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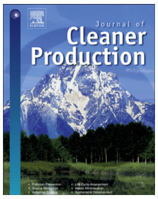

Carbon nanomaterials induce residue degradation and increase methane production from livestock manure in an anaerobic digestion system

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ABSTRACT

The present study investigated effects of two carbon-based nanomaterials, multiwall carbon nanotubes and fullerenes, on biogas and methane yield from sheep manure in an anaerobic digestion system over a 45 days period. The results show that the presence of 500 mg/kg multiwall carbon nanotubes or fullerenes increased the daily and accumulative production of methane (by approximately 46.8% and 33.6%), and decreased the total solid content (by approximately 12.8% and 10.4%) and pH. Exposure 50 mg/kg carbon-based nanomaterials had no impact on digestion. A high-throughput sequencing technique was used to analyze the microbial community diversity and composition in the digests across all treatments. The addition of 500 mg/kg fullerenes and multiwall carbon nanotubes notably altered the composition of the bacteria and archaea at the genus level. The change was particularly evident for *Methanobacterium*, whose relative abundance was significantly increased (by 67%, 53% and 120% upon with 50 mg/kg fullerenes, 50 and 500 mg/kg multiwall carbon nanotubes treatments, respectively), highlighting the positive effects of carbon-based nanomaterials on microorganisms and the subsequent acceleration of methane production. These findings provide important information on the potential use of carbon-based nanomaterials in methane production via altering or tuning the composition of the bacterial and archaeal communities and have relevance for exploring the use of carbon-based nanomaterials in clean energy and agricultural water recycling.

1. Introduction

With the rapid development of intensive animal farming in China, an increasing amount of manure is being produced every year. For example, 10-million tons of sheep manure is produced annually. The inappropriate disposal of these bio-wastes is not only a loss of valuable of resources, but also can cause environmental issues (Lin et al., 2018). Anaerobic digestion is considered a suitable and efficient method for waste treatment to produce renewable energy (Latha et al., 2019). In the process of anerobic digestion, different additives can modulate the digesting conditions. For example, pH can affect the microbial community, which can degrade and transform organic matter to methane. For example, amendments of trace elements (Fe, Mo, Se, Mn, etc.) (Cai et al., 2017) and buffering agents (Spasiano et al., 2017) have been used in anaerobic digestion process to improve the methane yields.

In recent years, engineered nanomaterials (ENMs) have been widely used in agriculture. Within an appropriate dose range, ENMs have shown potential for enhancing crop growth(Hao et al., 2016), suppressing plant disease (Hao et al., 2017), and efficiently facilitating agricultural waste recycling (Abdelsalam et al., 2016).

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For example, under hydroponic conditions, 10 mg/L multiwall carbon nanotubes (MWCNTs) increased the root length of rice (*Oryza sativa*) by regulating the carbon-nitrogen ratio and the level of phytohormones (Hao et al., 2016). CuO NPs increased tomato (*Lycopersicum esculentum*) and eggplant (*Solanum melongena*) growth during infection of *Fusarium oxysporum* f. sp. *Niveum*, largely through enhanced nutrition and plant defense (Elmer and White, 2016). Similarly, foliar application of MWCNTs and fullerene (C_{60}) enhanced tobacco (*Nicotiana benthamiana*) biomass and greatly inhibited Turnip mosaic virus (TuMV) proliferation in plant leaves (Hao et al., 2018).

With regard to agricultural waste resource utilization, Juntupally et al. (2017) compared the effects of $NiCl_2$, Fe_2O_3, $CoCl_2$, $(NH_4)_6Mo_7O_{24}$ micronutrients (MPs) and the corresponding NPs on methane production in a cattle manure slurry anaerobic digestion system, and demonstrated that $NiCl_2$ NPs and the MPs promoted biogas production as compared to other treatments. Abdelsalam et al. (2017a) reported that 20 mg/L Fe NPs and 10 mg/L magnetic Fe_3O_4 NPs significantly increased biogas and methane production in a batch anaerobic system during the processing of fresh raw manure. These positive effects can be ascribed to the release of Fe ions from the corresponding NPs, which played a key role in stimulating the digestion process. The addition of NP Co (1 mg/L) and Ni (2 mg/L) as additives significantly increased biogas and methane yield from the raw manure under the anaerobic conditions when comparing with the control, as well as the corresponding ionic treatments and other NPs at different doses (Abdelsalam et al., 2017b). Additional work comparatively evaluated biogas and methane production upon amendment with Co, Ni, Fe and Fe_3O_4 NPs at 1, 2 and 20 mg/L, and demonstrated that these additives significantly accelerated the production of biogas by 1.7, 1.8, 1.5 and 1.7-fold, respectively, and increased methane yield by 2, 2.17, 1.67 and 2.16-fold, respectively (Abdelsalam et al., 2016). Similarly, the addition of 50 mg/L Fe_3O_4 NPs significantly increased methane yield by 53% as compared to control in a waste anaerobic digestion system operating at mesophilic temperature ($37 \pm 0.5\,^{\circ}C$) (Ali et al., 2017). MWCNTs are carbon allotropes with cylindrical nanostructures and can form both naturally and artificially. C_{60} is nano-sized molecular cages of carbon allotropes, and the size varies from 28 to 100 carbon atoms. As carbon-based conductive materials, typical carbon-based nanomaterials (CNMs) obtained the shift anaerobic microbial community for promoting extracellular electron transfer which could effectively stimulates organic compounds degradation (Zhao et al., 2017). Notably, investigation on the effects of CNMs on anaerobically digesting agricultural waste with high solid content has not been reported.

