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Enhanced VFA degradation and methane production efficiency by biochar addition in food waste-sludge co-digestion: A step towards increased organic loading efficiency in co-digestion

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Abstract

This work investigated the effect of biochar addition to mitigate VFA accumulation and enhance methane production in mesophilic food waste/sludge co-digestion. Three different types of biochar derived from agricultural and forestry residues at two pyrolysis temperatures were tested. Results showed that wheat straw biochar 550°C supported the highest specific methane yield of 381.9 LCH₄/kg VS_{added} and VS removal efficiency of 41.62% among all treatments. Degradation of propionic acid and long-chain fatty acids such as valeric, caproic and isovaleric acids was observed. This also corresponded to an increase in methanogenic favorable substrates including acetic acid (>40%) and butyric acid (~20%) over the control. Consequently, a 24% increase in overall methane production was obtained as compared to control. This demonstrated that biochar addition had positive effects on VFA degradation and methane production which could be a useful strategy to increase the organic loading in co-digestions without the fear of process failure.

Keywords: VFA inhibition, mesophilic co-digestion, syntrophic oxidation, organic loading, food waste

1. Introduction

Food waste management is one of the most challenging environmental problems in the recent times. This is due to the huge quantities of food waste generated per year globally which amounts to 1.3 billion tonnes (Kaur et al., 2019). Landfilling remains the main disposal method, however it is not a sustainable option in view of greenhouse gas emission and breakdown of nutrient cycling. In order to provide a more efficient, attractive and sustainable solution, several governments including those in Hong Kong and United Kingdom are promoting the use of anaerobic digestion (AD) as the core food waste treatment method and develop a waste-to-energy technology (HK Policy address, 2016).

Food waste is a highly biodegradable organic material with a high energy potential of 0.25 KWh/kg. However, its mono-digestion suffers from the problems of quick acidification, long solid retention time and toxic inhibitors. Its co-digestion with sewage sludge which exhibits high alkalinity and improved buffering capacity has been considered to provide increased digestion efficiencies over respective mono-digestions of sludge and food waste (Wong et al., 2018). Furthermore, the low organic load of sewage sludge and the consequent unused capacity (30%) of sludge digesters at wastewater treatment plants are additional drivers for food waste/sludge co-digestions for reduced waste discharge and optimal methane production.

Although an attractive solution to divert food waste from landfills and make use of excess design capacity of sludge digesters, co-digestion is plagued with serious operational difficulties. Our recent studies on food waste/sludge co-digestion have shown that high concentrations of intermediate metabolites i.e. volatile fatty acids (VFA) including acetic acid, butyric acid and propionic acid existed in co-digestion system at the end of digestion period (Chakraborty et al., 2018; Kaur et al., 2019; Wong et al. 2018). Acetic acid followed by butyric acid is the most favourable substrate for methanogenesis while propionic acid inhibits methane production.

Additionally, higher concentration of long-chain VFAs e.g. valeric acid, caproic acid etc. which are difficult to metabolize to methane also existed in the co-digestion reactors. Consequently, low methane yields and volatile solids (VS) removal efficiency were obtained for food waste/sludge co-digestion. An additional impact of such reduced process performance was that only very diluted food waste systems i.e. containing low food waste loading could be operated in food waste/sludge co-digestion scheme (Wong et al., 2018).

Mechanistically, AD comprises a series of reactions which are performed by different groups of microbial populations. During AD, VFAs are decomposed to acetate, CO₂ and H₂ which are subsequently utilized as substrates for methanogenesis. However, due to the positive Gibbs energy value ($\Delta G_0 = 76.1$ kJ/mol), the conversion of C3-C6 VFAs to methane occurs by syntrophic methanogenesis i.e. it requires synergistic relation between fatty acid oxidizers and hydrogenotrophic methanogens (de Bok et al., 2004). This microbial interaction is associated with interspecies electron transfer (IET) via H₂/formate. It is the rate limiting step because of the low efficiency of electron transfer which allows both groups to cross their energy barriers and perform these reactions (Li et al., 2012). To solve this issue, direct interspecies electron transfer (DIET) has been recently identified as a faster alternative to H₂/formate-dependent IET. In DIET, high electrical conductivity and surface chemistry of DIET materials allows electrical connections on the cell surface of microbial species and mediate cell-to-cell electron transport between acetogens and methanogens (Barua and Dhar, 2017). To facilitate DIET during syntrophic VFA oxidation, carbon-based conductive materials such as granular activated carbon, cloth, magnetite, biochar have been introduced into AD.

