Comparative Effects of GAC Addition on Methane Productivity and Microbial Community in Mesophilic and Thermophilic Anaerobic Digestion of Food Waste

by

John Ryue

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science

In

Environmental Engineering
Department of Civil and Environmental Engineering
University of Alberta

Abstract

The abundance and potential of food wastes for high bio-methane potential has generated considerable interest in its application for anaerobic digestion (AD). The mechanism of direct interspecies electron transfer (DIET) was recently discovered which provided an alternative method of electron transfer and has been shown to greatly enhance methane production and improve kinetics. However, DIET-AD studies involving food waste are sorely lacking. Furthermore, DIET studies have typically operated in mesophilic conditions due to the intensive energy requirements at thermophilic conditions.

In this study, the relative effects of granular activated carbon (GAC) addition (25 g/L) in anaerobic digestion of food waste at mesophilic (37°C) and thermophilic (55°C) temperatures were investigated using biochemical methane potential (BMP) test. The addition of GAC significantly reduced lag phases for methane production in comparison with the unamended control at both temperatures. Microbial community analyses revealed that GAC addition increased the diversity and richness of both bacterial and archaeal communities. Besides, several known or potential electroactive fermentative bacteria (e.g., *Calorameter*, *Sporanaerobacter*, *Coprothermobacter*, etc.) were found in GAC-amended bioreactors at both temperatures, suggesting the possibility of DIET-based syntrophy in these reactors. At mesophilic temperature, GAC amendment increased methane productivity (L CH4/kg-VS) by almost two-fold in comparison with the control; however, methane production at the thermophilic temperature was unaffected by GAC addition. These results indicate that enhanced process kinetics at thermophilic temperature might diminish the visible impact on methane productivity due to the addition of GAC.

Preface

The findings presented in this thesis (Chapter 1, 3, 4, and 5) has been published as John, Ryue; Long, Lin; Yang, Liu; Wenjing, Lu; Daryl, McCartney; Bipro R., Dhar (2019) "Comparative effects of GAC addition on methane productivity and microbial community in mesophilic and thermophilic anaerobic digestion of food waste" in Biochemical Engineering Journal, vol. 146, pp. 79-87. J. Ryue was responsible for experimental design, laboratory experiments, data interpretation and analyses. L. Lin assisted in processing of microbial community analysis data. B.R. Dhar planned and directed the study. All authors contributed to the manuscript preparation.

Furthermore, a version of chapter 2 will be submitted as John Ryue and Bipro R. Dhar (2019) "A critical review of strategies for improving performance and stability of thermophilic anaerobic digestion: focusing on acidification and ammonia inhibition" for publication in a peer-review journal. For this manuscript, John Ryue was responsible for the data collection from the literature and manuscript preparation. B.R. Dhar was the supervisory author and directed the concept formation and manuscript preparation.

Acknowledgements

I would like to express my deep gratitude to my direct supervisor, Dr. Bipro Dhar, for the supervision and guidance he provided to me during this research work and to Dr. Daryl McCartney and Dr. Yang Liu for their active collaboration and for providing suggestions along the way. I would also like to thank Dr. Long Lin for her immediate supervision and in teaching me all the lab techniques and analyses that I have used throughout the entire duration of my program. In addition, thank you Dr. Long Lin for your assistance in processing the microbial ecology data from my first experiment.

Finally, I would also like to thank my colleagues, Basem Reda and Sajib Barua, who have mentored me and taught me about lab safety, lab techniques, report writing, etc. Thank you, I could not have done it without you guys.

This research project was financially supported by the Government of Alberta, Natural Sciences and Engineering Research Council of Canada (NSERC), and Future Energy Systems (FES). I would also like to thank Queen Elizabeth II Graduate Scholarship for partially supporting my master's program at the University of Alberta.

Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction

1.1 *Background*

Anaerobic digestion (AD) is a widely practiced waste-to-biomethane process that has been used for different organic waste streams, such as sewage sludge, agricultural/livestock residues, food wastes, etc. (Jiang et al, 2018; Li et al, 2018) (Junfeng Jiang et al., 2018; X. Li et al., 2018). Notably, methane production from food waste has attracted a lot of interest due to the sheer amounts that are generated each year globally. For instance, China produces nearly 195 million tons of food waste, followed by 61 million tons in the United States, 47 million tons in Europe (Braguglia et al, 2018) (Braguglia, Gallipoli, Gianico, & Pagliaccia, 2018). Based on the current trajectory of the global population, it is expected that the amount of food waste will grow over time. Wide availability and a high fraction of biodegradable organic matter in food waste makes it an attractive feedstock for bio-methane recovery through anaerobic digestion (Braguglia et al, 2018; Wang et al, 2018; Facchin et al, 2013; Stabnikova et al, 2008) (Braguglia et al., 2018; Facchin et al., 2013; Stabnikova, Liu, & Wang, 2008; G. Wang, Li, Gao, & Wang, 2018b).

Anaerobic digestion is a multi-step biological process that depends on the syntrophic partnership between fermentative bacteria and methane-producing archaea for methane production from complex organic substrates (see Figure 1.1 below). Over the course of this process, organic waste is broken down into simpler compounds like short-chain volatile fatty acids (SCVFAs) and residuals like H_2 and CO_2 before finally being utilized by methanogens (Barua & Dhar, 2017; Cheng & Call, 2016). Methanogenesis consists of a syntrophic partnership between fermentative bacteria and methanogens, with the latter acting as an electron sink for electrons generated by the bacteria. This process is known as mediated

interspecies electron transfer (MIET), which transfers electrons in the form of metabolites like H2 and formate. Until recently, it was believed that methanogenic communities utilized MIET as the dominant electron transfer mechanism. However, MIET is limited by the critical requirement that metabolites (i.e. H_2 or formate) must remain at a low concentration to ensure favorable thermodynamic conditions for additional metabolite generation, which can also affect the kinetics of methanogenesis (Cheng and Call, 2016) (Cheng & Call, 2016). In contrast, direct interspecies electron transfer (DIET) is a recently revealed form of interspecies electron transfer that was first discovered by Morita et al (2011) (Morita et al., 2011), providing evidence that bacteria could transfer electrons directly to methanogens without reliance on metabolites (e.g., H_2/formate). This was an exciting development for many researchers in the field of anaerobic digestion, as DIET opened the potential for the optimization of the methanogenesis step in the digester. The DIET allows for a more efficient transfer of electrons compared to MIET, which in turn dramatically improves the kinetics of methane production. Since the discovery of DIET, studies have reported significant enhancements to methanogenic kinetics by the supplementation of conductive materials (Zhao et al, 2016; Lin et al, 2017; Xu et al, 2018) (R. Lin et al., 2017; Xu, Han, Zhang, He, & Liu, 2018; Zhao et al., 2016). Most of these studies have utilized simple substrates like ethanol or acetate, mainly because SCVFAs and alcohols are known to be the preferred substrates for DIET-active electroactive bacteria, such as *Geobacter* sp. (Park et al, 2018). In contrast, DIET studies involving complex substrates like food waste are still scarce. Few recent studies suggested that mesophilic food waste digestion can be enhanced with the addition of various conductive additives, including GAC, biochar, and magnetite (Capson-Tojo et al, 2018; Dang et al, 2017; Peng et al, 2018).