In the present study, two types of CNMs, MWCNTs and C_{60}, at 50 and 500 mg/kg were used as exogenous additives in a model 1-L anaerobic digestion system containing sheep manure and microbial inoculum. The digestion process was allowed to occur 45 days at a mesophilic temperature (35 °C) to investigate potential of these CNMs to modulate the production of biogas and methane. After 45 days digestion, the production of biogas and methane was measured across all treatments, as well as the pH and total solids (TS) content. In addition, the bacterial and archaeal community composition in the digestion system was analyzed using the paired-end sequencing method to investigate the mechanisms underlying observed changes biogas and methane production upon CNMs exposure. These findings in this study provide new insights into use of CNMs in the anaerobic digestion process and highlight the potential use of these materials from a broader perspective in agricultural production and waste treatment. Considering the rapid development of nanomaterial science, the economic and environmental efficiency of agricultural waste anaerobic digestion at high solid content, it is of great significance to investigate the potential methods to improve digestion efficiency by adding novel nanomaterials.

2. Materials and methods

The experiment was conducted in the laboratory at China agricultural university. Detailed methods and materials used in this experiment are provided in the following paragraphs.

2.1. Characterization of CNMs

C_{60} was purchased from Puyang Yongxin Fullerene Technology Co. Ltd. (Puyang, China), and MWCNTs were kindly provided by Professor Wei Fei (Tsinghua University, China) after synthesis in a nanoagglomerate fluidized-bed reactor. The purity of both C_{60} and MWCNTs were 99.9%. MWCNTs and C_{60} suspensions were prepared in ethanol by sonicating for 30 min, and the suspension was then dropped onto Cu grids for evaluation by transmission electron microscopy (TEM, JEM-2100, JEOL, Japan) as described by Li et al. (2014). The zeta potential and the conductivity of 50 mg/L MWCNTs and C_{60} suspensions in deionized water were measured using a dynamic light scattering particle size analyzer (DLS, Zetasizer NanoZS, Malvern, UK).

2.2. Preparation of livestock manure and inoculum for anaerobic digestion

Sheep manure was collected from a sheep farm in Changchun (Jilin province, China). The inoculum was obtained from a local waste treatment company (Beilangzhong of Beijing, China), and that operates an industrial anaerobic digestion system under mesophilic conditions. The properties of feedstock and inoculum, including moisture content, TS, and volatile solid content (VS), were measured as described by Wang et al. (2018a).

2.3. Batch anaerobic digestion tests

For each anaerobic digestion batch test, the sheep manure and inoculum were thoroughly mixed to achieve a TS content of 20% and then resulting substrate was thoroughly blended with MWCNTs or C_{60} for a final CNMs concentration of 50 and 500 mg/kg based on a previous report (Zhang et al., 2017). The CNM amended digestion substrates were added into 1 L glass reactors and were inoculated in an incubator (WD-DZ-300L, Hongyuan, China) at 35 °C for 45 days. Each treatment was carried out in triplicate. Reactors without the addition of CNMs were used as controls. Biogas produced from each glass reactor was collected daily using Tedlar gas bags (Wang et al., 2018a). The biochemical reaction during anaerobic digestion processes could be described by the following formula:

$$C_aH_bO_cN_d + nH_2O \rightarrow xCH_4 + yCO_2 + wNH_4^+ + zHCO_3^-$$

where $C_aH_bO_cN_d$ represent the organic matters in digestion substrates.

2.4. Biogas production

The daily yield and composition of produced biogas were measured using a portable biogas meter (BIOGAS5000, Geotech, UK). pH value and TS content of the digested samples at Day 1, 3, 5, 7, 10, 15, 20, 25, 30 and 45 were measured by pH meter (ST2100, OHAUS, USA) (Wang et al., 2016) and the standard method (APHA, 2005) respectively.

2.5. SEM observation of digested samples

After a 45 days anaerobic digestion process, the residues across all the CNM treatments were dried in an oven to constant weight. The dehydrated samples were then fixed onto sample stage by conductive tape, gold sprayed, and a vacuum was applied in the chamber. The morphology and size of the digested residues were observed by scanning electron microscopy (SEM; GeminiSEM 300, Carl Zeiss, Germany). The diameter of digested particles treated with different concentrations of CNMs was measured by randomly selecting 140 particles/regions using Image J software.

2.6. DNA extraction and sequencing

The digestion reactors were sampled at Day 30 for analysis of the bacterial and archaeal community composition across all treatments. Briefly, a 1-g sample of digest was used for total DNA extraction with a DNA extraction kit (MO BIO Laboratories, Carlsbad, CA, USA). The isolated DNA was amplified using universal primers: for bacteria, 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), targeting the V4 region of the 16S rDNA gene; for archaea, 516F (5′-TGY-CAGCCGCCGCGGTAAHACCVGC-3′) and 806R (5′-GGAC-TACHVGGGTWTCTAAT-3′), targeting the V4 region of the 16S rDNA gene. The polymerase chain reactions were conducted using the following temperature profile: denaturation at 94 °C for 5 min, followed by 30 cycles of amplification at 94 °C for 1 min, 48 °C for 1 min and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min, and held at 4 °C. The paired-end sequencing was conducted using the Illumina sequencing platform (Illumina, San Diego, CA, USA) by Epsilon Bio-Technique Co. Ltd., Beijing, China.

