

Biogas Enhancement Using Iron Oxide Nanoparticles and Multi-Wall Carbon Nanotubes

John Justo Ambuchi, Zhaohan Zhang, Yujie Feng

Abstract—Quick development and usage of nanotechnology have resulted to massive use of various nanoparticles, such as iron oxide nanoparticles (IONPs) and multi-wall carbon nanotubes (MWCNTs). Thus, this study investigated the role of IONPs and MWCNTs in enhancing bioenergy recovery. Results show that IONPs at a concentration of 750 mg/L and MWCNTs at a concentration of 1500 mg/L induced faster substrate utilization and biogas production rates than the control. IONPs exhibited higher carbon oxygen demand (COD) removal efficiency than MWCNTs while on the contrary, MWCNT performance on biogas generation was remarkable than IONPs. Furthermore, scanning electron microscopy (SEM) investigation revealed extracellular polymeric substances (EPS) excretion from AGS had an interaction with nanoparticles. This interaction created a protective barrier to microbial consortia hence reducing their cytotoxicity. Microbial community analyses revealed genus predominance of bacteria of Anaerolineaceae and Longilinea. Their role in biodegradation of the substrate could have highly been boosted by nanoparticles. The archaea predominance of the genus level of Methanosaeta and Methanobacterium enhanced methanation process. The presence of bacteria of genus Geobacter was also reported. Their presence might have significantly contributed to direct interspecies electron transfer in the system. Exposure of AGS to nanoparticles promoted direct interspecies electron transfer among the anaerobic fermenting bacteria and their counterpart methanogens during the anaerobic digestion process. This results provide useful insightful information in understanding the response of microorganisms to IONPs and MWCNTs in the complex natural environment.

Keywords—Anaerobic granular sludge, extracellular polymeric substances, iron oxide nanoparticles, multi-wall carbon nanotubes.

I. INTRODUCTION

NANOPARTICLES have recently attracted enormous attention in the manufacture of commercial industrial and consumer products readily available in the market. Their unique physiochemical properties such as nano-size, structure, surface area, solubility and catalytic characteristics have enabled their application in the fabrication of products like coatings, antimicrobials, paints, cosmetics, medicines, foods, catalysts and environmental processes useful [1], [2]. However, continuous utilization of this products leads to more release of nanoparticles (NPs) into wastewater treatment plants and the environment at large [3], [4]. This in return is

developing human health and environmental safety concerns with great impacts directed towards aquatic life, cells and microscopic community [5], [6]. So far studies have investigated the impact of NPs in the environment and their associated relevant risks [7]-[9], but, it is still necessary to have an in-depth study of the influences of different NPs (such as iron oxide (Fe_2O_3) and MWCNTs) on AGS during wastewater treatment processes. On the other hand, the looming global energy crisis orchestrated by high demand of highly depleted fossil fuel and the dangers of global warming and air pollution associated with it insinuates that an alternative cleaner and more sustainable energy production source, such as energy from biomass, is an imminent need [10], [11]. This is key to transition of the world's energy source from fossil fuel to sustainable energy supply [12], [13]. In this regard biomass from agricultural products (beet sugar industrial wastewater) constitute key secondary source for energy production [14]. This is because beet sugar industrial wastewater (BSIW) is known to be very degradable due to high concentrations of hydrocarbons and sucrose [15]. Previous studies have shown that anaerobic biodegradation of BSIW produces much bioenergy in terms of methane [16]. However, there is much potential for improving biogas and methane production using NPs.

The usefulness of iron ions is embedded upon their tendency to up-take or loose electrons, a characteristic that boosts biomethanation process. But its occurrence in excess is known to easily degenerate to toxicity creating deleterious effects to microorganisms [17]. Studies have shown the usage of iron oxides increasing biomethanation in biodegradation of aquatic plant curly leaf pondweed [13]. IONPs (Fe_2O_3) provide unique source of Fe^{2+} known to enhance methane production [18]. Even in their insolubility state at times, they are reported to increase methane production at lower concentrations [19]. Their potential to increase methane production cannot therefore be underestimated. On the other hand, carbon nanotubes (CNTs) have been useful owing to their miniaturized form, fortified nature and excellent physical properties [20]. Studies have revealed that they have adversely affected aquatic organisms, cells and bacterial community [5], [6]. This puts in great danger natural microbial systems and engineered processes at work [21]. For example, MWCNTs have caused damage to the bacterial cells and significantly reduced biogas generation in UASB microbial floc [22]. However, there is limited information available on the response of AGS to MWCNTs.