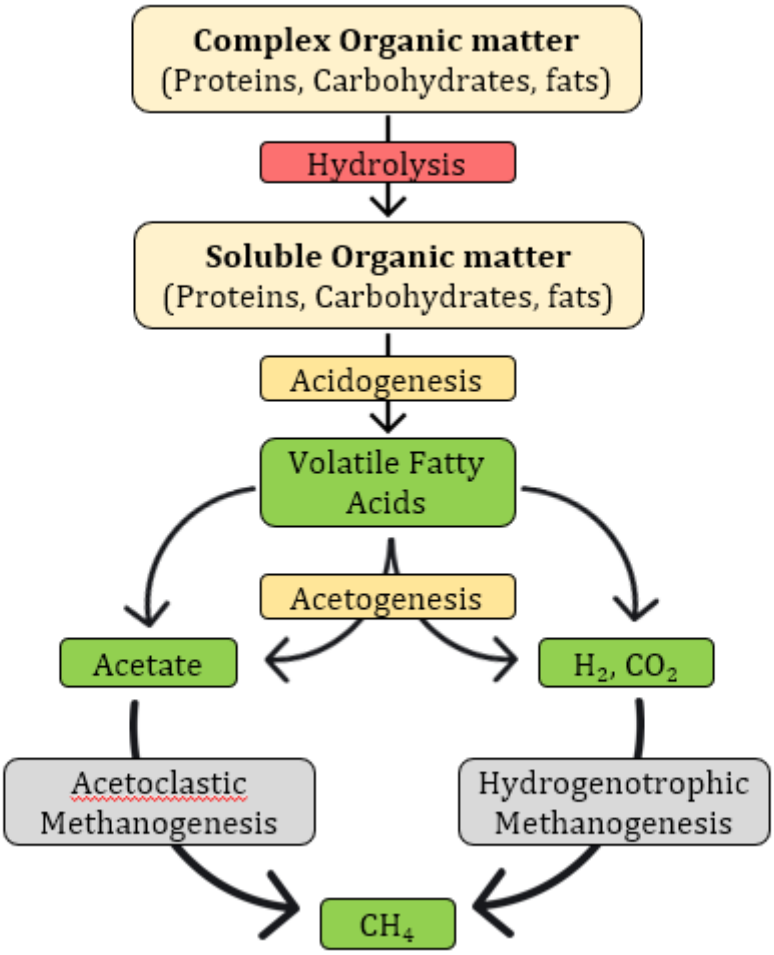

Fig. 1.1. Diagram illustrating the biological steps involved in the anaerobic digestion process.

To date, most of the studies involving DIET have been carried out at mesophilic temperature, which has left open a wide research gap in understanding the effects of conductive additives on thermophilic digestion (Barua and Dhar, 2017; Lin et al, 2018) (Barua & Dhar, 2017; R. Lin, Cheng, Ding, & Murphy, 2018a). Most importantly, limited information is available on the influence of conductive material addition at mesophilic and thermophilic anaerobic digestion of complex organic wastes. Lin et al (2018) (R. Lin et al., 2018a) recently carried out a study using graphene to compare the impact of DIET on anaerobic digestion at mesophilic and thermophilic conditions; graphene amendment improved methanogenesis from ethanol at both mesophilic and thermophilic temperatures. Their results showed similar

levels of improvement from methanogenesis of ethanol (mesophilic: 25%; thermophilic: 26.4%), indicating the trivial effect of temperature on DIET flux. Similarly, Jang et al. (2018) (Jang, Choi, & Kan, 2018) conducted anaerobic digestion of dairy manure under psychrophilic, mesophilic and thermophilic conditions with biochar supplementation; all these conditions led to comparable improvement in methane yield (24.9-26.47%). However, the potential role of DIET was not evident due to the lack of microbial community analyses in their studies. Based on extensive literature search, no studies could be found on the comparative effects of conductive materials addition on anaerobic digestion of food waste.

1.2 *Specific objectives*

The application of DIET in AD studies is a relatively novel concept. Most DIET studies have been conducted in mesophilic temperatures and a wide gap in knowledge remains regarding its application in thermophilic conditions. Based on the research gaps identified, the objective of this thesis was to assess the comparative effects of GAC addition on anaerobic digestion of food waste at mesophilic and thermophilic temperatures. First, the impact of GAC addition was assessed on methane productivity, and accumulation of various SCVFAs and ammonium were examined using biochemical methane potential (BMP) test. Following that, the results from the mesophilic AD (M-AD) and thermophilic AD (T-AD) were compared. Lastly, microbial community analyses were also performed to understand the impact of GAC on microbial diversity, richness, and enrichment of potential DIET-active methanogenic biomass.

1.3 *Thesis organization*

This dissertation shows how temperature effects can influence the performances of anaerobic digestion of food wastes coupled with DIET syntrophy. The organization of this thesis is as follows. Chapter 2 provides a literature on the current status of anaerobic digestion studies using DIET mechanism. The review discusses common inhibition scenarios (SCVFAs and

TAN accumulation) that is encountered during operation of anaerobic digesters and looks at the role that temperature plays. DIET is also discussed as a practical strategy to promote stability in digesters and enhance methane production. Chapter 3 details the materials and methods that were used to conduct the BMP tests for the anaerobic digestion of food wastes at mesophilic (37°C) and thermophilic (55°C) conditions. Furthermore, this section documents the analytical methods used in this experiment, including the microbial community analysis and statistical methods used. Following this, Chapter 4 presents the results and discussion on the experimental work conducted within the scope of this thesis. The performances of both M-AD and T-AD are discussed and compared based on methane productivity and the impacts of temperature on stability indicators (VFAs and TAN). Evidence of DIET-based syntropy were detected in both temperature conditions; however, the methane production in T-AD was unaffected by GAC addition which suggested that enhanced process kinetics at thermophilic temperature might have diminished the visible impact on methane productivity from GAC addition. Microbial communities were analyzed to understand the impacts on diversity and richness from GAC addition. Finally, Chapter 5 presents the conclusions from the BMP experiment and the learning experiences from this study are used to discuss the implications of the DIET-AD process for food wastes (and other complex organic wastes). A summary of the take-home messages and directions for future work are provided.

Chapter 2

Literature Review

2.1 *Challenges in conventional anaerobic digestion*

As a biowaste-to-energy process, anaerobic digestion has been given increasing attention over the years. However, anaerobic digestion still suffers from problems such as low methane yield and process instabilities that prevent this process from being more widely used for waste management. In particular, digesters often encounter operational issues such as the accumulation of short-chain volatile fatty acids (SCVFAs) or ammonia. The accumulation of SCVFAs and ammonia leads to an uncoupling of the acidogenic and methanogenic phases inside the digester and inhibits methanogenic activity, which results in unstable AD operation and reduced methane yields (Zhang, Hu, & Lee, 2016).

Total ammonia nitrogen (TAN) is a by-product that is produced during the anaerobic digestion of proteinaceous compounds and is required by microorganisms for growth up to a certain concentration (Sung & Liu, 2003). Besides its role as a macro-nutrient for microbial growth, ammonia is integral to anaerobic digestion due to its effect on the performance and stability of the process. Excess ammonia is a primary cause of digester failure due to its inhibitory effect on microbial activity (Rajagopal, Massé, & Singh, 2013a). Elevated levels of ammonia increases the digester's pH, which proportionately increases the amount of free ammonia nitrogen (FAN) present in the digester. FAN is widely regarded as the more inhibitory compound due to its toxic affect on methanogens, which has been attributed to changing the intracellular pH and causing methanogens to expend more energy for proton balancing (J. L. Chen, Ortiz, Steele, & Stuckey, 2014). The inhibition of methanogenic activity is of crucial concern due to the sensitivity of the methanogenesis phase, as compared to the previous stages (i.e. Fermentation). Not only does this lead to reduced methane yields, but the diminished

activity also results in a steady accumulation of SCVFAs, which are the by-products from the preceding fermentation step.