2.7. Data analysis

All the experimental data were analyzed statistically by the software SPSS 19.0 using the one-way analysis of variance and Duncan's test and shown significant difference among all treatments when the P value was less than 0.05.

3. Results and discussion

In this section, the main results obtained in this study were analyzed and discussed in detail. Firstly, two CNMs were characterized including the NM sizes and zeta potentials. Secondly, methane yields, pH and TS alterations were presented after various CNM treatments. Finally, microbial community changes were detailed combined with specific functions of main microbes in anaerobic digestion system.

3.1. Characterization of CNMs, livestock manure and inoculum

TEM images of MWCNTs and C_{60} are shown in Fig. 1. MWCNTs were aggregated and mingled, the length of MWCNTs varied from 2 to 20 μm, and the average diameter of the individual tubes was approximately 20–30 nm (Fig. 1A). The size of C_{60} ranged from 40 to 60 nm (Fig. 1B). The zeta potential of MWCNTs and C_{60} were -19.17 ± 0.42 and -25.37 ± 0.52 mV, respectively; the conductivity of MWCNTs and C_{60} were 18.03 ± 8.71 and 5.12 ± 0.19 μS/cm, respectively, which were similar to previous study (Hao et al., 2019).

The moisture content, TS content and VS content of the inoculum used in the solid-state anaerobic digestion system were approximately 76.8%, 23.2% and 12.9%, respectively. For the sheep manure, the moisture content, TS content and VS content were 59.7%, 40.3%, and 27.2% respectively, which were proper for anaerobic digestion (Wang et al., 2018a).

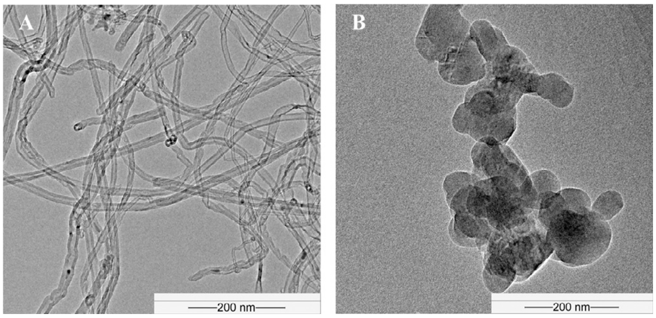

Fig. 1. TEM images of MWCNTs (**A**) and C_{60} (**B**). The scale bar in each panel represents 200 nm.

3.2. Daily and accumulative methane yield

The methane content across all the treatments increased sharply and reached the peak at approximately 50% in the first 10 days and then plateaued at 40–60% until the end of the digestion period (Fig. 2A). The average methane content in the 500 mg/kg MWCNTs treatment (1.32 ml/g, VS) was higher than other treatments and the daily yield of methane was also more stable over time, suggesting that the nanotubes could facilitate the methane production. This positive effect could be ascribed to the stable tubular structure and higher electric conductivity of the MWCNTs, both of which could promote the syntrophic oxidation rate of butyrate to methane, and consequently accelerate the transformation and degradation of organic matter (Ambuchi et al., 2017).

Similar to the methane content, the daily methane yield increased over the first 10 days. The maximum daily methane yield occurred at Day 9 across all the treatments, except the control, where the maximum daily yield was at Day 10 (Fig. 2B). After that time point, the daily methane yield decreased dramatically with time. The highest peak (3.269 mL/g VS) of daily methane yield was evident in the 500 mg/kg C_{60} treatment, followed by the 50 and 500 mg/kg MWCNT treatment, in which the daily methane yield was 3.01 and 2.95 mL/g VS and significantly different to control at the same day, respectively. The lowest daily methane yield was 2.72 mL/g VS in the 500 mg/kg C_{60} treatment. The maximum daily methane yield in the control was 2.16 mL/g VS; this values is significantly lower than all CNM treatments. During the digestion process, the presence of CNMs caused several methane peaks at Day 20, 25 and 34; all of these levels were 50%–80% higher than the control, suggesting that CNMs addition could uniquely increase daily methane yields in the middle and later stages of the digestion process. Along with the sheep manure digesting processes, some intermediate products including propionic acid and acetic acid were yield especially in the middle and later periods, which could limit the manure digestion and the daily methane yields (Molaey et al., 2018). The addition of NMs may promote the disintegration of intermediate products, alleviate their inhibited effects, and promote daily methane yields in these stages (Yang et al., 2018b).

The accumulative methane yield upon exposure to CNMs were considerably higher than the control (Fig. 2C), particularly the 500 mg/kg CNM treatments. As evident in the cumulative methane production curves, 50 mg/kg MWCNTs or C_{60} slightly and insignificantly promoted the accumulative values to 45.60 and 49.61 ml/g VS, whereas treatment with 500 mg/kg MWCNTs or C_{60} significantly increased the accumulative yields up to 59.42 and 54.06 ml/g VS respectively, as compared to 40.47 ml/g VS in the control.