To gain in-depth understanding of the potential to improve biomethanation, this study focused on the role of IONPs and

This work was supported by National Science Foundation of China (51308150, 51125033), State Key Laboratory of Urban Water Resource and Environment (Harbin Institute of Technology) (2016TS05).

John Justo Ambuchi, Zhaohan Zhang, and, Yujie Feng* are with the a State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, 73 Huanghe Road, Harbin 150090, P.R. China (* Corresponding author; phone: 86-451-86287017; fax: 86-451-86287017; e-mail: yujief@hit.edu.cn).

MWCNTs in anaerobic degradation of BSIW. In addition, substrate utilization was studied, influence of NPs under study on microbial community involvement was examined, metabolites in terms of acetic and propionic acid utilization was investigated and the interaction of the microbes, NPs and EPS was observed using SEM. The results obtained provide insight into maximization of biomethanation while describing the effect of an interaction between the microorganisms and MWCNTs and IONPs in a complex natural setting.

II. MATERIALS AND METHODS

A. Preparation

NPs utilized in this experiment, IONPs and MWCNTs, were obtained from Alpha Nano Technology Company (Chengdu, China). They were prepared by mixing with deionized water, sonication for 1 hour using a sonicator (VCX130-USA) at a power output of 130 w 20 KHz with the aim to disperse the particles in order to ensure complete mixture.

The seed sludge was AGS obtained from Beer industry in Harbin-Heilongjiang province in China. Before the inoculum was used in a batch experiment, acclimatization in an expanded granular sludge bed reactor (EGSB) of volume 6.5 liters for at least 30 days was done. This reactor operated in mesophilic condition of 36°C, 12 h hydraulic retention time and 3.2 kg COD m⁻³d⁻¹ organic loading rate. Synthetically prepared wastewater with sucrose as main carbon source had concentration of 2000 mg/L COD realizing its removal efficiency of 90% and above at steady state conditions.

Exposure of sludge to IONPs and MWCNTs was done in 250 ml duplicate glass serum bottles. BSIW substrate obtained from Sugar Beet Factory in Nehe, Heilongjiang Province, China was used. Substrate volume of 120 mL and inoculum 100 mL with pH of 6.9 after adjustment was used. The bottles were fed with IONPs at 750 mg/L and MWCNTs at 1500 mg/L concentration and some serum bottles without NPs were used as the control. They were sealed using rubber stoppers together with plastic screw caps, sparged with nitrogen gas for 15 minutes to maintain anaerobic condition. Sparged serum bottles were incubated in a 36 ± 1°C mechanical shaker with 150 rpm. Sample collection of headspace biogas and substrate samples for COD and VFA measurements was carried out at set time intervals. At the end of degradation period inoculum samples were collected for the archaea and bacterial community composition analysis and EPS distribution observance using SEM.

B. Analytical Methods

Substrate samples were collected after 6 hours at the onset of the experiment and periodically after 12 h thereafter for soluble COD (sCOD) and VFA determinations. They were centrifuged for 5 min (13000 rpm) and supernatant filtered using 0.45µm pore sized filters. VFAs were analysed by a gas chromatograph (Agilent GC 7890A, USA) with flame ionization detector (FID) equipped with a HP-INNOWAX column (HP-Innowax 19095N-123, Agilent, USA). The

temperature of injection, detector, and column was set as 170, 240 and 240 °C, respectively. Nitrogen was used as the carrier gas with a flow rate of 60 mL mm⁻¹. After filtering the samples for VFA, they were then acidified with concentrated formic acid (98% purity) to adjust pH below 2 in order to convert fatty acids to their undissociated forms.

Collected biogas samples composition determination was done using a gas chromatograph (Agilent GC 7890A, USA) with thermal conductivity detector (TCD) equipped with a stainless column packed with Porapak Q. The operating temperatures were 200 and 35 °C for detector and column, respectively. The gases produced were mainly separated as Methane (CH₄), carbon dioxide (CO₂) and hydrogen (H₂). At the end of the experiment, collected inoculum samples were immediately stored in -40 °C and thereafter used for microbial community analysis process. Agarose gel was run to check the integrity and concentration of extracted genomic DNA. Genomic DNA was quantified using Qubit 2.0 DNA kit for PCR reaction. This process was done using Sangon agarose recovery kit at Sangon Biotech (Shanghai, China) Co., Ltd. .

C. Multiple Fluorescent Staining of AGS and Imaging

After 150 hours exposure of AGS to nanomaterials, collected sample sludge was fixed with 2.5% glutaraldehyde in phosphate-buffered saline of pH 7.2 for CLSM observation. This was followed by fluorescent staining with FITC, Con A and calcofluor white (Invitrogen Life Science, USA) for protein, α-D-glucopyranose polysaccharides and β-D-glucopyranose polysaccharides, respectively, as described by [23]. Meanwhile SEM samples collected and pretreated as described in the given procedure for measurement [24]. This was done together with Energy Dispersive Spectroscopy (EDS).