Another challenge commonly faced in anaerobic digestion is the accumulation of SCVFAs. As evident by its nomenclature, SCVFAs are acidic in nature and will decrease the digester's pH when accumulated in great amounts. Process pH is a great indicator of the digester's stability due to the sensitivity of methanogens to changes in pH. Methanogens operate at an optimal pH range of 6.8 to 7.2 (Yuan & Zhu, 2016a), and even slight fluctuations of the pH can lead to destabilization of methanogenesis. The decreased pH creates a feedback loop where methanogens are unable to degrade SCVFAs, causing greater accumulation of SCVFAs to further decrease the pH. Eventually, the system will deteriorate to the point where complete failure of the digester occurs. Inhibition by SCVFAs accumulation is a huge concern when dealing with substrates that are highly biodegradable and easily hydrolyzed, such as food wastes. From an economic standpoint, this is undesirable due to the limitations on the organic loading rates that can be achieved in a digester. Low loading rates in digesters decreases the overall productivity of the anaerobic digestion process and makes it less economically feasible due to lower methane yields.

While the AD process has been practiced for years, it still remains a research focus in literature due to the stability issues identified above. Successful operation of digesters requires developing strategies that can mitigate inhibitors (SCVFAs, ammonia).

2.2 *Short-chain volatile fatty acids*

SCVFAs are produced during the acidogenesis stage of the AD process by fermentative bacteria and is mainly represented by acetic acid, propionic acid, butyric acid and valeric acid. These intermediary products are then broken down into acetate before being used by methanogens to produce methane (Wang et al., 2009). Yuan & Zhu (2016) estimates that 72% of methane

production is from the degradation of acetate, where the acetate has been converted from other SCVFAs like ethanol, propionate and butyrate. SCVFAs serve multiple roles in the AD process. First, SCVFAs are used as nutrients in the digester and are the building blocks for the end product, methane. Additionally, SCVFAs are a useful tool that can be used to indicate system instability. Due to its transition from the acidogenesis and methanogenesis steps, an accumulation of SCVFAs can point to an imbalance between the fermentation and methanogenesis steps (X. Shi et al., 2017). SCVFA accumulation is a concern as it is associated with severe drops in pH that put it well below the optimal range required for methanogens to operate in. Methanogens are notably sensitive to even the slightest disturbances in pH, and decreasing the pH below the optimal pH of 6.8 – 7.2 can result in severe inhibition to the AD process (Vavilin et al., 2008). Low pH inhibits the growth of methanogens and leads to a decline in methanogenic activity, causing further accumulation of SCVFAs due to the imbalance between the fermentation and methanogenesis steps. Ultimately, this creates a feedback loop where an accumulation of SCVFAs continues to decrease the pH, inhibiting the activity of methanogens and so on, eventually deteriorating the system and creating process conditions that make it unsuitable for AD to take place (X. Shi et al., 2017; Yuan & Zhu, 2016b).

Table 2.1. Impact of SCVFA levels on instability of anaerobic digestion

Substrate	Operation Mode	Temperature (°C)	pH	SCVFA specific acid	Threshold Conc.	Impacts	Reference
OFMSW	Batch	50	5.5 – 7.6	n/a	25.1 meq/L	Decreased pH and high SCVFA generation (no inhibition observed)	(Sajeena Beevi, Madhu, & Sahoo, 2015)
Corn stover	Batch	55	Lowest pH was 7.3	Acetic acid (propionate and butyrate also observed)	Peaked at 16 g/kg digestate	Decreased methane content (high levels of propionate could be concern due to inhibition of process)	(J. Shi, Wang, Stiverson, Yu, & Li, 2013)
SS + Rice Straw	Batch	55	As low as 6.1	Propionate, Acetate	Total SCVFA = 803 mg/L	Slight inhibition of methanogens causing lower methane yield	(Kim et al., 2013)
Rice straw	Batch	55	As low as 4.9	Mostly acetate, some propionate/butyrate	Total SCVFA = 6511 mg/L	Drop in pH, and digester failure	(Kim et al., 2013)
Ensiled grass	Batch (F/M = 4)	55	5.53	Acetate, propionate	14032 mg/L	Digester failure (stopped producing methane)	(Andriamanohiarisoamanana et al., 2017)
Cow manure + Dog food	Continuous	37, 55		Acetate and propionate	6500 mg/L	Digester failure	(Labatut, Angenent, & Scott, 2014)
Fruits/Veggies + FW	Semi-continuous	35	7.8	Severe acetate accumulation due to increasing ammonia	Total SCVFA = 9900 mg/L	High TAN and SCVFA accumulation caused stable pH (7.2) with low biogas yields → "inhibited steady state"; Inhibition to acetoclastic methanogens	(X. Shi et al., 2017)
Acetate, Propionate, Butyrate, Ethanol	Semi-continuous	35	7.0	Propionate	900 mg/L	Highest conc of acetate, butyrate and ethanol were 2400, 1800, 2400 mg/L, respectively → no significant inhibition; However when propionate reached 900 mg/L → significant inhibition (low growth rate, low methane yield)	(Y. Wang et al., 2009)

High-strength molasses-based wastewater	Continuous	55	5.5 and 7.2 (H and M-reactor)	Acetate, Butyrate	Acetate = 42.2-44.2% of total SCVFA Butyrate = 41.2-48.3% of total SCVFA	Total SCVFA almost doubled when OLR was increased from 5.1 to 8.1	(Wijekoon, Visvanathan, & Abeynayaka, 2011)
FW	Batch	35, 45, 55	5, 6, 7	Acetate (pH = 5) Butyrate (pH = 6.7)		Total SCVFA = 39.5 g/L at pH=6	(Jianguo Jiang et al., 2013b)

Among the four most common SCVFAs, acetate and propionate usually have the biggest role in methane production and an accumulation of both are used as an indication of system instability. The threshold concentrations of acetate and propionate before system failure varies from study to study. Kim et al (2013) carried out a batch study on AD of rice straw and observed that the SCVFAs that were produced were mostly acetate, which eventually led to decreasing pH and digester failure. The system was able to tolerate up to 5.0 g/L of acetate (from 6.5 g/L of total SCVFAs) before failing to produce methane. Others have reported higher tolerance levels up to 6.8 g acetate/L (Andriamanohiarisoamanana et al, 2017) before system failure during thermophilic AD of ensiled grass. On the other hand, some researchers believe propionate is a better indicator for system instability. Wang et al. (2009) reported that no significant inhibition occurred when acetate and butyrate levels exceeded 2.4 and 1.8 g/L, respectively, while significant inhibition caused the decline of the biomass when propionate levels hit 0.9 g/L.

A key factor influencing inhibition by SCVFA accumulation is the organic loading rate (OLR). OLR is the amount of organics – volatile solids – that is fed into a digester per day. Operation of reactors at high loading rates result in higher SCVFA generation, which eventually leads to inhibition of methanogens. In their two stage T-AD study, Wijekoon et al. (2011) saw an increase in SCVFA generation by a factor of 1.9 when the OLR was increased from 5.1 to 8.1 kg COD/(m^3-d). Jiang et al (2013) studied the effects of increasing OLRs (5, 11, and 16 kg TS/(m^3-d)) on the total SCVFA concentrations. The SCVFA concentrations were able to reach steady state for the OLR of 5 and 11 kg TS/(m^3-d); however, an OLR of 16 kg TS/(m^3-d) led to failure of the reactor due to higher concentration of SCVFAs (24.9 g/L). Increasing the OLR leads to higher SCVFA concentrations which causes pH drops and inactivates metabolic pathways due to their dependence on process pH, leading to inhibited methane yields and/or total failure (Jiang et al., 2013a; Jang et al, 2015). Jang et al. (2015) carried out co-

digestion of WAS and food wastes under thermophilic conditions while varying the OLR from 2.83 to 8.21 kg COD/(m3-d); with each successive increase in OLR the total SCVFAs increased from 0.79 to 55.26 kg COD/(m3-d). Up to the OLR of 6.88 kg COD/(m3-d), stable operation was observed although total SCVFAs accumulated to 42.88 g COD/L. However, once they increased the OLR to 8.21 kg COD/(m3-d), the severe accumulation of SCVFAs led to irreversible acidification, causing methane production to cease completely.