Biochar, as a conductive material obtained from low temperature pyrolysis of plant and animal based bioresources is a low-cost alternative to other expensive materials. Until now, biochar-assisted AD studies have been focussed on defined co-cultures (e.g. *Geobacter* or *Methanosarcina sp.*) or simple substrates (e.g. VFA, ethanol, glucose) (Dang et al., 2016; Liu

et al., 2012; Zhang and Lu, 2016). Two recent studies have reported the effect of biochar addition on thermophilic food waste and sludge co-digestion (Li et al., 2018; Wang et al., 2019). Li et al. (2018) demonstrated a 50% shortening of lag phase and 1.8-fold higher methane production by biochar addition, however the observed enhancement was not directly related with syntrophic oxidation during the course of digestion. Thus, it remains unclear as to how biochar affects VFA metabolism and digestion kinetics. Further, as per our knowledge, there have been no study on biochar-assisted food waste/sludge co-digestion at mesophilic temperature which would be a more energy efficient process. Therefore, it is the objective of the present study to investigate the impact of addition of various types of biochar on mesophilic food waste/sludge co-digestion and elucidate the relation between VFA decomposition and methane enhancement. This study forms the first step towards the development of a high OLR food waste/sludge co-digestion system using biochar.

2. Materials and methods

2.1. Biochar sources and characterisation

Biochars derived from soft wood pellet (SWP), oil seed rape (OSR) and wheat straw pellet (WSP) were obtained from United Kingdom Biochar Research Centre (UKBRC), Edinburgh. Pyrolysis was performed in a pilot scale rotary kiln unit, at 550°C and 700°C for different biochars which are named SWP700, SWP550, OSR550, WSP550, according to the biochar raw material and pyrolysis temperature. Details about the system operations and some properties can be found in Mašek et al. (2018). Selected physiochemical properties are also provided in Table 1. Scanning electron microscopy was used for determination of surface properties.

2.2. Substrates and inoculum

Food waste was artificially prepared using 35% bread, 25% boiled rice, 25% cabbage and 15% boiled pork to simulate the food waste composition in Hong Kong, as in our previous studies (Chakraborty et al., 2018). Anaerobically digested (non-saline) sludge was used as inoculum while primary and secondary (activated) sludge was used as co-substrate along with food waste in co-digestion experiments. These were collected from wastewater treatment plant in Shek Wu Hui, Hong Kong. Both sludge and food waste substrates were individually characterized for various physiochemical parameters prior to use in the experiment, as per the protocols in our previous publications (Chakraborty et al., 2018). The sludges were preserved in a 10 L tank at a temperature 4°C before use. The total organic carbon content (TOC) and total Kjeldahl nitrogen (TKN) in food waste was $42.45 \pm 0.07\%$ and 2.97 ± 0.5 respectively. The characteristics of food waste, inoculum and sludge co-substrate are shown in Table 2.

2.3. Setup and design of biochar-amended food waste/sludge co-digestion experiments

Based on our previous studies, a sludge: food waste ratio of 1:7 (w/w) as identified to be the most suitable for co-digestion (Chakraborty et al., 2018; Kaur et al., 2019) and therefore, this ratio was used in the present study. To investigate the effects of biochar addition on co-digestion and screen the best biochar, a biochar dosage of 10 g/L which is a moderately high concentration for AD was used (Jang et al., 2018). Reactor bottles contained a TS ratio of substrate to inoculum (S/I) at 6.048. Control reactors with no biochar were used to investigate the effect of biochar addition on VFA degradation and methane production.

At every 12 h of co-digestion, the pH of the reactor content was measured and adjusted to near natural pH (~6.0-6.5) using lime (CaO), if required, to overcome the quick acidification of co-digestion mixture. In any case, not more than 2% of lime (on food waste wet weight basis) was added to avoid inhibition due to the introduced high alkalinity (Chakraborty et al., 2018). The reactors were incubated in an Automated Methane Potential Testing System (AMPTS-II)

(Bioprocess control, Sweden). Fifteen reactors in the AMPTS-II system for four biochar treatments and one control with no biochar and containing a total volume of 500 mL were used. According to the manufacture instructions, they were connected to CO₂ traps (3M NaOH) and to gas flow meters to determine continuously the methane flow rates. The reactors were purged with nitrogen gas for 10 min to remove the oxygen and then placed in a digital water bath and stirred at 80 rpm under mesophilic (35°C) conditions for a digestion period of 30 days. All the experiments were conducted in triplicate.