III. RESULTS AND DISCUSSION

A. Substrate Utilization

The presence of NPs in the reactors had significant effect on the substrate utilization. Fig. 1 shows substrate utilization in different reactors. In the initial 24 h, COD concentration decreased rapidly in all the reactors with IONPs, MWCNTs and control reactors dropping to 151, 226 and 189 mg/L respectively. Interestingly substrate utilization was much faster in the reactors with IONPs in comparison with the others. The performance of the MWCNTs reactor was however unexpectedly lower than the control for initial 36 h. However, this continuously showed steady rise in the substrate utilization as compared to other reactors registering higher COD removal efficiency than control by 48 h and highest than all reactors after 60 h. Reactors with NPs levelled off after 84 h and after 96 h, MWCNTs, IONPs and control reactors registering 51.0, 60.5 and 64.5 mg/L COD concentrations equivalent to 97.0, 96.5 and 96.3% removal efficiencies respectively. This results suggests that microbes responded swiftly to the availability of IONPs increasing catabolic reaction while microorganisms in the reactors with MWCNTs

took longer to acclimatize to the new environment which increased performance thereafter.

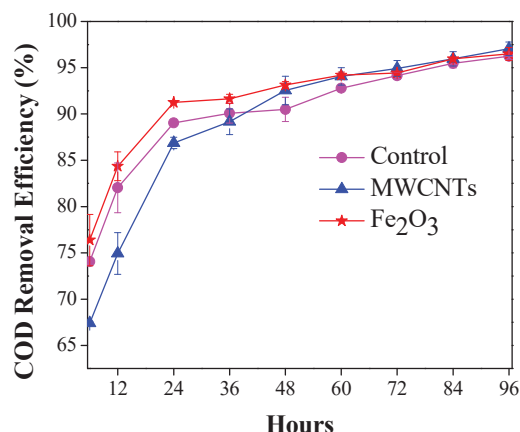


Fig. 1 COD removal efficiency for control, MWCNTs and Fe₂O₃ reactors. Error bars represent the standard deviations of duplicate tests

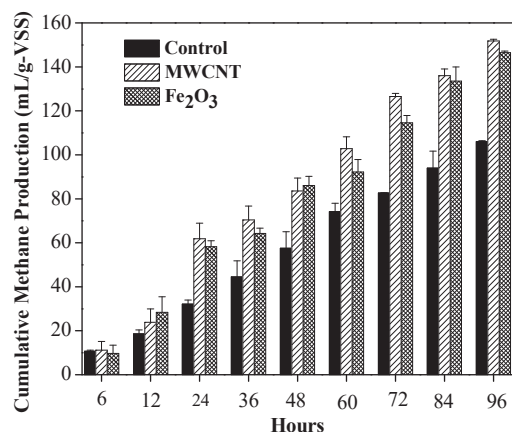


Fig. 2 Effects of MWCNTs and IONPs on methane production during anaerobic BSIW digestion. Error bars represent standard deviation of duplicate tests