Due to enhanced production rates and kinetics at increased temperatures, T-AD elevates the risk of instability even further due to higher generation of SCVFAs. As a result, increased SCVFA production will decrease pH faster and puts the system at risk of acidification, leading to the inhibition of methanogens, and repeating in a feedback loop. Compared to mesophilic AD, SCVFA production in thermophilic digesters are enhanced several times over. Franke-Whittle et al (2014) compared the performances of mesophilic and thermophilic semi-continuous reactors by monitoring the SCVFA concentrations over time. The results of their study showed that the thermophilic reactor contained the highest amounts of total SCVFAs measured at 16.86 g/L compared to the 7.22 g/L in the mesophilic digester. In another study, Shi et al. (2013) observed that peak SCVFA concentrations in the thermophilic batch were 5-fold higher than its mesophilic counterpart. The thermophilic reactor saw a sharp decrease in pH which also correlated to a rapid decrease in biogas production and methane content. The thermophilic regime is especially sensitive to environmental changes compared to mesophilic AD. Sudden changes to the pH and accumulation of intermediate products like SCVFAs can bring about rapid instability and shutdown of the AD process (Labatut et al, 2014).

2.3 *Ammonia inhibition*

During anaerobic digestion, proteins and nitrogen-rich organic substrates released during the intermediate stages result in the formation of ammonia. Total ammonia nitrogen (TAN) plays

several key roles in AD. TAN is an essential nutrient source that is required by microorganisms for growth. Sung and Liu (2003) suggested that ammonia concentrations up to 200 mg/L were necessary to provide the essential nitrogen requirements for microorganisms in anaerobic processes. Furthermore, TAN is a natural buffer source for the AD system, which helps to resist acidification from SCVFA accumulation (Yuan & Zhu, 2016b). While certain amounts of ammonia are required for healthy anaerobic digestion, exceeding a threshold concentration for the system will result in strong inhibition of the AD process. Inhibition of the system is usually indicated by external factors such as the decrease in methane production rates and an accumulation of intermediate products like volatile fatty acids (Rajagopal et al, 2013). The threshold concentration of TAN in literature varies from as low as 1500 mg-N/L up to 11,000 mg-N/L (Kayhanian, 1994; Nakakubo et al, 2008; Lauterböck et al, 2012; H. Wang et al, 2016). Certain AD systems are able to tolerate higher TAN concentrations due to factors such as acclimation of inoculum, inoculum source, microbial communities, temperature, pH, etc.

In concentration, TAN exists as both ammonium (NH_4^+) and free ammonia (NH_3) ions. Of the two species, free ammonia nitrogen (FAN) is the main concern due to the ability of FAN to diffuse passively into cells and cause proton imbalance and potassium deficiencies (Chen et al., 2008). According to Sprott et al (1984), small weak bases like free ammonia (NH_3) can cause a shift in intracellular pH, leading to free ammonia ions absorbing protons and turning into ammonium ions. This forces the cells to expend energy to restore protons, thus decreasing the energy required for other cellular activities.

While many factors can influence the fluctuation of TAN levels, the pH and temperature of the system are especially important; an increase of TAN will proportionally increase the FAN concentrations. Kayhanian (1994) proposed the following equation to calculate the FAN concentration, Eq (1), given the process temperature and pH:

$$FAN = (TAN * K_a)/(C_H * (K_a/C_H + 1)) \quad (1)$$

where TAN is the total ammonia nitrogen concentration (mg/L), K_a is the temperature dependent dissociation constant (1.097×10^{-9} at 35°C and 3.77×10^{-9} at 55°C), and C_H is the concentration of hydrogen ions (Kayhanian, 1999). Another equation was proposed by Chen et al (2014) which can be used to estimate the FAN levels given the pH of the system. As shown below (Eq 2), the pH determines the shift in equilibrium to form either ammonium or free ammonia ions; higher pH results in a higher concentration of FAN:

$$NH_4^+ + OH^- \rightleftarrows NH_3 + H_2O \quad (2)$$

When ammonia nitrogen begins to accumulate, the pH increases, causing the equilibrium to shift towards FAN production. Studies have observed that AD of wastes with high ammonia potential were more inhibited at thermophilic temperatures than at lower temperatures (Bayr, Rantanen, Kaparaju, & Rintala, 2012a; Y. Chen et al., 2008b; Hidaka, Wang, Togari, Uchida, & Suzuki, 2013a).

While TAD brings many benefits in terms of accelerated digestion rates and increased kinetics, a key drawback that must be addressed is the poor process stability that results from ammonia accumulation. Due to increased digestion efficiencies, TAD accelerates the rate at which ammonia nitrogen is produced, tipping the scale in favor of ammonia production which leading to accumulation and eventually inhibition of microbiomes. The threshold concentration of total ammonia nitrogen (TAN) for TAD specified in literature is very wide-ranging, starting as low as 1500 mg-N/L up to 11,000 mg-N/L (Kayhanian, 1994); (Nakakubo et al., 2008); (Lauterböck et al., 2012); (H. Wang et al., 2016). Certain AD systems can tolerate higher TAN concentrations due to various factors such as acclimation of inoculum, inoculum source,

microbial communities, temperature, pH, etc. TAN exists as both ammonium (NH_4^+) and free ammonia (NH_3) ions. Thermophilic studies utilizing manure as substrate have reported issues ranging from 50% inhibition of methane yields to complete inhibition when TAN levels reached up to 7 – 11 g-N/L (Nakakubo et al., 2008); (Wang et al., 2016); (Sun et al., 2016). Several studies have reported that anaerobic digestion of wastes at thermophilic temperatures results in increased inhibition of methane production due to the effects of FAN (Bayr et al., 2012a; Hidaka, Wang, Togari, Uchida, & Suzuki, 2013b; Karthikeyan, Visvanathan, Zeshan, Karthikeyan, & Visvanathan, 2012; Mace, 2009).

As mentioned previously, temperature plays a key role in FAN production. FAN negatively impacts the biogas process in several ways: causes inhibition of methanogenic cultures due to change in process pH, affect the specific methane production, and diffusing into cells to cause proton imbalances (Y. Chen, Cheng, & Creamer, 2008a). The study by Hidaka et al. (2013) reported that the free ammonia concentration in the high-solids degradation of sewage sludge did not exceed 600 mg-N/L at thermophilic conditions. Their study related the decreased COD removal rates to the increased TAN levels; this was more pronounced in the thermophilic reactors, which had higher TAN/FAN concentrations. Methanogenic activity ceased when TAN levels exceeded 2000 mg-N/L (FAN was just under 600 mg-N/L). Additionally, they noted that it took weeks for methane activity to recover after the inhibition. Treating synthetic wastewater at thermophilic conditions, Sung & Liu (2003) reported that a 50% of inhibition of methanogenesis occurred when FAN concentrations reached about 600 mg-N/L. Several authors agree with this threshold concentration of FAN at thermophilic conditions: Siles et al (2010) observed a 50% inhibition of hydrogenotrophic methanogens when FAN was 620 mg-N/L; Bayr et al (2012) saw 50% inhibition to SMP at 635 mg-N/L FAN; Karthikeyan et al (2012) observed decreased methane yields when FAN reached as high as 660 mg-N/L. However, threshold concentrations can vary depending on the type of feedstock used

(ie. Nitrogen rich feedstocks like animal manure, etc.), the source of the inoculum (ie. taken from digester treating nitrogen-rich feedstocks), or whether the inoculum has been acclimatized to high ammonia levels.