2.4. Analytical methods

For all co-digestion reactors, samples were withdrawn every second day and major parameters including pH, VFAs (acetate, propionate, butyrate, isobutyrate, isovalerate, valerate, caproate), chemical oxygen demand (COD) were monitored, using the standard protocols (APHA, 2005) and those described in Kaur et al. (2019). Total solids (TS) and volatile solids (VS) were measured on first and last day of digestion and VS removal % was calculated. Cumulative methane production data recorded by online data acquisition unit of AMPTS was analyzed and compared for different biochars and control.

2.5. Statistical analysis

All statistical analyses were performed using SPSS program by one-way analysis of variance (ANOVA). Changes were considered to be significant with p -value < 0.05.

3. Results and Discussion

3.1. Biochar characteristics and their implications in AD

Biochar with varying physical and chemical properties can be produced depending on the initial material and pyrolysis temperature used for their production (Tan et al., 2015). In the present study, two different feedstocks i.e. agricultural residue-based (WSP and OSR) and forestry residue-based (SWP) were used. Additionally, biochars at two different pyrolysis temperatures

of 550°C and 700°C were used. The physiochemical properties of biochars of different feedstocks and temperatures are shown in Table 1. pH, porous structure and morphology, ash content, presence of organic functional groups and nutrient content are some of the key properties of biochar that influence the AD reactions (Wang et al., 2018).

Among all biochars, agri-based biochars had a higher pH than soft wood biochar (Table 1). Additionally, significantly higher ash contents were present in WSP550 and OSR700 biochars than in SWP biochar. These are reported to the increased amounts of some inorganic salts and metallic oxides, thereby resulting in higher biochar pH and contribution to enhanced buffering capacity upon their addition to AD. Similarly, nutrient elements (N, P, K, S) are usually abundant in biochar derived from agri-based residues than forestry residues (Shen et al., 2017). This was also evident in the present study in which WSP550 and OSR700 contained a 2-3-fold higher total P than SWP550 biochar. Furthermore, a 6.2-fold and 11.9-fold higher total K was present in WSP550 and OSR700 biochar respectively as compared to SWP550 biochar. This also contributes to making the biochar surface more alkaline. Another useful property is biochar conductivity. As can be seen in Table 1, agri-based biochars had a much higher conductivity as compared to soft wood biochar which can be an important determining factor for facilitating VFA oxidation and assimilation into methane via DIET in AD (Wang et al., 2019) (see results on VFA metabolism in Section 3.2). Finally, the presence of AD-beneficial properties in agri-based biochars could contribute to improved VFA degradation and methane production efficiency in co-digestion systems containing these biochars.

3.2. Degradation of organics during biochar-assisted co-digestion

3.2.1. Influence of biochar addition on total soluble products formation

The presence of food waste with sludge in a co-digestion system leads to a better hydrolysis and acidogenesis in the system, thereby resulting in generation of soluble products in the form

of VFAs. As important precursors of acetogenesis and methanogenesis, their concentration and speciation play an important role in determining the overall digestion and methane production efficiency (Wong et al., 2018). Particularly, in a single-phase co-digestion system, the intrinsic balance between acidogenesis and methanogenesis indicating the mechanistic coupling of acid producers and consumers is crucial for a good digestion performance.

Considering the above, temporal changes in VFA profiles were monitored throughout the course of co-digestion in this study. The results of total VFA (tVFA) concentrations during a period of 30 days are shown in Fig. 1. As can be seen in the figure, the tVFA production during the first five days of digestion under all treatments was around 15 g/L. The VFA concentration remained at a high level until the lag time ended and this **increased as the pH became more stable (around pH 7.0) in the following days**. pH is an important process parameter which influences the microbial activity, and consequently the prevailing acidogenic metabolic pathways for solubilization of organic matter (Kaur et al., 2019). The tVFA concentrations peaked around day 9 after which a gradual decrease in levels was observed due to their concomitant assimilation into methane from this point onwards (see methane production results). The tVFA concentrations were 10% higher ($p\text{-value}<0.05$) in OSR700 and WSP550 biochar treated co-digestions as compared to the control with no biochar addition. As can be seen in Fig. 1, following day 16, the VFA concentration in the control reactor remained at a higher level until the end of co-digestion period as compared to biochar amended reactors. The best performance for enabling VFA consumption was exhibited by WSP550 biochar and VFA concentrations in this reactor remained lower by 35.5-67.5% ($p\text{-value}<0.05$) as compared to the control.