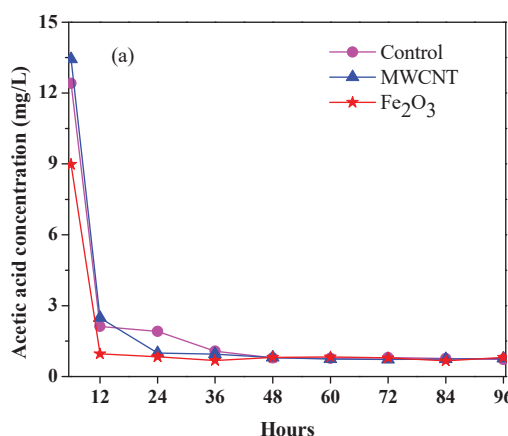
B. CH₄ Production after Exposure to NPs

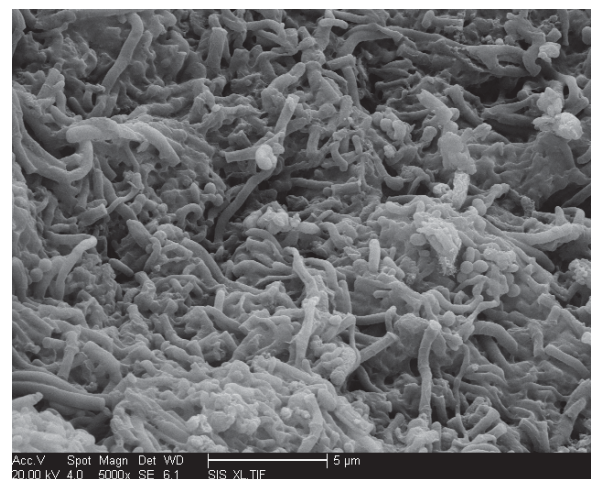
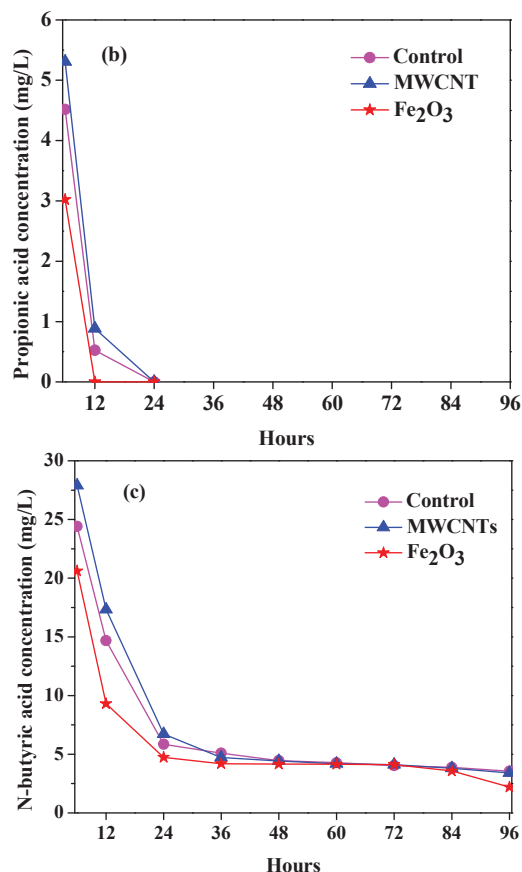
The end of anaerobic degradation process is evidenced by the production of CH₄ gas. During the 96 h incubation period, methane gas production was determined from the headspace at given time intervals. Fig. 2 shows cumulative CH₄ gas production profiles (mL/g-VSS) in the degradation of BSIW anaerobically. After 6 h exposure to NPs, MWCNTs response was immense both in biogas generation and methane gas content. This resulted to methane production of 10.6, 11.2 and 9.7 mL/g-VSS for control, MWCNTs and IONPs respectively. Cumulative methane production levels for MWCNTs kept further increasing while IONPs induced higher methane production than the control reactors. There was no much difference in the CH₄ production rate among the reactors with nanomaterials and after 48 h, IONPs had higher cumulative CH₄ production. This resulted to 86.0, 83.6 and 57.6 mL/g VSS methane gas for IONPs, MWCNTs and control reactors respectively. After 96 h of anaerobic digestion cumulative CH₄ production was highest in the reactors with MWCNTs

(151.8 mL/g VSS) as compared to IONPs (146.5 mL/g VSS) while control had lowest (106.0 mL/g VSS). This is equivalent to 143.19% and 138.16% of control. This results show that MWCNTs at a concentration of 1500 mg/L induced higher biogas production and increased methane content in the biogas as compared to IONPs at concentration of 750 mg/L. Despite of lower COD removal efficiency as compared to IONPs at initial stages, MWCNTs could increase methane production rate better than IONPs. This suggests that MWCNTs might be a better enhancer of direct interspecies electron transfer increasing biogas production.

