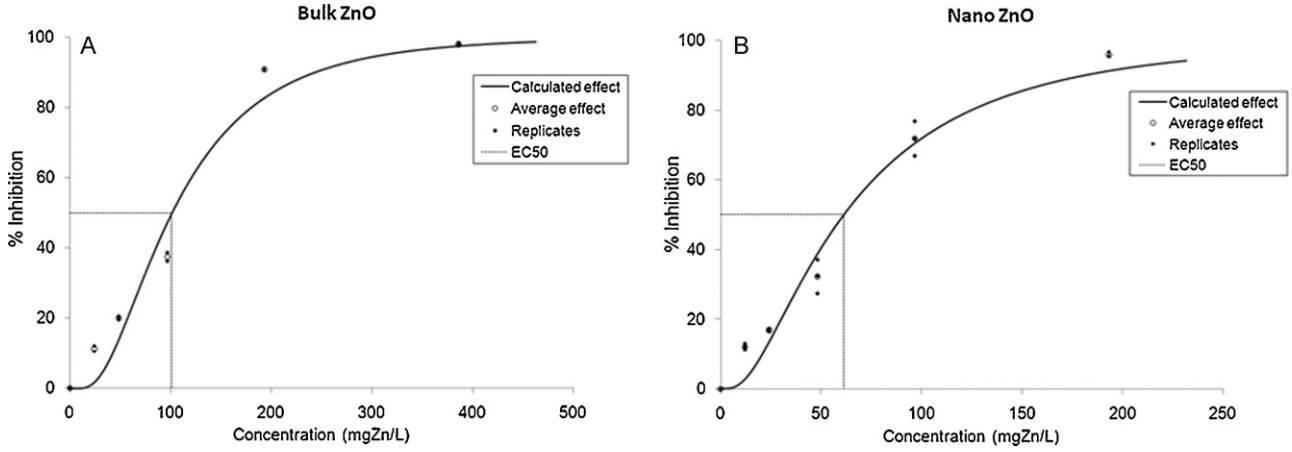

Fig. 4. Methane production dose–response curves during exposure to bulk ZnO (A) and nano ZnO (B) particles.

Table 3
Copper concentrations in the liquid phase of the reaction mixture.

Element	Inhibitor	Inhibitor concentration (mg/L)	Concentration in digestate	
			Average[a] (mg/L)	SD
Cu	Nano CuO	480	47.7	5
		240	34.8	2.3
		120	7.9	0.1
		<60	<1	–

[a] Average from 3 replicates.

process in anaerobic reactors. One of the problems with heavy metal compounds is that these elements are not biodegradable. Due to this, these compounds are known to accumulate, reaching potentially toxic concentrations for anaerobic bacteria [26].

In our experiments, the Cu and Zn ion concentrations in the liquid phase of reaction mixtures were analysed. Quantification limits of the method used for the determination of Zn and Cu were 10 mg/L and 1 mg/L, respectively. Zn concentrations in the liquid phase of the reaction mixture were less than 10 mg/L in all tests. Cu concentrations were less than 1 mg/L in the control bottles and also when bulk CuO was used as the test material. Cu ion concentrations in the reaction mixtures containing CuO nanoparticles are presented in Table 3.

According to the technical data sheets, aqueous solubility of CuO and ZnO bulk and nanoparticles is very low. However, the results presented in Table 3 demonstrate a higher solubility of CuO nanoparticles when compared to bulk CuO. These results suggest that CuO and ZnO nanoparticle toxicity to anaerobic bacteria can be attributed to the dissolved bioavailable fractions of these metals. An ecotoxicological study conducted by Aruoja et al. [19] also concluded that CuO nanoparticle toxicity is attributed to a higher solubility of nanoparticles in the test medium. However, a comparison of Cu ion concentrations in the reaction mixture (Table 3) with the EC50 values obtained by Zayed and Winter [35] for Cu ions from $CuCl_2$ shows that the toxicity of nanoparticles can only be partially explained by the dissolution of CuO nanoparticles to Cu ions. Most likely, different adverse effects of nano- and micro-sized particles to the anaerobic process remain partially due to different surface areas and surface characteristics [23].

4. Conclusions

The results of this study reveal that CuO and ZnO particle-size directly influence the toxicity of these compounds to anaerobic bacteria, and thus affect the production of biogas including methane yield.

Inhibition of biogas and methane production by CuO nanoparticles can be partially attributed to the soluble bioavailable fraction of the metal found in the liquid phase of the reaction mixture after a 14-day incubation period. However, high CuO nanoparticle toxicity cannot only be explained by the release of toxic Cu ions. Zinc oxide formulations were equally toxic, resulting in alterations of biogas production. However, methane production was highly inhibited in the presence of ZnO nanoparticles. From the compounds studied, the most toxic to anaerobic bacteria were CuO nanoparticles (~30 nm) followed by ZnO nanoparticles (50–70 nm), bulk ZnO and bulk CuO.