Similarly, recent studies demonstrated that typical CNMs, such

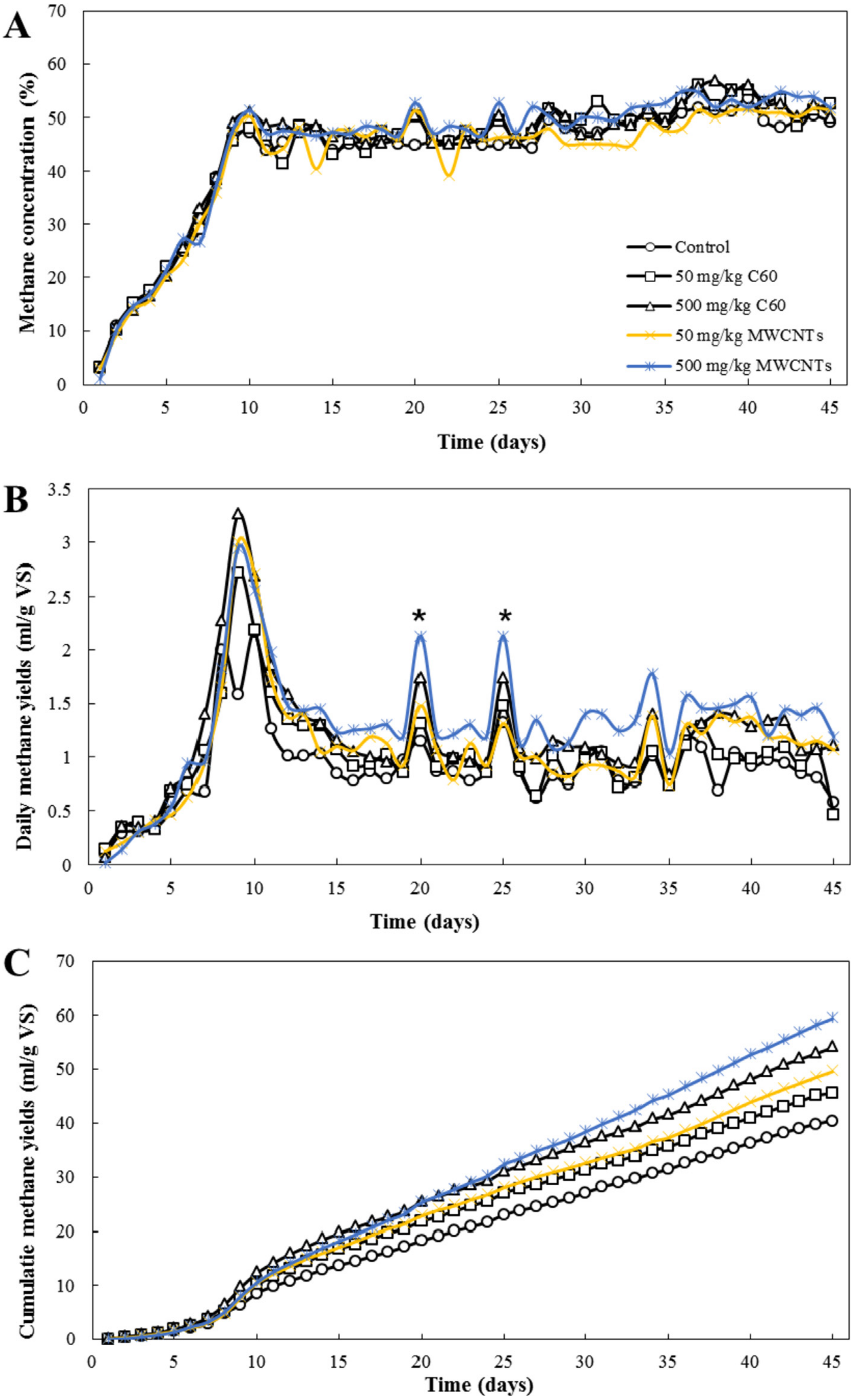

Fig. 2. The methane concentration (A), daily methane yield (B) and cumulative methane yield (C) in the digestion system affected by the addition of CNMs at different concentrations. Significant difference ($p < 0.\ 05$) between the 500 mg/kg CNM treatments (MWCNTs and C_{60}) and the control was marked with "asterisk".

as graphene, single-walled carbon nanotubes (SWCNTs), and MWCNTs, could effectively promote anaerobic digestion performance resulted from their unique properties (Zhang et al., 2018). Specifically, 1.0 g/L graphene nanomaterial promoted the biomethane yield by 25.0% and production rate by 19.5%, because of high electron transfer flux provided by graphene-based direct interspecies electron transfer (DIET) (Lin et al., 2017). The addition of 30 and 120 mg/L graphene significantly increased methane production rate by 17.0% and 51.4%, respectively in anaerobic digestion by enhancing acetate-consuming methanogenesis (Tian et al., 2017). Similarly, as a carbon-based conductive material, MWCNTs could promote direct the interspecies electron transfer in microbial communities, consequently, improve the production of methane (Salvador et al., 2017). Besides, MWCNTs could also act as a habitation for microbial immobilization, provide essential elements for anaerobes, enrich the bacterial community, and enhance the hydrogen or methane production (Ambuchi et al., 2017). As for SWCNTs, Yan et al. (2017) demonstrated that SWCNTs could significantly promote the methane production, shorten start-up period and enhance the microbial activity in a thermophilic anaerobic system by the formation of DIET using the CNMs as electron conduits. Aligning with previous studies, it is demonstrated that the addition of CNMs within appropriate concentration could significantly enhance the total methane production during anaerobic digestion processes.