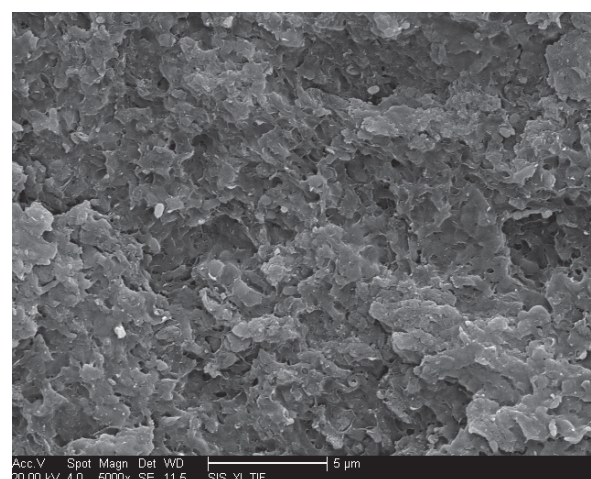
C. Volatile Fatty Acids (VFAs) Profiles

During the anaerobic digestion process, the major metabolites realized were acetic, propionic and n-butyric acid. Fig. 3 shows how their profiles changed over the BSIW degradation period. At the initial stages of anaerobic digestion, reactors with MWCNTs had the highest concentrations of all the three acids. This is however consistent with COD removal efficiency (as observed in Fig. 1) where the reactors with MWCNTs had the lowest removal rate as compared with the others. Acetic and propionic acids concentrations dropped drastically to 1.9, 2.5, 0.9 and 0.5, 0.9 and 0.0% for control, MWCNTs and IONPs reactors respectively after 12 h. This results show that most of the acetic and propionic acids had been converted to methane gas while IONPs had completely converted all propionic acid into biogas. After 12 h, Propionic acid could not be detected further while acetic acid was scintilla in all the reactors. N-butyric acid which had highest concentration after 6 h followed the same trend of reduction. It can clearly be deduced that the reactors with IONPs utilized metabolites at a faster rate than other reactors, although, it is intriguing to find MWCNTs predominance in methane production surpassing IONPs. This warrants further investigation to ascertain the mechanism behind biomethanation process.

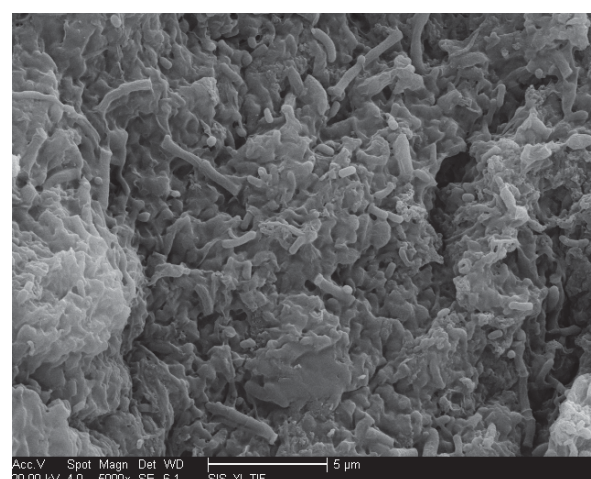




(a)



(b)



(c)

Fig. 4 SEM images for sludge grains in (a) control, (b) MWCNTs and (c) IONPs

Fig. 3 The profile of VFAs showing the changing of (a) acetic, (b) propionic and (c) n-butyric acid in the control, MWCNTs and IONPs reactors

D. Microscopic Images of AGS

After 96 h of incubation in anaerobic condition, sludge granules from control, MWCNTs and IONPs were obtained and observed using SEM. Fig 4 shows structural images of different bacteria. Images in the control reactors (Fig. 4 (a)) showed bacteria as dispersed loose aggregates, while bacteria appeared densely packed in the reactors with MWCNTs (Fig. 4 (b)) and IONPs (Fig. 4 (c)) reactors. This dense-packed structural configuration probably suggests that there was an interaction among the NPs, microbes and their EPS. Microorganisms are well known to excrete complex high-molecular weight substances which attach themselves on the surface of the microbes forming a formidable protective band against any adverse external influence [25]. These successful shielding might have protected NPs from piercing through to the microorganisms which could have had adverse effects on their functionalities, preventing cytotoxicity. This is because methanogens are known to be hyper-sensitive to toxicants [26], [27] with their its effect inhibiting anaerobic degradation process. Higher enhancement of COD removal efficiency and biomethanation process (as observed in Figs. 1 and 2) suggests that there was no inhibitory effects and if any, they were too mild to cause noticeable effects.