Analyses of biogas production kinetics showed a possible bacterial adaptation to the medium. We therefore recommend future studies surrounding the inhibition of these chemicals for a longer period to assess possible recovery rates. This may allow for the discovery of suitable mechanisms for re-establishing the anaerobic digestion process. Further research on intermediate products (e.g., hydrogen and volatile fatty acids) of the anaerobic digestion process is needed to obtain more information on the toxicity of these nanoparticles.

The results of our study are an important complement to published data on the ecotoxicity of nanoparticles that are currently used in industry. Data showing high toxicity of nanoparticles indicate that nanolevel particle sizes should also be of concern for the anaerobic digestion processes.

Acknowledgements

We would like to thank the Archimedes Foundation for the PhD grant and Eesti Energia for funding the research project. This research was co-funded by the Estonian Targeted Funding projects SF0690063s08 and SF1090050s07. We greatly thank Dr. Anne Kahru and Dr. Margit Heinlaan for their continuous support and useful comments, and the anonymous reviewers for their helpful comments on the manuscript.

References

[1] G. Schmid, Nanoparticles: From Theory to Application, first ed., John Wiley, Weinheim, 2004.
[2] S.K. Brar, M. Verma, R.D. Tyagi, R.Y. Surampalli, Engineered nanoparticles in wastewater and wastewater sludge – evidence and impacts, Waste Manage. 30 (2010) 504–520.
[3] Organisation for Economic Co-operation and Development (OECD), Safety of Manufactured Nanomaterials. http://www.oecd.org/about/0,3347,en_2649_37015404_1_1_1_1_1,00.html.
[4] R. Paull, J. Wolfe, P. Herbert, M. Sinkula, Investing in nanotechnology, Nat. Biotechnol. 21 (2003) 1144–1147.
[5] A. Baun, N.B. Hartmann, K. Grieger, K.O. Kusk, Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing, Ecotoxicology 17 (2008) 387–395.
[6] C. Blaise, F. Gagné, J.F. Férard, P. Eullaffroy, Ecotoxicity of selected nanomaterials to aquatic organisms, Environ. Toxicol. 23 (2008) 591–598.
[7] R.J. Griffitt, J. Luo, J. Gao, J.-C. Bonzongo, D.S. Barber, Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms, Environ. Toxicol. Chem. 27 (2009) 1972–1978.
[8] M.N. Moore, Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environ. Int. 32 (2006) 967–976.
[9] E. Oberdorster, Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of juvenile largemouth bass, Environ. Health Perspectives 112 (2004) 1058–1062.
[10] A.J. Velzeboer, A.M.J. Hendriks, D. Ragas, Van de Meent, Aquatic ecotoxicity tests of some nanomaterials, Environ. Toxicol. Chem. 27 (2008) 1942–1947.
[11] S.Q. Zhu, E. Oberdorster, M.L. Haasch, Toxicity of an engineered nanoparticle (fullerene, C60) in two aquatic species, *Daphnia* and fathead minnow, Mar. Environ. Res. 62 (2006) S5–S9.
[12] X. Zhu, Y. Chang, Y. Chen, Toxicity and bioaccumulation of TiO_2 nanoparticles aggregates in *Daphnia magna*, Chemosphere 78 (2010) 209–215.
[13] J. Valant, D. Drobne, K. Sepčić, A. Jemec, K. Kogej, R. Kostanjšek, Hazardous potential of manufactured nanoparticles identified by in vivo assay, J. Hazard. Mater. 171 (2009) 160–165.
[14] M. Farré, K. Gajda-Schrantz, L. Kantiani, D. Barceló, Ecotoxicity and analysis of nanomaterials in the aquatic environment, Anal. Bioanal. Chem. 393 (2009) 81–85.
[15] W. Liu, Y. Wu, C. Wang, H.C. Li, T. Wang, C.Y. Liao, L. Cui, Q.F. Zhou, B. Yan, G.B. Jiang, Impact of silver nanoparticles on human cells: effect of particle size, Nanotoxicology 4 (2010) 319–330.
[16] Z. Yang, Z.W. Liu, R.P. Allaker, P. Reip, J. Oxford, Z. Ahmad, G. Ren, A review of nanoparticle functionality and toxicity on the central nervous system, J. R. Soc. Interface 7 (2010) S411–S422.
[17] A.G. Cattaneo, R. Gornati, M. Chiriva-Internati, G. Bernardini, Ecotoxicology of nanomaterials: the role of invertebrate testing, Invert. Surviv. J. 6 (2009) 78–97.
[18] I. Blinova, A. Ivask, M. Heinlaan, M. Mortimer, A. Kahru, Ecotoxicity of nanoparticles of CuO and ZnO in natural water, Environ. Pollut. 158 (2010) 41–47.
[19] V. Aruoja, H.C. Dubourguier, K. Kasemets, A. Kahru, Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae *Pseudokirchneriella subcapitata*, Sci. Total Environ. 407 (2009) 1461–1468.
[20] N.M. Franklin, N.J. Rogers, S.C. Apte, G.E. Batley, G.E. Gadd, P.S. Casey, Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and $ZnCl_2$ to a freshwater microalga (*Pseudokirchneriella subcapitata*): the importance of particle solubility, Environ. Sci. Technol. 41 (2007) 8484–8490.
[21] M. Heinlaan, A. Ivask, I. Blinova, H.C. Dubourguier, A. Kahru, Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*, Chemosphere 71 (2008) 1308–1316.
[22] K. Kasemets, A. Ivask, H.C. Dubourguier, A. Kahru, Toxicity of nanoparticles of ZnO, CuO and TiO_2 to yeast *Saccharomyces cerevisiae*, Toxicol. In Vitro 23 (2009) 1116–1122.