3.3. *pH value and TS*

Fermentative bacteria and archaea are sensitive pH in the process of anaerobic digestion (Dai et al., 2017). The appropriate pH in the substrate not only benefits methanogen growth but can also inhibit ammonia production (Ramírez-Sáenz et al., 2009). As shown in Fig. 3A, pH in the control was higher as compared to that of the CNM treatments during the 45 days of anaerobic digestion, considering the lowest cumulative methane yields in control, the results suggested that CNMs amendment could decrease the substrate pH and establish the beneficial environment for microbial growth that indirectly enhances methane production. A common finding across all the treatments is that high pH corresponds with low methane production in the anaerobic digestion system (Fig. 3A). The TS content in all treatments shows a decreasing trend over time (Fig. 3B). Both CNM treatments at 500 mg/kg induced the largest and significantly decrease in TS content compared with control, which occurred at Day 20 and then became stable until the end of the process. The lowest impact on TS content was found in the control, whose pH was too high to be suitable for microbial growth (Wang et al., 2016). As for the 50 mg/kg CNM treatments, the changing trends of TS was similar to the control during the 45-day fermentation processes, indicating that the low-dose of CNMs had no impact on the anaerobic digestion. A low microbial biomass will obviously result in low biodegradation of organic matter in the substrates and consequently produce less methane in the anaerobic digestion system.

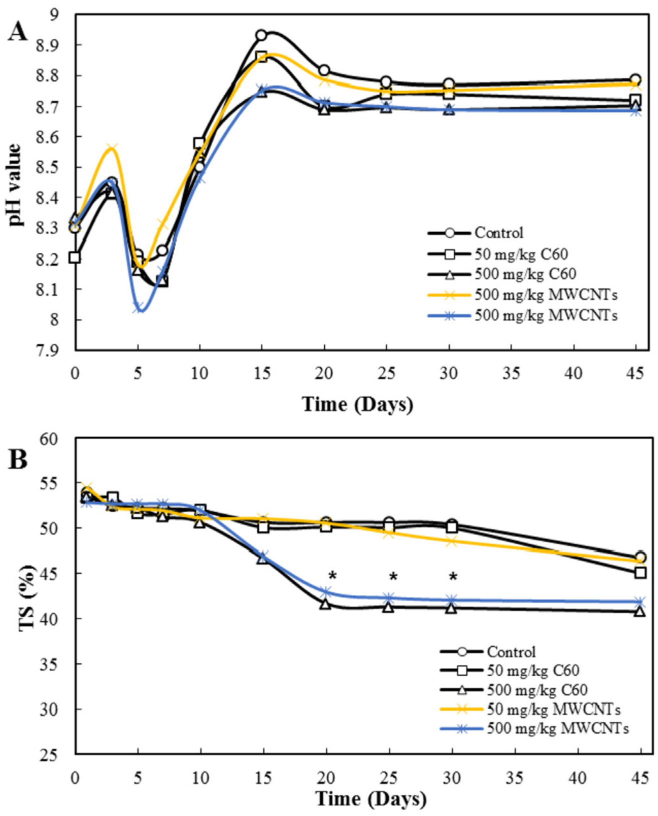

Fig. 3. Changes of pH (A) and TS (B) in a high solid content anaerobic digestion system upon CNMs exposure. Significant difference ($p < 0.05$) between the 500 mg/kg CNM treatments (MWCNTs and C_{60}) and the control was marked with "asterisk".

3.4. *SEM observation of digested residues upon ENM exposure*

SEM images of the residue after the 45 days anaerobic digestion process show that the presence of CNMs altered feedstock biodegradation (Fig. 4). The particle size of the digested residues treated with high concentration of C_{60} and MWCNTs (Fig. 4C and E) was smaller and more homogenous than the control (Fig. 4A). However, the low concentration of both CNMs had little impact on residue biodegradation in terms of the particle size and overall uniformity (Fig. 4B and D). The dimension of the residue particles in each treatment was quantitatively analyzed as shown in each panel of Fig. 4. The addition of 500 mg/kg C_{60} and MWCNTs significantly decreased the average diameter of digested particles to 3.64 and 2.84 μm, respectively, which represented 16.3 and 34.7% decreases from the control. The particle size was not significantly changed by the low dose CNMs treatments.

Size of the digested residues particles was an important index to determine the digesting level (Wang et al., 2007). The small and homogenous digested particles upon 500 mg/kg MWCNTs and C_{60} treatments may resulted from the activation of bacteria and

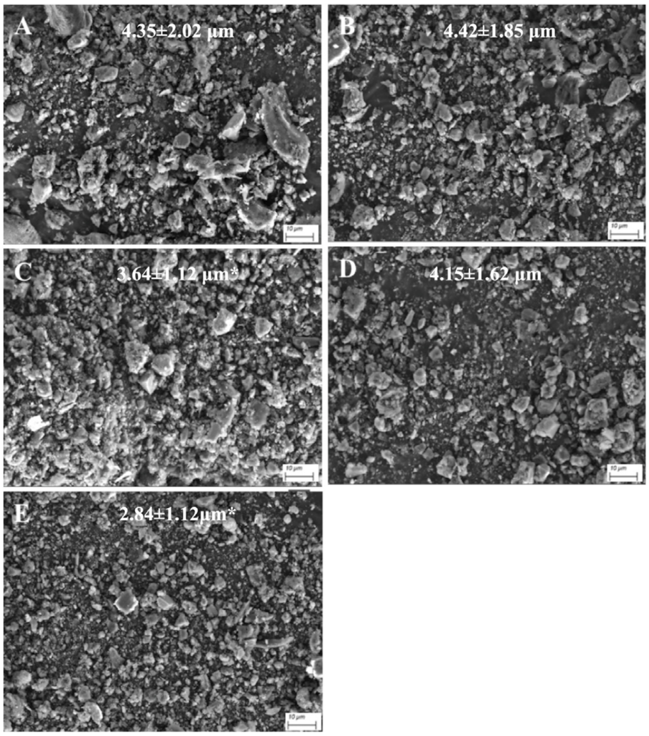

Fig. 4. SEM images of anaerobic digested residual treated with two CNMs at different concentrations: Control (A); C_{60} at 50 mg/kg (B); C_{60} at 500 mg/kg (C); MWCNTs at 50 mg/kg (D); MWCNTs at 500 mg/kg (E). The average diameter of residue particles in digested samples upon CNM exposures is shown in each panel. The asterisk indicates the significant difference between the control and each CNM treatment at $p < 0.05$ based on the measurement of 140 particles randomly selected from each SEM images using ImageJ software.

archaea associated with carbon sources and proteins ferment and the changes of corresponding microbe's proportion (Diaz et al., 2018). Coincided with the results of methane yields and TS contents, high-dose CNMs exhibited obvious promoting effects on anaerobic digestion processes.