E. Microbial Community Compositions

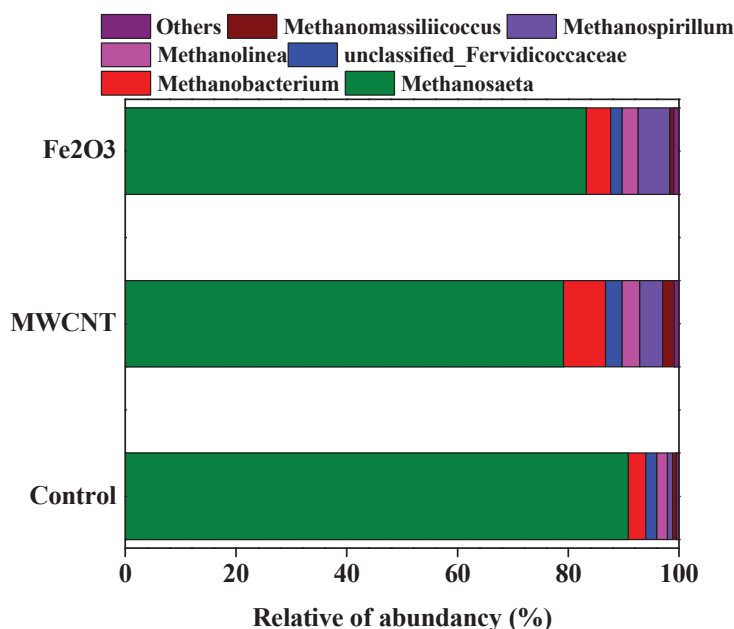
In order to demystify microbial community structure of anaerobic sludge involved in digestion process of BSIW after exposure to MWCNTs and IONPs, bacterial and archaeal analysis was done using pyro-sequencing procedure. The results are as shown in Fig 5. The archaea analysis revealed predominance of over 98% of microorganisms belonging to the phylum Euryarchaeota with the rest belonging to Crenarchaeota. Further analysis of the genus level (as shown in Fig 5 (a)) revealed predominance of Methanosaeta which was above 80% in all reactors followed by Methanobacterium. Close examination shows that MWCNTs and IONPs negated the growth of Methanosaeta while they significantly induced the growth of all others where Methanobacterium abundance was 3.2, 7.6 and 4.4% in the control, MWCNTs and IONPs reactors respectively. However, this seemed to have had no negative influence on the functionalities of the microorganisms as reactors with the NPs produced higher methane gas in both volume and content.

Bacterial analysis, on the other hand, revealed phylum predominance of Chloroflexi followed by Proteobacteria, Bacteroidetes and Firmicutes (as shown in Table I). Further focus on the genus level revealed predominance of Anaerolinea followed by Longilinea (Fig. 5 (b)). The presence of Geobacter was also observed. It is quite clear that MWCNTs and IONPs significantly induced the growth of Anaerolinea and Clostridium bacteria. For example, Anaerolinea was 35.1, 47.8 and 47.5% in control, MWCNTs and IONPs reactors respectively. The dominance of Anaerolinea, Longilinea and the presence of *Clostridium* bacteria is associated with efficiency in hydrolytic and

acidogenetic fermentation processes. They produce cellulosomes which aid in the degradation of recalcitrant microcrystalline cellulose while releasing compounds such as H₂, CO₂, formate and acetate [28] that acetogens and methanogens utilize in biomethanation. The availability of Geobacter bacteria suggests that interspecies electron transfer between bacteria and archea could have been made possible. The inducement of Methanobacterium and the aforementioned bacteria groups by MWCNTs and IONPs could have greatly influenced substrate utilization and biomethanation process. This results clearly demonstrate that MWCNTs has greater influence to biomethanation process than IONPs. The extent of influence to different microbial community therefore needs more research to establish and if there can be synergistic effect in the utilization of both of them.

TABLE I
 BACTERIAL PHYLUM LEVEL

Relative abundance in different reactors (%)			
Name	Control	MWCNT	Fe2O3
<i>Chloroflexi</i>	49.0	54.7	54.6
<i>Proteobacteria</i>	15.9	12.4	13.6
<i>Bacteroidetes</i>	10.4	4.9	5.0
<i>Firmicutes</i>	8.7	16.7	13.8
<i>Synergistetes</i>	3.1	2.2	2.8
<i>Verrucomicrobia</i>	1.9	0.3	0.4
<i>Thermotogae</i>	1.8	1.3	1.5
<i>Planctomycetes</i>	1.7	2.8	3.8
<i>Spirochaetae</i>	1.0	0.7	0.6
<i>Actinobacteria</i>	0.9	1.2	1.0
Others	5.7	2.9	3.0



(a)