[23] H.L. Karlsson, J. Gustafsson, P. Cronholm, L. Möller, Size-dependent toxicity of metal oxide particles – a comparison between nano- and micrometer size, Toxicol. Lett. 188 (2009) 112–118.
[24] M. Mortimer, K. Kasemets, A. Kahru, Toxicity of ZnO and CuO nanoparticles to ciliated protozoa *Tetrahymena thermophila*, Toxicology 269 (2010) 182–189.
[25] L. Atlas, Inhibitory effect of heavy metals on methane-producing anaerobic granular sludge, J. Hazard. Mater. 162 (2009) 1551–1556.
[26] Y. Chen, J.J. Cheng, K.S. Creamer, Inhibition of anaerobic digestion process: a review, Bioresour. Technol. 99 (2007) 4044–4064.
[27] C.Y. Lin, M.L. Lin, Effect of heavy metals on the upflow anaerobic sludge blanket process, J. Environ. Sci. Health A 32 (1997) 1933–1944.
[28] J.M. Sánchez, L. Valle, F. Rodriguez, M.A. Morinigo, J.J. Borrego, Inhibition of methanogenesis by several heavy metals using pure cultures, Lett. Appl. Microbiol. 23 (1996) 439–444.
[29] I.R. Leighton, C.F. Forster, The effect of heavy metal ions on the performance of a two-phase thermophilic anaerobic digester, Process Saf. Environ. 75 (1997) 27–32.
[30] ISO 13641-1, 2003 ISO 13641-1 (2003-05-15), Determination of inhibition of activity of anaerobic bacteria. Part 2. Test at low biomass concentration, ISO/TC 147/SC 5: 29/12/99.
[31] R.F. Hickey, J. Vanderwielen, M.S. Switzenbaum, The effect of heavy metals on methane production and hydrogen and carbon monoxide levels during batch anaerobic sludge digestion, Water Res. 23 (1989) 207–219.
[32] D.C. Stuckey, W.F. Owen, P.L. McCarty, G.F. Parkin, Anaerobic toxicity evaluation by batch and semi-continuous assays, J. Water Pollut. Control Fed. 52 (1980) 720–729.
[33] G.F. Parkin, R.E. Speece, Modeling toxicity in methane fermentation systems, J. Environ. Eng. Div. 108 (1982) 515–531.
[34] G.M. Gadd, A.J. Griffiths, Microorganisms and heavy metal toxicity, Microb. Ecol. 4 (1978) 303–317.
[35] G. Zayed, J. Winter, Inhibition of methane production from whey by heavy metals – protective effect of sulfide, Appl. Microbiol. Biotechnol. 53 (2000) 726–731.
[36] A.L. Neal, What can be interfered from bacterium-nanoparticle interactions about the potential consequences of environmental exposure to nanoparticles? Ecotoxicology 17 (2008) 362–371.
[37] A. Ivask, O. Bondarenko, N. Jepihhina, A. Kahru, Profiling of the reactive oxygen species-related ecotoxicity of CuO, ZnO, TiO_2, silver and fullerene nanoparticles using a set of recombinant luminescent *Escherichia coli* strains: differentiating the impact of particles and solubilised metals, Anal. Bioanal. Chem. 398 (2010) 701–716.