3.5. *The composition and diversity of microbial community*

Microorganisms are critical to the process of anaerobic digestion. The composition of microbial community in the digested samples treated with different CNMs was analyzed to investigate the effects of amendment on the microbial community and subsequent methane production. The samples used for analysis of microbial community were collected at Day 30, where the accumulative methane production showed the notable differences as a function of treatment.

At the phylum level for the bacteria, *Proteobacteria* was dominant across all the treatments, although the relative abundance varied with CMN exposure and dose (Fig. 5A). A common finding is that the high dose of both CNMs decreased the relative abundance of *Proteobacteria* and that the low dose increased abundance as compared to the control. For example, the addition of 500 mg/kg C_{60} and MWCNTs decreased the relative abundance of *Proteobacteria* to 48.13 and 48.26%, respectively, as compared to 51.20% in the control. However, the low dose of both CNMs increased the relative abundance of *Proteobacteria* by 1.09−3.45%. The addition of 50 mg/kg C_{60} and MWCNTs shifted the relative abundance of *Bacteroidetes* from 6.28% in control to 6.47% and 5.23%, respectively. However, 500 mg/kg C_{60} and MWCNTs dramatically increased the relative abundance of *Bacteroidetes* by 104.9 and 19.9%, respectively. It has been reported that *Proteobacteria* is one of most commonly dominant groups in mesophilic anaerobic digestion systems (Raliya et al., 2017), and can utilize glucose and some volatile fatty acids through organic waste digestion (Liu et al., 2016). As a major phyla in the digested system, *Bacteroidetes* is essential heterotroph containing hydrolytic, acidogenic and fermentative bacteria, which play important roles in hydrolyzing and fermenting various carbon sources and proteins to acetic and propionic acid during the anaerobic digestion process (Diaz et al., 2018), high relative abundance of this group can potentially enhance biogas production (Westerholm et al., 2016). This finding is consistent with the observed increases in methane production and accumulative yield. As for other bacteria such as *Acidobacteria*, *Thaumarchaeota*, etc., CNMs amendment had no impact on their abundance at the phylum level. Similar to a previous study (Yang et al., 2018a), at the phylum level of archaea (Fig. 5B), *Euryarchaeota* was dominant in all the CNM treatments with the high proportions varied from 70.7% to 93.6%, except 500 mg/kg C_{60}, where *Woesearchaeota* was dominant and its relative abundance was almost 80%. Conversely, the relative abundance of *Euryarchaeota* in this treatment was only 19.5%, indicating that the addition of the high-dose of CNMs induced competition between *Euryarchaeota* and *Woesearchaeota*. *Euryarchaeota* is the largest archaeal group and is considered to be the major methane-producing microbial group during the process of anaerobic respiration. Notably, this biological process accounts for approximately 74% of methane released into the atmosphere (Liu and Whitman, 2010). *Woesearchaeota* can enable carbon and hydrogen metabolism within anoxic conditions, which results from symbiotic or fermentation-based lifestyles (Castelle et al., 2015). These findings align with considerable elevation of methane production that was observed with the addition of C_{60} and MWCNTs at the high doses, and might also explain the measurable decrease in the size of CNM-treated digested residues (Fig. 5). Clearly, at the phylum level, the addition of two typical CNMs increased the proportion of *Bacteroidetes* in the bacterial community while *Euryarchaeota* and *Woesearchaeota* were predominate groups in the archaea community.

At the class level, *Betaproteobacteria* within the phylum *Proteobacteria* was the most abundant group across all treatments (Fig. 6A). The relative abundance of *Betaproteobacteria* increased slightly with CNM treatment. The addition of 500 mg/kg CNMs decreased the relative abundance of *Deltaproteobacteria* from 7.55% in control to 5.10 and 6.43% in the C_{60} and MWCNT treatment, respectively. Conversely, the low dose of both CNMs had no impact on *Deltaproteobacteria*. The relative abundance of *Alphaproteobacteria* decreased to 11.25 and 9.86% in the C_{60} and MWCNT treatment, respectively, as compared to 12.75% in the control; however, no change was found in the 500 mg/kg CNM treatments. At the