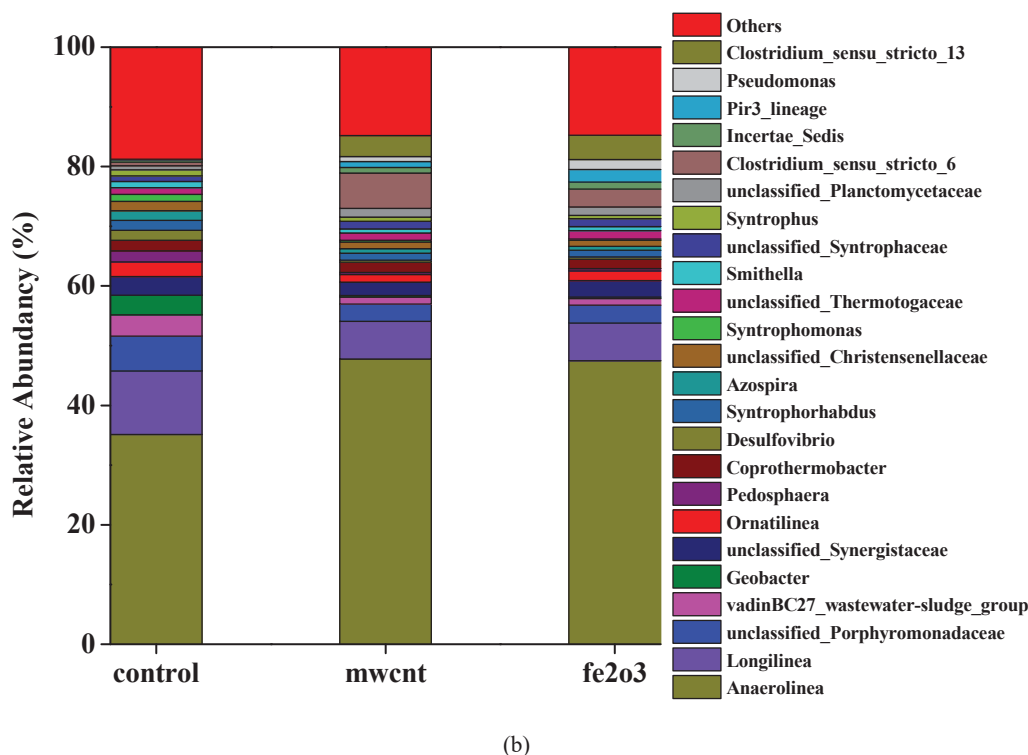


Fig. 5 Microorganisms predominance in different reactors in a genus level as shown in microbial community analysis (a) archaea (b) bacterial

REFERENCES

- [1] A. A. Keller, S. McFerran, A. Lazareva, and S. Suh, "Global life cycle releases of engineered nanomaterials," *Journal of Nanoparticle Research*, vol. 15, pp. 1-17, 2013, pp. 1-17.
- [2] Z.-Y. Zhou, N. Tian, J.-T. Li, I. Broadwell, and S.-G. Sun, "Nanomaterials of high surface energy with exceptional properties in catalysis and energy storage," *Chemical Society Reviews*, vol. 40, pp. 4167-4185, 2011, pp. 4167-4185.
- [3] T. M. Benn and P. Westerhoff, "Nanoparticle silver released into water from commercially available sock fabrics," *Environmental science & technology*, vol. 42, pp. 4133-4139, 2008, pp. 4133-4139.
- [4] H.-J. Kim, T. Phenrat, R. D. Tilton, and G. V. Lowry, "Fe0 nanoparticles remain mobile in porous media after aging due to slow desorption of polymeric surface modifiers," *Environmental Science & Technology*, vol. 43, pp. 3824-3830, 2009, pp. 3824-3830.
- [5] A. P. Roberts, A. S. Mount, B. Seda, J. Souther, R. Qiao, S. Lin, *et al.*, "In vivo biomodification of lipid-coated carbon nanotubes by *Daphnia magna*," *Environmental science & technology*, vol. 41, pp. 3025-3029, 2007, pp. 3025-3029.
- [6] J. N. Mwangi, N. Wang, C. G. Ingersoll, D. K. Hardesty, E. L. Brunson, H. Li, *et al.*, "Toxicity of carbon nanotubes to freshwater aquatic invertebrates," *Environmental Toxicology and Chemistry*, vol. 31, pp. 1823-1830, 2012, pp. 1823-1830.
- [7] A. D. Maynard, R. J. Aitken, T. Butz, V. Colvin, K. Donaldson, G. Oberdörster, *et al.*, "Safe handling of nanotechnology," *Nature*, vol. 444, pp. 267-269, 2006, pp. 267-269.
- [8] T. Satterfield, M. Kandlikar, C. E. Beaudrie, J. Conti, and B. H. Harthorn, "Anticipating the perceived risk of nanotechnologies," *Nature nanotechnology*, vol. 4, pp. 752-758, 2009, pp. 752-758.
- [9] L. H. Alvarez and F. J. Cervantes, "Assessing the impact of alumina nanoparticles in an anaerobic consortium: methanogenic and humus reducing activity," *Applied microbiology and biotechnology*, vol. 95, pp. 1323-1331, 2012, pp. 1323-1331.
- [10] A. Distaso, "For an integrated and sustainable management of solid urban waste: an approach based on the theory of social costs," *International Journal of Sustainable Development*, vol. 15, pp. 220-248, 2012, pp. 220-248.
- [11] C. Wolfram, O. Shelef, and P. Gertler, "How will energy demand develop in the developing world?," *The Journal of Economic Perspectives*, vol. 26, pp. 119-137, 2012, pp. 119-137.
- [12] L. Adelard, T. G. Poulsen, and V. Rakotoniaina, "Biogas and methane yield in response to co-and separate digestion of biomass wastes," *Waste Management & Research*, vol. 33, pp. 55-62, 2015, pp. 55-62.
- [13] C. Mao, Y. Feng, X. Wang, and G. Ren, "Review on research achievements of biogas from anaerobic digestion," *Renewable and Sustainable Energy Reviews*, vol. 45, pp. 540-555, 2015, pp. 540-555.
- [14] M. Hoogwijk, A. Faaij, R. Van Den Broek, G. Berndes, D. Gielen, and W. Turkenburg, "Exploration of the ranges of the global potential of biomass for energy," *Biomass and bioenergy*, vol. 25, pp. 119-133, 2003, pp. 119-133.
- [15] B. Wang, J. Sui, R. Liu, G. Yang, and P. Qi, "Anaerobic reactors treating beet sugar effluents," *Effluent & water treatment journal*, vol. 26, pp. 150-162, 1986, pp. 150-162.
- [16] A. J. Justo, L. Junfeng, S. Lili, W. Haiman, M. R. Lorivi, M. O. Mohammed, *et al.*, "Integrated expanded granular sludge bed and sequential batch reactor treating beet sugar industrial wastewater and recovering bioenergy," *Environmental Science and Pollution Research*, pp. 1-9, 2016, pp. 1-9.
- [17] E. Casals, R. Barrena, A. García, E. González, L. Delgado, M. Busquets-Fité, *et al.*, "Programmed iron oxide nanoparticles disintegration in anaerobic digesters boosts biogas production," *Small*, vol. 10, pp. 2801-2808, 2014, pp. 2801-2808.
- [18] S. Kato, K. Hashimoto, and K. Watanabe, "Methanogenesis facilitated by electric syntrophy via (semi) conductive iron-oxide minerals," *Environmental microbiology*, vol. 14, pp. 1646-1654, 2012, pp. 1646-1654.
- [19] T. Wang, D. Zhang, L. Dai, Y. Chen, and X. Dai, "Effects of Metal Nanoparticles on Methane Production from Waste-Activated Sludge and Microorganism Community Shift in Anaerobic Granular Sludge," *Scientific reports*, vol. 6, 2016.
- [20] T. W. Odom, J.-L. Huang, P. Kim, and C. M. Lieber, "Atomic structure and electronic properties of single-walled carbon nanotubes," *Nature*, vol. 391, pp. 62-64, 1998, pp. 62-64.
- [21] A. Simon-Deckers, S. Loo, M. Mayne-L'hermite, N. Herlin-Boime, N. Menguy, C. Reynaud, *et al.*, "Size-, composition- and shape-dependent toxicological impact of metal oxide nanoparticles and carbon nanotubes