Table 2.2. Impact of total ammonia nitrogen on instability of anaerobic digestion

Substrate	Operation Mode	Temperature (°C)	pH	TAN content	FAN Threshold	Impacts	Reference
Paper and yard waste	Semi-continuous	54-60		750 mg-N/kg 1500 mg-N/kg 2500 mg-N/kg	n/a	No inhibition 50% CH4 decrease Completely failed	(Kayhanian, 1994)
Corn stover	Batch	35-40	8.2-8.8	6000 mg-N/kg	n/a	50% CH4 decrease	(Z. Wang, Xu, & Li, 2013)
Sewage Sludge (SS)	Semi-continuous	35	7-8	3 g-N/L	6 g-N/L	Intense SCVFA accumulation and big decrease in biogas prod	(Duan, Dong, Wu, & Dai, 2012)
Pig manure	Continuous	51	8	11 g-N/L	1.45 g-N/L	50% inhibition	(Nakakubo et al., 2008)
SS	Continuous	35 55	7-8 6-7	4 g-N/L 3 g-N/L	<600 mg-N/L	Mesophilic digestion not affected Methane fermentation unstable when TAN over 2 g/L	(Hidaka et al., 2013a)
OF-MSW	Continuous	55	6.7-8	3 g-N/L	660 mg-N/L	Reactor inhibited by FAN toxicity; decreased methane yield	(Obuli et al., 2012)
Glucose	Batch	52	7.8	7 g-N/L	620 mg-N/L	50% inhibition of hydrogenotrophic methanogens; 21% decrease in biogas production	(Siles et al., 2010)
Slaughter-house wastes	Semi-continuous	55	7.5	5600 mg-N/L	635 mg-N/L	50% inhibition of methane production	(Bayr et al., 2012b)
Chicken manure + maize silage	Semi-continuous	36	7.3-8.0	9 g-N/L	630 mg-N/L	Acetic acid accumulation	(Chen Sun et al., 2016)
Dairy Manure	Batch	37 55	7.8-8.1 7.9-8.2	7 g-N/L 7 g-N/L	n/a	65% decrease in methane yield from 1 to 7 g-N/L (meso) 44% decrease in methane yield from 1-7 g-N/L (thermo)	(H. Wang et al., 2016a)
Fruits and Veggies + FW	Batch	35	7.2-7.8	1 – 4 g-N/L	45 mg-N/L	Significant SCVFAs accumulation and low BPR, "inhibited steady state" (mainly acetate under ammonia inhibition)	(X. Shi et al., 2017)

Kitchen Waste	Continuous	38	7.5-7.9	2 – 4.5 g-N/L	413 mg-N/L	Reactor adapted to stepwise ammonia stress by increased abundance of Firmicutes bacteria, community shift from acetotrophic to hydrogenotrophic methanogens, and enhanced activity of CoF_{420}.	(Gao, Zhao, Chen, Yu, & Ruan, 2015)
Slaughter-house wastes	Semi-continuous	38	7.9-8.2	6 g-N/L	1-1.2 g-N/L	Complete inhibition	(Lauterböck et al., 2012)
Dairy manure	Batch	37 55	7.8-8.1	1-7 g/L	n/a	-methane yield decreased by 65% when TAN raised from 1 to 7 g/L -tolerance to ammonia toxicity was enhanced by hydrogen addition -methane yield affected less by increasing ammonia levels compared to mesophilic -hydrogenotrophic thermophilic methanogens can tolerate higher ammonia and FAN compared to mesophilic methanogens	(H. Wang, Zhang, & Angelidaki, 2016b)
WAS	Semi-continuous	55		Up to 7.6 g/L	n/a	-methane production ceased when TAN reached 7600 mg/L	(Nakashimada & Ohshima, 2008)

2.4 Direct Interspecies Electron Transfer

As known, AD is comprised of multi-step biological processes that builds upon the syntrophic synergy between bacteria and methanogens to degrade organic wastes (Barua & Dhar, 2017). Methanogenesis is facilitated by mediated interspecies electron transfer (MIET) between fermentative bacteria and methanogens where the oxidation and reduction of key elements results in methane formation (Cheng & Call, 2016). A well-known form of MIET is interspecies hydrogen transfer (IHT), which is present in numerous methanogenic communities that relies on the transfer of hydrogen to shuttle electrons between syntrophic partners (Lovley, 2017). However, MIET is limited by the critical requirement that hydrogen partial pressure must remain at a low enough to ensure favorable conditions for continuous fermentation of complex organics (Cheng & Call, 2016), which introduce thermodynamic and kinetic barriers to methanogenesis (see Fig. 2.1 below). Recent studies have discovered a new type of syntrophy within the anaerobic microbiome, where bacteria could transfer electrons directly to methanogens, called direct interspecies electron transfer (DIET) (Barua, Zakaria, & Dhar, 2018a; Dang et al., 2016; Liu et al., 2012; Morita et al., 2011).

Currently, DIET syntrophy can be reproduced in one of two ways: 1) physical cell-to-cell contact between electrically conductive bacteria and methanogens, where they expend energy to construct conductive appendages – called nanowires – for electron transfer, or 2) inducing DIET-like effects by introducing conductive materials that act as a conduit for electron transfer in the digester (Cheng & Call, 2016). Due to a limited number of bacteria and methanogens that are capable of producing nanowires for the first method of DIET, many DIET-studies have focused on the practical application of conductive materials supplementation into digesters (Dang et al, 2016; Yang et al, 2017; Lin et al, 2018; Xu et al, 2018; J. H. Park et al, 2018). The use of conductive materials as a conduit for electron transfer can substitute the requirements of microbial nanowires or cytochromes for electron transfer,

which makes this a more inclusive approach for various bacteria to participate in DIET with methanogens. In addition, the biosynthesis of nanowires and cytochromes require significant amounts of energy investment by microbes and so the presence of conductive materials allows microbes to conserve energy from electron transfer process (Barua & Dhar, 2017).

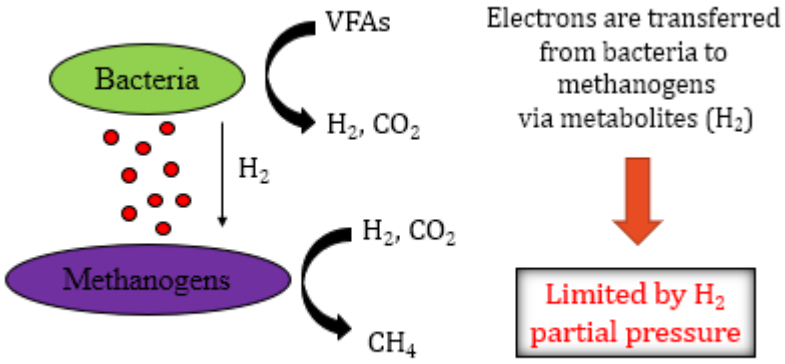

Fig. 2.1. Mediated interspecies electron transfer (MIET).

Stimulating DIET in digesters can enhance the methane production rate by promoting the rate at which SCVFAs are degraded, simultaneously relieving the system from increasing acidity and increasing methane yields. Among the available studies, supplementing digesters with conductive materials such as GAC, biochar, graphene, magnetite, etc., has shown to greatly increase methane production rates and substantially lower SCVFA accumulation (Lin et al, 2017; Yang et al, 2017a; Wang et al, 2018). Others have reported that in addition to enhancing methane production, DIET syntrophy allows digesters to operate at higher OLRs and achieve higher system stability. Dang et al (2016) evaluated the performance of increasing OLR on the anaerobic digestion of commercial dog food by supplementing their digesters with various carbon-based conductives (i.e. carbon cloth, granular activated carbon (GAC)). The reactors supplemented with conductive materials showed superior performance compared to the control digester even as the OLR was increased to 8.5 kg COD/(m^3-d); however, increasing

the OLR more caused system failure of all reactors. Interestingly, once the OLR was decreased following system failure, the reactors supplemented with conductive materials recovered faster than the control, indicating that DIET could promote faster recovery of soured reactors. Furthermore, DIET syntrophy has been shown to reduce the start-up period in anaerobic digestion, resulting in shorter times to peak production. By supplementing their reactors with conductive carbon nanotubes, Yan et al (2017) were able to shorten the start-up period by up to 40% and also achieved lower SCVFA accumulation and higher methane production rates than the control.