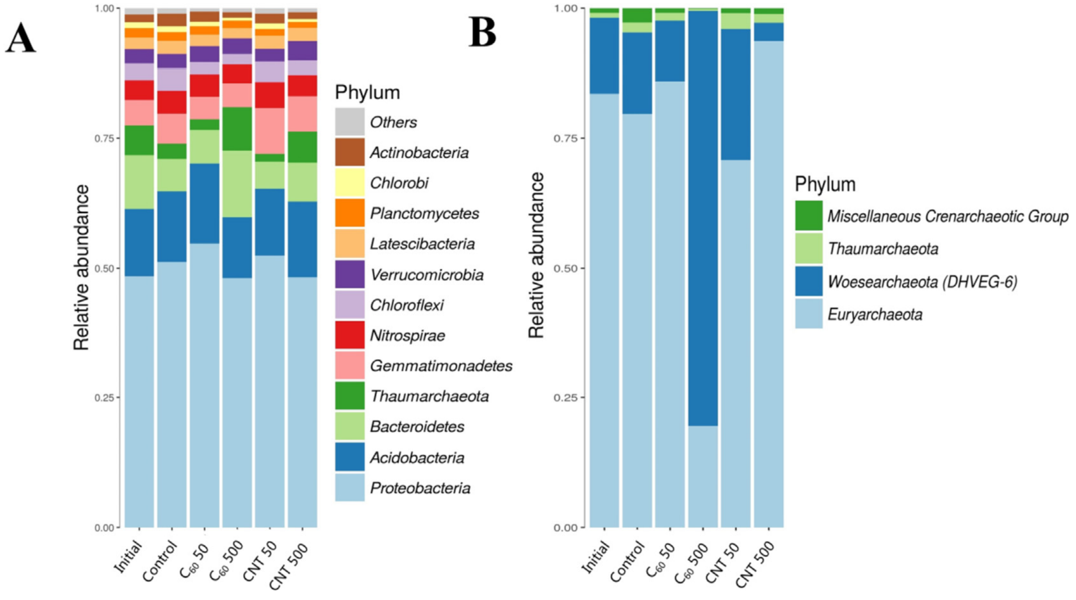

Fig. 5. The relative abundance of bacteria (A) and archaea (B) at the phylum level in the CNM amended feedstocks, including the control at Day 1 and Day 30, and the CNM treatments at 50 and 500 mg/kg at Day 30.

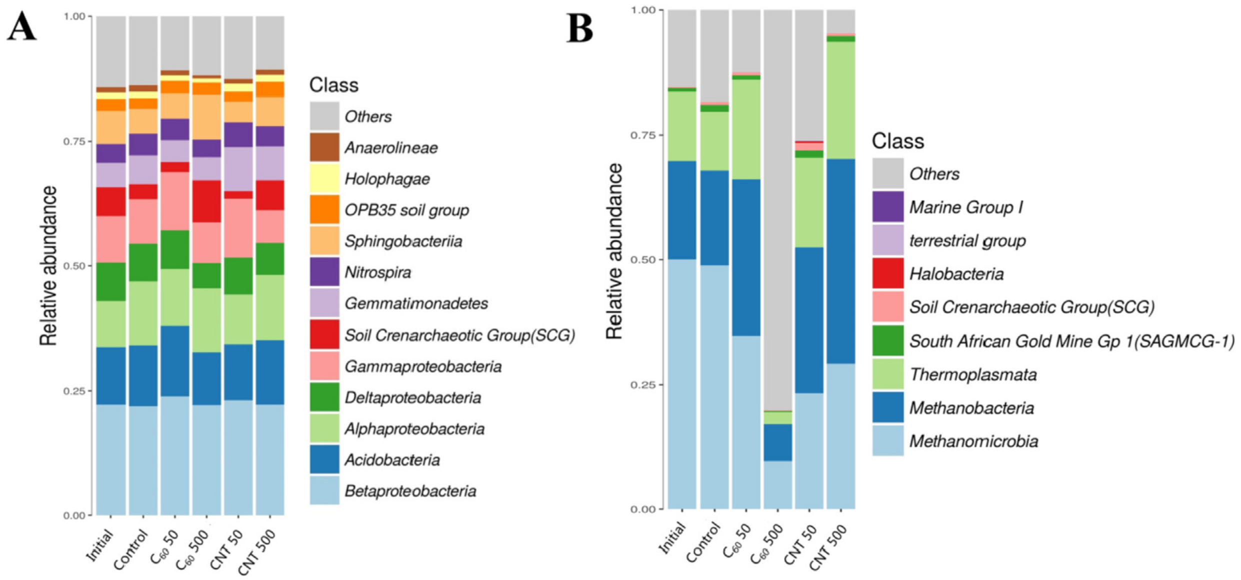

Fig. 6. The relative abundance of bacteria (A) and archaea (B) at the class level in the CNM amended feedstocks, including the control at Day 1 and Day 30, and the CNM treatments at 50 and 500 mg/kg at Day 30.

class level within the archaea, with the exception of the 500 mg/kg C_{60} treatment, the relative abundance of *Methanobacteria* was increased by 10.2−22.0% as compared to the control (Fig. 6B). Similarly, the relative abundance of *Thermoplasmata* was greatly increased across nearly treatments, the exception being 500 mg/kg C_{60}. The addition of 50 and 500 mg/kg C_{60} decreased the proportion of South African Gold Mine Gp1 (*SAGMCG-1*) from 1.35% in control to 0.85% and 0.18%, respectively; while its relative abundance was slightly elevated by 0.15 and 0.23% in the 50 and 500 mg/kg MWCNT treatment, respectively. *Methanobacteria* are a class of *Euryarchaeota* that can use hydrogen efficiently to reduce CO_2 into methane (Zhao et al., 2018). Notably, at the class level, CNMs increased the relative abundance of methane production relating archaea *Methanobacteria*, activated the carbonaceous oxidation-reduction process, which also explained the promotion of methane generation and accumulative production.