At the end of co-digestion, a high tVFA concentration of 8.8 g/L was observed in the control reactor which implied that such a high VFA concentration remained unconsumed in control while it declined rapidly to a meagre concentration of ~2 g/L in the WSP550 biochar treated

reactor. The next best performing biochar was OSR700. Such high unconsumed VFA concentrations remaining at the end of digestion period in co-digestion systems is usually seen, as also reported in our previous studies (Chakraborty et al., 2016; Wong et al., 2018) and other similar studies (Wang et al., 2017).

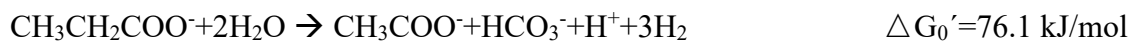
Although co-digestion is considered as a better strategy than mono-digestion, this partial consumption of intermediate substrates is a major drawback which decreases its treatment efficiency and methane production potential. Additionally, the decreased VFA degradation implies greater inhibition on methanogenesis by accumulated VFA. Consequently, only diluted food waste-based co-digestions employing only a small food waste ratio can be operated. The alleviation of VFA-induced inhibition and their degradation is thus facilitated by biochar addition, as seen in Fig. 1.

3.2.2. Influence of biochar addition on VFA degradation

Further demonstration of the effect of biochar-assisted VFA degradation can be seen from analyses of individual VFA profiles under different treatments. The individual distributions of VFA in the total product stream majorly dictate the subsequent methane production. Thus, the variations in VFA distribution were measured throughout the co-digestion to understand the effect of addition of different biochar types on VFA degradation and consequent methane production efficiency. Fig. 2 shows the VFA kinetics under different biochar treatments and control co-digestion conditions. VFAs such as acetic acid, butyric acid, propionic acid, valeric acid, caproic acid, isovaleric acid and iso-butyric acid were detected under all conditions. Acetic acid and butyric acid are the most important methanogenic substrates (Capson-Tojo et al., 2018). The most important effect of biochar addition was seen in terms of VFA degradation and their assimilation into methane.

As shown in Fig. 2A and 2B, the WSP550 biochar amended reactor contained the highest concentrations of these two VFA during acidogenesis period of day 7-15. Particularly on day 11-13, their highest concentrations were observed which were 40% and 20% higher than the control reactor (p -value<0.05). Following the acid production period, the following days manifested a faster degradation of both acetic and butyric acids which was facilitated by biochar addition. The synergistic effects of biochar on stimulation of degradation of these important methanogenic VFA promoted a higher methane production along with lower accumulation of these VFA (see methane production results).

Another key short-chain VFA in digestion is propionic acid which appeared in higher concentrations towards the later period in a food waste/sludge co-digestion, as also reported in our previous studies (Kaur et al., 2019; Wong et al., 2018). In the present study, increasing propionic acid concentrations were observed from day 18 onwards in all reactors (Fig. 2C). Mechanistically, syntrophic oxidation of propionic acid during digestion is relatively slow and is a limiting step for methanogenesis. The conversion of propionic acid is thermodynamically unfavourable as given by the following reaction (de Bok et al., 2004):



However, in the presence of biochar, propionic acid oxidation was greatly facilitated resulting in a rapid decline in its concentrations following day 21. The concentration decreased rapidly from 9.3 g/L to a final concentration of 1.46 g/L at the end of co-digestion in WSP550 biochar amended reactor. Similar degradation of propionic acid was also observed in OSR700 biochar treated co-digestion in which the acid concentration decreased to 3.7 g/L. On the other hand, the concentration in the control reactor continued to remain very high from day 21 until day 28 (9.3-7.8 g/L). As compared to the biochar amended reactors, the control reactor has ~4-fold higher propionic acid concentration during this period (p -value<0.005). This conversion of

propionic acid to acetate by syntrophic oxidation at this time would mean utilization of available acetate for methane production. This is similar to the results for VFA syntrophic oxidation reported by Wang et al. (2019) by the addition of biochar in food waste/sludge thermophilic co-digestion. Giwa et al. (2019) also reported that propionate fermentation was stimulated by biochar addition which resulted in accumulation in the initial start-up phase. Later, the oxidation of the propionate and iso-butyrate to acetate was induced which is a favourable substrate for methanogenesis. Therefore, biochar-assisted acid oxidation is expected to enhance methane production under these treatments as compared to the control (see methane production results).