The literature review on DIET studies conducted at thermophilic temperatures shows that it is a relatively novel concept and large gaps in knowledge remain. In addition to the enhanced degradation efficiencies and higher methane yields for T-AD, the introduction of carbon additives reveal certain trends as shown from the available studies. Past AD studies comparing the performance at different temperatures have shown that increasing the temperature enhances the reaction kinetics of all AD steps; however, the fermentation step being the most thermodynamically favorable and fastest step in the AD process leads to SCVFAs accumulation and acidification in thermophilic conditions. Recent studies have shown that supplementing reactors with conductive carbon materials at thermophilic temperatures provided the ability to control acidification and control the accumulation of common inhibitors, such as ammonia. Biochar supplementation in several studies have shown the benefits of increased alkalinity due to natural alkali functional groups present in the biochar and the neutralization of generated SCVFAs which prevented acidification of reactors, even at increasing OLRs (Jang et al., 2018; Q. Li et al., 2018; Caiyu Sun et al., 2019; G. Wang, Li, Gao, & Wang, 2018a). For example, G. Wang et al (2018) conducted a semi-continuous experiment on the codigestion of food waste and sewage sludge while varying the OLR from 1.6 to 5.4 gVS/(L-d). Among the two types of biochars used, the sawdust-derived biochar (SDBC)

showed optimal performance by mitigating SCVFAs and controlling acidification up to the highest OLR studied (5.4 gVS/(L-d)). Concurrently, stable methane production was observed at the highest OLR in the SDBC-amended reactor, demonstrating robust AD performance. Others, like Li et al (2018) and Sun et al (2019), also noted that total SCVFAs were higher in the control reactors and attributed the lower total SCVFAs in biochar-amended cultures to the alkalinity provided from biochars.

Furthermore, conductive materials supplemented into AD systems have shown to be favorable for propionate degradation. The oxidation of propionate is well-known to be a limiting factor of methanogenesis and has shown to limit the efficiency of AD due to its slow metabolism (Stams & Plugge, 2009; Y. Yang et al., 2017b). The addition of magnetite in batch studies of acetate and propionate showed that the time to degrade propionate was reduced 3-fold compared to reactors without magnetite reinforcement (150 d to 50 d). The authors, Yamada et al (2015), attributed this phenomenon to the induced electric syntrophy between the acetate- and propionate-oxidizing bacteria and methanogens. Jang et al (2018) tested the effects of adding dairy-manure derived biochar to their batch tests on dairy manure. Likewise, they observed that the biochar supplementation resulted in lower propionate levels compared to control reactors.

Table 2.3. Enhancement of process stability by conductive materials supplementation

Substrate	Operational Conditions	Conductive material	Impacts on stability (SCVFA, pH, TAN)	Impacts on methanogenesis/methane yield	Reference
Commercial dog food (DF)	Semi-continuous HRT = 10 d Temp = 37 deg	5 materials: Carbon cloth, carbon felt, GAC, Graphite rods, polyester cloth	- SCVFAs remained at low concentrations when OLR increased to 6.7; - SCVFAs accumulated when OLR further increased and pH > 6.0	- Reactors with carbon cloth, GAC, and carbon felt performed better than control, graphite rods, and polyester cloth at OLR = 6.7 - Reactors with carbon cloth, GAC, and carbon felt performed stably at OLR = 8.5, whereas control, graphite rods, and polyester rods-amended reactors declined in performance - Carbon cloth and GAC enhanced methane production from complex organic waste, permitted higher OLRs, and promoted faster recovery after souring	(Dang et al., 2016)
WAS	Batch; 20 d operation; WV = 0.25 L Temp = 37 deg	GAC at different doses: 0, 0.5, 1, 2, 5 g	- Control reactor (< 1000 mg COD/L); GAC-amended reactors (below 100-200 mg COD/L); - Higher acetate and propionate degradation rates in GAC-amended reactors; - pH was higher in all GAC-amended reactors	- Highest increase in methane production from 5.0 g GAC dosage compared to control; - Highest VSS removal rate of 50.6% at 5.0 g GAC dosed reactor (6% increase compared to control)	(Y. Yang et al., 2017a)
Ethanol	Batch; Temp = 37 & 55 deg pH adjusted to 7.5 WV = 0.4 L	Graphene	M-AD: With graphene, 50% of ethanol was consumed in first 48 hr, compared to only 29% in control T-AD: With graphene, 55% of ethanol consumed in first 48 hr, compared to only 12.4% in control	M-AD: 1.0 g/L of graphene saw 13.8% increase in methane production, reduced time to peak production from 54 to 42 hr, peak production rate increased from 4.8 to 6.0 mL/g/hr T-AD: 1.0 g/L graphene saw peak production rate increased from 8.7 to 11.0 mL/g/hr, time for peak rate reduced from 60 to 48 hr	(R. Lin et al., 2018b)
Acetate (HAc), Propionate (HPr),	Batch Temp = 35 deg	GAC doses: 0, 0.5, 5, 25 g/L	-At high organic loading rates (5 g/L), GAC supplementation increased degradation rates of propionate (4.7 times) and butyrate (7 times)	Methane production rates significantly enhanced when GAC increased to 5 g/L for propionate and butyrate, but not so much for acetate	(Xu et al., 2018)

Butyrate (HBu)				Propionate: Reduced lag phase from 3.4 to 0.9 d Butyrate: Reduced lag phase from 12.7 to 7.8 d	
Acetate + Ethanol	Batch; WV = 0.25 L S/I = 1.0 Temp = 35 deg pH maintained at 7.5	GAC, Powdered activated carbon (PAC): 0.6 g and 1.2 g		Acetate: Supplemented with 1.2g GAC had best ultimate methane volume (31% better than control), methane production rate, and smallest lag phase Ethanol: Best ultimate methane volume and methane production rate with 1.2g GAC, but least lag phase with 0.6g GAC	(Park et al., 2018)
Glucose	Batch; WV = 0.36 L Temp = 55 deg	Carbon nanotubes (CNT) at 1 g/L, GAC at 10 g/L	Acetate degradation rates were much higher in reactors supplemented with carbon materials Within 22 hr, 80 and 81% of acetate removed by GAC and CNT, respectively, compared to only 8.5% in control DIET pathways increased stability of the system by replacing H2 as a dominant pathway → system can tolerate high H2 partial pressure → there is less IHT (interspecies hydrogen transfer), implying DIET was main mode of electron transfer	GAC → SMP = 0.67 mL CH4/g-VSS, which was more than twofold of control CNT → SMP = 0.48 mL CH4/g-VSS, which was almost twofold of control (0.25) With conductive materials, the lag phase was reduced by almost 40%	(Yan, Shen, Xiao, Chen, Sun, Kumar Tyagi, et al., 2017)
Sewage sludge	Batch M-AD (37 deg) vs. T-AD (55 deg) WV = 0.55 L F/M = 0.5	Pine biochar: Loading = 2.49 and 4.97 g/g dry sludge White oak biochar:	- Digester pH increased due to alkaline nature of biochar; total alkalinity in biochar-amended digesters increased due to release of alkali metals (K, Ca, Mg) and ammonia generation which	- At lower loading, both biochars produced similar methane volumes compared to control; however, higher loadings decreased methane volume relative to control - Cumulative biogas volume was almost double in T-AD compared to M-AD	(Shen, Linville, Ignacio-de Leon, Schoene, & Urgun-Demirtas, 2016)