At the genus level, the presence of 50 and 500 mg/kg C_{60} increased the relative abundance of *Ramlibacter* by 1.26 and 0.77%, respectively, as compared to the control. Similarly, MWCNTs increased the proportion of *Ramlibacter* to 3.02% and 2.92% at the low and high doses, respectively (Fig. 7A). The relative abundance of *Massilia* declined across all CNM treatments, particularly with the 500 mg/kg C_{60} and MWCNT treatments, where the proportion of *Massilia* was decreased by 1.07 and 0.93%, respectively. The relative abundance of *Gemmatimonas* was altered upon exposure to C_{60} and MWCNTs, although in the opposite way. The presence of

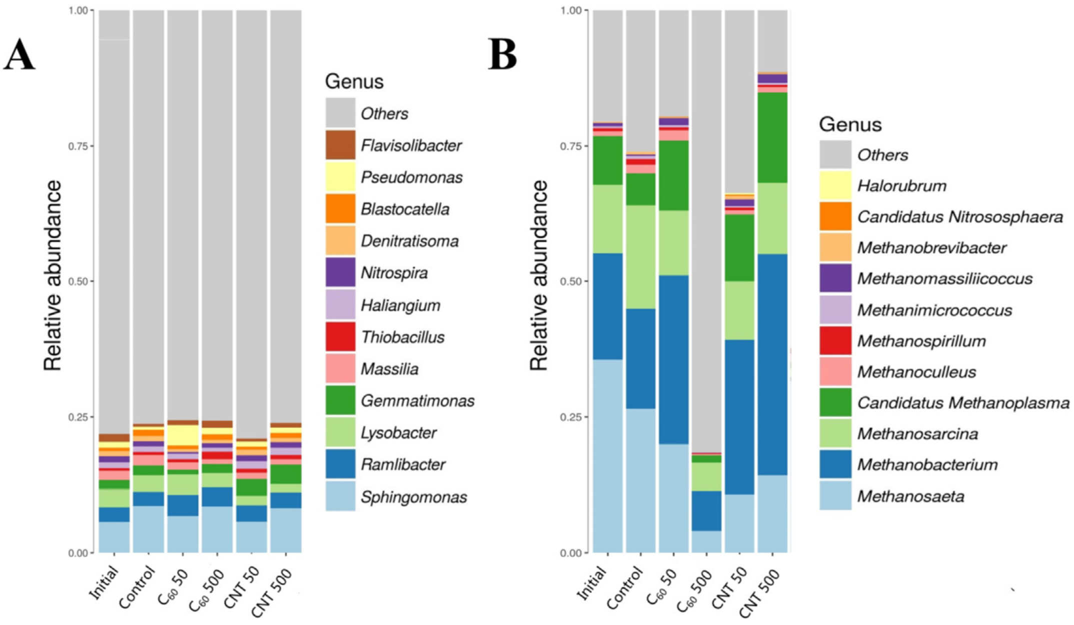

Fig. 7. The relative abundance of bacteria (A) and archaea (B) at the genus level in the CNM amended feedstocks, including the control at Day 1 and Day 30, and the CNM treatments at 50 and 500 mg/kg at Day 30.

MWCNTs increased the abundance of *Gemmatimonas* by 1.16–1.73%; however, C_{60} decreased its relative abundance by 0.27–1.03%. *Ramlibacter* is anaerobic bacteria growing by reducing oxyanions, including sulfate, perchlorate, nitrate and nitrite (Byrne-Bailey and Coates, 2012). While *Massilia* widely exists in rhizosphere soil, refuse landfill, lacustrine sediments, etc., and played essential roles in depredating and utilizing carbon sources (Bru-Adan et al., 2009). *Gemmatimonas* is a major genus in consuming and removing phosphorus sources (Takaichi et al., 2010). These findings are consistent with the positive effects on methane production and accumulative yield. As for the composition of archaea at the genus level, the proportion of *Methanosaeta* was 4.06–20.0% and 10.8–14.2% in the C_{60} and MWCNT treatments, respectively, as compared to 26.5% in the control (Fig. 7B). Similar results were also found with the relative abundance of *Methanosarcina* upon exposure to both CNMs. Notably, the relative abundance of *Methanobacterium* upon treatment with 50 mg/kg C_{60}, 50 and 500 mg/kg MWCNTs was 1.67-, 1.53- and 2.20-fold that of the control, respectively. *Methanosaeta* and *Methanosarcina* belong to acetoclastic methanogens, while *Methanobacterium* is an important hydrogenotrophic methanogen (Wang et al., 2018b). Evidently, the results suggest that the addition of C_{60} and MWCNTs enhanced the proportion of archaea *Methanobacterium* at the genus level, and subsequently increased interspecies hydrogen transfer to facilitate the generation of methane during the anaerobic digestion. (Yuan et al., 2016).

4. Conclusion

Taken together, CNMs accelerated the methane production and yield in an anaerobic system by positively altering the composition of the residential microbial community, in particular, the abundance of *Bacteroidetes*, *Methanobacteria*, and *Methanobacterium* at the level of phylum, class, and genus, respectively. So far, this is the first report on the role of CNMs in anaerobically digesting livestock manure with high solid content, which could provide important information for use of CNMs for enhancing anaerobic digestion and agricultural waste recycling.

Author contributions

Y.R and G. L proposed the present study. Y.H, Y.W, C.M, B.X and Y.R designed the experiments. Y.H, Y.W, C.D, Y.Z and Z.Z performed the experiments and analyzed the data. Y.H, C.M and Y.R wrote the manuscript. C.M and J.C.W commented on the statistical analysis, presentation of the results and revised the manuscript. All authors have read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

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