The biochar effect on degradation of long-chain VFAs particularly valeric acid, caproic acid and iso-valeric acid was also observed (Fig. 2D-2G). For example, a clear low availability of these long-chain VFAs was observed following day 12 in WSP550 biochar treated reactor as compared to control which showed a high accumulation of these acids almost throughout the digestion period. Valeric acid concentration in WSP550 reactor was ~2.5-fold lower (p -value<0.05) than control on day 15 onwards. Similarly, caproic acid concentration was 3-fold lower in WSP550 reactor as compared to control during this time. The degradation of iso-valeric acid was also significant and steeper decline in its concentration was observed in WSP550 amended reactor (Fig. 2), indicating a higher stability of this system. The second best performing biochar was OSR700 which led to an increased VFA degradation as compared to control. Furthermore, as can be seen for caproic acid and iso-valeric acid profiles, the almost complete consumption of these acids occurred at least 2-4 days before as compared to the situation in control reactor.

The percentage distribution profile of all soluble products is shown in Fig. 3 which compares the WSP550 and control reactors. As can be seen in the figure, in WSP550 reactor, ~80% total VFA was dominated by acetate and butyrate up to day 16, both of which are favourable

substrates for methanogenesis. Furthermore, in WSP550 reactor, the long chain VFA seen in the initial period started to decrease from day 13. On the other hand, the control reactor contained only 50% acetate and remaining 50% was composed of long-chain VFA. In fact, long-chain VFA such as caproic acid was seen from day 9 which continued to increase until day 16. Increasing predominance of long-chain and inhibitory VFA was seen from day 19. Propionate continued to increase from day 22 until the end of co-digestion accounting for 60-90% of total VFA in the control reactor.

3.3. Effect of biochar addition on methane production during co-digestion

Most extensive use of biochar in literature until now has been reported in defined co-cultures (e.g. *Geobacter sp.* and *Methanosarcina sp.*) or simple substrate cultures (Liu et al., 2012; Zhang and Lu, 2016), while the detailed studies on the effect of biochar addition on mesophilic food waste/sludge co-digestion did not exist in literature. In our study, we have studied syntrophic oxidation during co-digestion, and correlated the effect of biochar addition on VFA metabolism and digestion kinetics with methane production.

The profile of daily methane production is illustrated in Fig. 4A. The daily methane production of the all treatments exhibited three peaks, whereas the time of occurrence and duration of these peaks differed. The first production peaks of all groups appeared during the first two days of AD, which might result from the use of dissolved and easily degradable substances in methanogenesis. During this period, hydrolytic acidification continued and produced more micromolecular organic acids by decomposition of macromolecules. These organic acids accumulated and further assimilated into methane, resulting in a gradual increase in the daily methane production after the first peak period. It is important to note that the emergence of a second peak in both WSP550 and OSR700 biochar amended co-digestion systems was sooner than in the control and higher methane production values were obtained. For example, the daily methane production was 20-24% higher (p -value<0.05) during day 15-day 19 in these biochar

treated systems relative to control. This increase can be directly related to the higher availability of methanogenic substrates acetic and butyric acids during this period in these reactors as compared to the control reactor. Higher degree of oxidation of long-chain VFA (Fig. 2D-2G) is another contributing factor for increased methanogenesis in biochar containing reactors particularly WSP550 and OSR700. Maximum difference between control and WSP550 biochar reactor was observed during day 16-day 20 which amounted to 1.3-fold.