		Loading = 2.20 and 4.40 g/g dry sludge	consumed CO2 to generate HCO3/CO3 buffer - The ammonia concentration of control digester increased by 67%, whereas biochar-amended digesters fluctuated in range of -7.2 to 4.7% → biochar can alleviate ammonia accumulation	- Biochars increased the methane content in the biogas - Reaction rates were much higher in T-AD due to faster degradation of sludge and higher growth rate of thermophilic methanogens	
Dairy Manure	Batch WV = 0.18 L F/M = 1 (VS basis) 3 temperatures: psychrophilic (20 deg), Mesophilic (35), thermophilic (55)	Dairy-manure derived biochar (M-BC) Dosage = 1 or 10 g/L	- Biochar supplementation alleviated SCVFAs accumulation and enhanced degradation rate of SCVFA; controls all showed higher SCVFA concentrations than those with biochar - Oxidation of propionate is relatively slow for AD compared to acetate; the addition of biochar also resulted in lower propionate levels compared to control reactors	- Methane production and yield were enhanced at all temperatures with the addition of biochar - Higher loading (10 g/L) resulted in greatest increases to cumulative methane and yield - Gompertz modelling showed that the max methane production rate, Rmax, and max methane potential, P, were significantly enhanced compared to control; biochar addition also shortened lag phase for all temperatures	(Jang et al., 2018)
WAS + FW	Batch, F/M = 0.25 to 3 (VS basis) T-AD (55 deg) WV = 0.1 L	Sawdust-derived biochar (SBC)	- high buffer capacity provided by the biochar (alkalis and organic alkali functional groups) neutralized the generated SCVFAs and prevented decline of pH at higher F/M reactors - biochar supplementation improved methanogen's ability to recover from acid shock and produce methane with short lag times → methanogens benefit from large specific surface areas that provide "safe spaces" for microbe attachment without direct exposure to low pH	- While the lag times for control groups ranged from 2 to 19 d (with increasing F/M ratios), the lag time of biochar supplemented groups were all maintained at ~2 d - Higher methanogenic activity was reported for biochar-supplemented groups, as the max methane production rates were 1.86 times greater than the control group - The control group with highest F/M = 3 had decreased methane production due to overloading, but biochar groups at same F/M was able to tolerate the high organic loading	(Q. Li et al., 2018)
Beer Lees	Batch WV = 0.15 L	Cow-manure derived Biochar (CBC):	- The total SCVFAs in control cultures were all higher than biochar-amended cultures	- Cultures amended with biochar had shorter lag phases and higher methane productions compared to control, at both temperatures	(Caiyu Sun et al., 2019)

	F/M = 3 (TS basis) M-AD (35 deg) vs T-AD (55 deg)	Dosage rate = 2, 6, 10, 14 g/L	- Cultures with 14 g/L biochar had high propionate concentrations, which caused lower methane production compared to the other biochar loadings → could be cause for lower cumulative methane production at 14 g/L biochar reactor - pH and alkalinity were higher in cultures with biochar addition	- Increasing biochar loading from 2 to 10 g/L led to increases in cumulative methane production and yield, but highest biochar loading of 14 g/L decreased in T-AD	

Chapter 3

Materials and Methods

3.1 *Food Waste*

Food waste collected from a student residence building at the University of Alberta campus (Edmonton, Alberta, Canada) was used for this study. The food waste was mainly composed of fruit and vegetable wastes, bread, salad dressings, eggshells, and paper wastes. Once received, the food waste sample was immediately blended using an electric mixer and stored at 4˚C prior to the experiment. The average characteristics of food waste are as follows: total solids (TS): 18.5 ± 0.5, volatile solids (VS): 73.4 ± 1.2% of TS, total chemical oxygen demand (TCOD): 366.7 ± 41.7 g/L, total nitrogen (TN): 3.1 ± 0.6 g/L, total ammonia-nitrogen (TAN): 0.15 ± 0.1 g/L, pH: 5.5 ± 0.1.

3.2 *Biochemical Methane Potential (BMP) test*

The anaerobic biodegradability of food waste was assessed using biochemical methane potential (BMP) test. Eighteen glass anaerobic bioreactors with a working volume of 0.7 L were used in this study. These bioreactors were equipped with a mechanical mixer coupled with electric motors to provide continuous mixing (See Fig. 3.1 and Fig. S2). For the BMP test, the inoculum was obtained from mesophilic sludge anaerobic digesters at the Gold Bar Wastewater Treatment plant in Edmonton, Alberta, Canada. The average characteristics of anaerobic digester sludge are as follows: total solids (TS): 2.64 ± 0.04, volatile solids (VS): 59.79 ± 0.37% of TS, total chemical oxygen demand (TCOD): 27.21 g/L, total nitrogen (TN): 5.72 ± 0.16 g/L, total ammonia-nitrogen (TAN): 1.28 g/L, pH: 8.12 ± 0.02. Prior to the start of the BMP test, the inoculum used for mesophilic (37°C) and thermophilic (55°C) conditions were acclimated at their respective conditions for one week. BMP tests were performed using

control (inoculum + food waste) and GAC-amended (inoculum + food waste + GAC) bioreactors operated at mesophilic (37±1ºC) and thermophilic (55±1ºC) temperatures. To estimate the gas produced from inoculum, blank tests were conducted with inoculum only (Dhar, Nakhla, & Ray, 2012). Triplicate bioreactors were operated for each condition. GAC (8-20 mesh) used in this study was purchased from Sigma-Aldrich (Saint Louis, MO). Prior to the addition of GAC in bioreactors, they were thoroughly washed with de-ionized (DI) water. Then, they were dried in the oven at 105°C prior to use. A loading rate of 25 g GAC/L was used in GAC-amended reactors, which is within the range of optimum GAC loadings previously reported in the literature (Barua & Dhar, 2017; Park et al., 2018; Y. Yang et al., 2017a). BMP tests were conducted using a food-to-microorganism (F/M) (kg $VS_{Food\ Waste}$/kg $VS_{inoculum}$) ratio of 3; thus, all reactors initially contained 24.7±0.4 g volatile solids (VS) of feedstock. The initial pH values in all reactors were around ~7.4. The operating temperature of bioreactors was maintained using water baths (General Purpose Water Bath, Digital, 20 L, PolyScience, Illinois, USA); the liquids in the bioreactors were continuously stirred at 300 rpm with the agitator. The volume of produced methane gas was monitored on a daily basis. Here, methane production was reported per gram of VS of food waste added to the bioreactor.

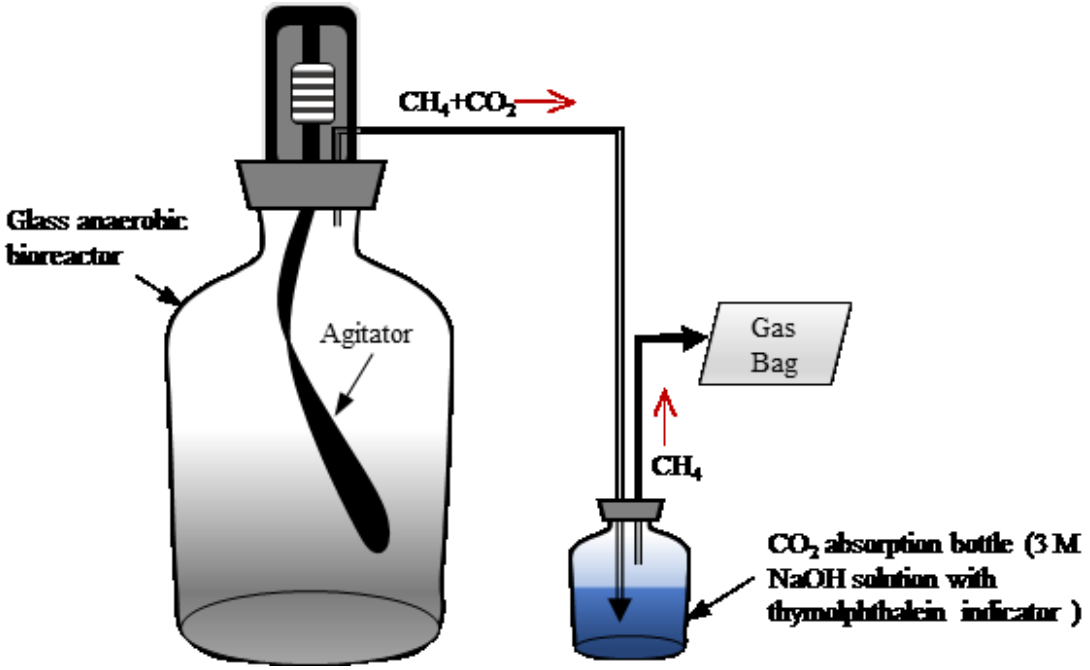

Fig. 3.1. Schematic diagram of experimental set-up for biochemical methane potential (BMP) test and methane volume measurement.