- toward bacteria," *Environmental science & technology*, vol. 43, pp. 8423-8429, 2009, pp. 8423-8429.
- [22] T. Yadav, A. A. Mungray, and A. K. Mungray, "Effect of multiwalled carbon nanotubes on UASB microbial consortium," *Environmental Science and Pollution Research*, pp. 1-10, 2015, pp. 1-10.
- [23] L.-L. Li, Z.-H. Tong, C.-Y. Fang, J. Chu, and H.-Q. Yu, "Response of anaerobic granular sludge to single-wall carbon nanotube exposure," *Water research*, vol. 70, pp. 1-8, 2015, pp. 1-8.
- [24] W. Jin, Z.-j. Zhang, Z.-f. Zhang, M. Qaisar, and P. Zheng, "Production and application of anaerobic granular sludge produced by landfill," *Journal of Environmental Sciences*, vol. 19, pp. 1454-1460, 2007, pp. 1454-1460.
- [25] G.-P. Sheng, H.-Q. Yu, and X.-Y. Li, "Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review," *Biotechnology advances*, vol. 28, pp. 882-894, 2010, pp. 882-894.
- [26] Y. Chen, J. J. Cheng, and K. S. Creamer, "Inhibition of anaerobic digestion process: a review," *Bioresource technology*, vol. 99, pp. 4044-4064, 2008, pp. 4044-4064.
- [27] H. Mu, X. Zheng, Y. Chen, H. Chen, and K. Liu, "Response of anaerobic granular sludge to a shock load of zinc oxide nanoparticles during biological wastewater treatment," *Environmental science & technology*, vol. 46, pp. 5997-6003, 2012, pp. 5997-6003.
- [28] L. R. Lynd, P. J. Weimer, W. H. Van Zyl, and I. S. Pretorius, "Microbial cellulose utilization: fundamentals and biotechnology," *Microbiology and molecular biology reviews*, vol. 66, pp. 506-577, 2002, pp. 506-577.