The last peak in daily methane production could be attributed to the oxidation of propionic acid to acetate and its further utilization for methane production. Correlating this finding to Fig. 2C, the highest level of propionic acid oxidation in WSP550 resulted in the highest peak of daily methane production value of 290 mL/d for WSP550 reactor during this time. On the other hand, a 1.9-fold lower daily methane production was observed for control reactor at this time (from day 23 onwards). During a 30-day co-digestion, the highest cumulative biomethane production of 381.92 ± 5.3 L/kg VS_{added} was obtained with the addition of WSP550 at a concentration of 10 g/L (Fig. 4B), which was $24.0 \pm 0.84\%$ higher than that in control group without biochar addition (p -value <0.05). Our results exhibited that the supplement of biochar significantly reduced the lag phase of co-digestion process, which could be attributed to the accelerated degradation of long chain fatty acids and propionic acid to methanogenic favourable acetic and butyric acids (Ko et al., 2018). Lü et al. (2019) reported that powdered biochar doubled the microbial growth in anaerobic digestion of oil and increased the capability of microorganisms to utilize long chain fatty acids in anaerobic digestion. After 23 days, the control turned into the inhibition stage, while the test group did not exhibit crisis until the end of the experiment. The control was negatively affected by the high propionic acid accumulation immediately, showing unstable daily methane production. Our results indicated that biochar supplement had positive effects on VFA degradation and enhanced methane production. Table 3 summarizes the overall performance of co-digestion with biochar addition and its comparison with control.

Finally, conditions such as acetic acid and propionic acid concentrations, carbon conversion or VS removal efficiency corresponded well to methane production. As shown in Table 3, the VS removal efficiency for WSP550 biochar co-digestion system was the highest and equalled to 41.6%. This was 1.2-fold higher as compared to control reactor (p -value <0.05).

Food waste/sludge co-digestion has been greatly emphasized in the recent years to address the problems of food waste and generate renewable energy. Our previous co-digestion studies have indicated that co-digestion indeed resulted in greater production over sludge mono-digestion (Kaur et al., 2019). However, the presence of considerable amounts of total soluble products (VFA) at the end of co-digestion implied that assimilation of these available substrates to methane was not complete. In the present study, a similar situation was observed for the control reactor with no biochar addition which showed a very slow degradation of all VFA i.e. an increased accumulation of VFA at higher levels than in biochar treatments during most of the co-digestion period and higher tVFA levels (Fig. 1) at the end of co-digestion.

The use of biochar in co-digestion in this study resulted in improved syntrophic VFA degradation and their subsequent methanation. WSP550 was the best biochar resulting in the highest methane production. The results of this study have significant implications in terms of increasing the food waste organic loading rate (OLR) in co-digestion which remains a challenge. A high OLR is desirable since it would allow higher quantities of organic matter to be treated in a given time and volume of a digester. However, the fear of overloading, accumulation of VFA and process failure always exists. With the increased VFA degradation and higher carbon conversion efficiency to methane by biochar addition established in this study, it should allow such biochar containing co-digestion systems to efficiently mitigate VFA accumulation and facilitate process stability under high food waste loading conditions. The operation of such high OLR biochar-assisted co-digestions is the topic of our future studies.

4. Conclusions

Implications of addition of different biochar types for enhanced VFA degradation and methane production efficiency in mesophilic food waste/sludge co-digestion was investigated. Agricultural residue-derived biochars promoted a higher VS removal efficiency and methane recovery as compared to both forestry residue-derived biochar and control with no biochar addition, by virtue of their beneficial physiochemical properties. Comparing with control, up to 24% increase in specific methane yield was obtained with biochar amendment. Therefore, biochar addition provides a useful means of VFA mitigation and an opportunity to increase the organic loading in co-digestion without any fear of VFA accumulation and process failure.

Supplementary data

E-supplementary data of this work can be found in online version of the paper.

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Competing interests

Authors declare no competing interests.

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Fig. 1. Influence of addition of different biochar types on total VFA kinetics during food waste/sludge co-digestion and its comparison with control.

Fig. 2. Individual VFA profiles and their degradation upon various biochar amendment conditions versus control co-digestion. (A) acetic acid, (B) butyric acid, (C) propionic acid, (D) valeric acid, (E) caproic acid, (F) iso-valeric acid, (G) iso-butyric acid.

Fig. 3. VFA percentage distribution profile for (A) WSP550 biochar co-digestion treatment and its comparison with (B) control.

Fig. 4. Influence of biochar addition on methane production in co-digestion system. (A) Daily and (B) Cumulative methane production profile during co-digestion, in biochar amended reactors and control reactor with no biochar addition.