3.3 *Analytical methods*

TS and VS concentrations were measured using standard methods (APHA, 1999). The pH was measured with a pH meter (AR15 pH meter, Fisher Scientific, Pittsburgh, PA). COD, TAN, and TN concentrations were measured using HACH reagent kits (HACH, Loveland, Colorado, USA). Free ammonia nitrogen (NH_3) concentrations were computed from the experimentally measured TAN concentrations, as previously described in the literature (Belmonte, Hsieh, Figueroa, Campos, & Vidal, 2011). The concentrations of various SCVFAs (acetate, propionate, and butyrate) were measured using an ion chromatograph (Dionex ICS-2100, Dionex, Sunnyvale, CA) equipped with an electrochemical detector (ECD) and microbore AS19 column. The volume of methane produced from bioreactors was monitored on a daily basis using gas bags connected with carbon dioxide sequestration chamber (ISES-Canada,

Vaughan, ON, Canada); 3 M NaOH solution with thymolphthalein indicator was used to absorb the CO_2 from biogas as previously described in the literature (Barua, Zakaria, & Dhar, 2018b).

3.4 *Microbial community analysis*

At the end of the BMP experiments, the microbial communities in control and GAC-amended bioreactors were characterized using high throughput 16S rRNA gene sequencing. For the control reactor, suspended biomass was collected from triplicate reactors and a composite sample was used for DNA extraction. For GAC amended reactors, both suspended and GAC attached biomass from triplicate reactors were sampled for DNA extraction. Total metagenomic DNA extraction of the biomass sample was carried out using the PowerSoil® DNA Isolation Kit (Mobio Laboratoies, Carlsbad, CA, USA). The DNA concentrations were measured using a NanoDrop spectrophotometer (NanoDrop 2000C, Thermo Fisher Scientific, Waltham, MA, USA). The extracted DNA samples were stored at -70 °C before submitting to the Research and Testing Laboratory (Lubbock, TX, USA) for 16S rRNA gene amplicon library preparation and subsequent gene sequencing. Briefly, amplicon libraries were prepared by amplifying 16S rRNA gene with the bacterial primer pair 341F/805R and archaeal primer pair 517F/909R. Then, the amplicon libraries were sequenced on an Illumina Miseq using a 2×300 bp paired-end protocol.

3.5 *Processing 16S rRNA sequence data*

The demultiplexed sequencing data were processed and analyzed using the Quantitative Insights Into Microbial Ecology (QIIME v2) software (Caporaso et al., 2010). First, the forward and reverse primers were trimmed (cutadapt) (Martin, 2011). Then the trimmed read pairs were joined into single sequences followed with quality filtering based on a threshold Q score of 20. The denoised sequences were assigned to species-equivalent operational

taxonomic units (OTUs) at a 97% sequence similarity level using the open-reference OTU picking method (vsearch), followed by de novo chimera checking (Uchime) and removal of singleton OTUs (Rideout et al., 2014) . Alpha diversity metrics were calculated for observed number of OTUs, Chao1 richness, phylogenetic distance, Shannon index, and good's coverage. Beta diversity was calculated with the weighted UniFrac distance matrix (phylogenetic-based method). The distribution of major bacterial and archaea genera (each represented by >0.5% of their population) was further analyzed using heatmap with a double hierarchical dendrogram (Babicki et al., 2016).

3.6 *Statistical analysis*

Statistical analyses of experimental data were performed using Analysis of Variance (ANOVA) and Student's t-test with a threshold value of 0.05 in software R project (version 3.5.1).

Chapter 4

Results and Discussion

4.1 *Impact of GAC addition on methane productivity*

Fig. 4.1 illustrates the effects of GAC addition on methane production from food waste at mesophilic and thermophilic temperatures. At mesophilic temperature, both control and GAC-amended bioreactors exhibited substantial lag phases before methane production began (Fig. 4.1(a)). However, the lag phase was considerably shorter for the GAC-amended bioreactor. In GAC-amended bioreactor, methane production commenced after 12 days, as opposed to the 17 days for the control. The peak methane production rates in control and GAC-amended reactors were 20.3 and 25.6 L CH_4/(kg-VS-d), respectively (Fig. S.2(a)), which equates to a 26% increase in the peak methane production rate that was achieved with GAC addition. Another notable benefit was the shorter time to reach a peak production rate with the addition of GAC. Ultimately, GAC addition resulted in a significant improvement to methane productivity ($p < 0.05$). The total cumulative methane production from the GAC-amended mesophilic bioreactor was 242 ± 36 L CH_4/kg-$VS_{substrate}$ corresponding to a 2-fold increase compared to the control.

As shown in Fig. 4.1(b), both control and GAC-amended thermophilic reactors exhibited minimal methane production for the first few days. Similar to mesophilic condition, the GAC-amended reactor showed relatively shorter lag phase and started producing methane after 7 days, which was three days earlier than the control reactor. Additionally, the time to reach peak production was 20 days for the control reactors compared to 13 days for the reactors supplemented with GAC (Fig. S.2(b)), albeit peak methane production rates from both reactors were comparable. The cumulative methane production from the control and GAC-amended thermophilic bioreactors were 700 ± 48 and 714 ± 9 L CH_4/kg-$VS_{substrate}$, respectively. As

expected, both control and GAC-amended thermophilic bioreactors showed relatively shorter lag phases and higher methane productivity in comparison with mesophilic conditions. However, there was no significant difference between methane productivities from the control and GAC-amended bioreactors ($p > 0.05$); thus, the most important indication of enhancement appears to be the decreased lag phase in the GAC-amended bioreactors.

The results of this study suggested that the addition of GAC (25 g/L) significantly improved bio-methane production from food waste at mesophilic temperature, while GAC addition did not have a beneficial effect on methane productivity and kinetics at thermophilic temperature. According to two recent studies on comparative impacts of conductive materials addition in anaerobic digestion at different temperatures (Jang et al., 2018; R. Lin et al., 2018a), it was reported that the addition of conductive materials (e.g., graphene, biochar) can equally improve both mesophilic and thermophilic digestion. In contrast, the results of this study emphasized that the addition of conductive additives may not necessarily produce equal benefits for anaerobic digestion of complex feedstocks at different temperatures. One possible reason is the different F/M ratios used for these studies. The F/M ratio used in this study was 3 (VS basis), which was much higher than the F/M ratio of 0.5-1 used in their studies (Jang et al., 2018; R. Lin et al., 2018a). Another likely reason for this observation is that an optimized feature of conductive additive (e.g., type, specific surface area or loading of additives) for a particular feedstock would be different depending on the operating temperature. Temperature has been reported to be a decisive factor in shaping the microbial communities in anaerobic digestion (Guo, Wang, Sun, Zhu, & Wu, 2014; R. Lin et al., 2018a). In addition, microbial growth rates almost double with approximately every 10°C increase in temperature (Tchobanoglous, Theisen, & Vigil, 1993). Thus, temperature can significantly increase microbial growth rate, shift microbial community, and improve subsequent kinetics.

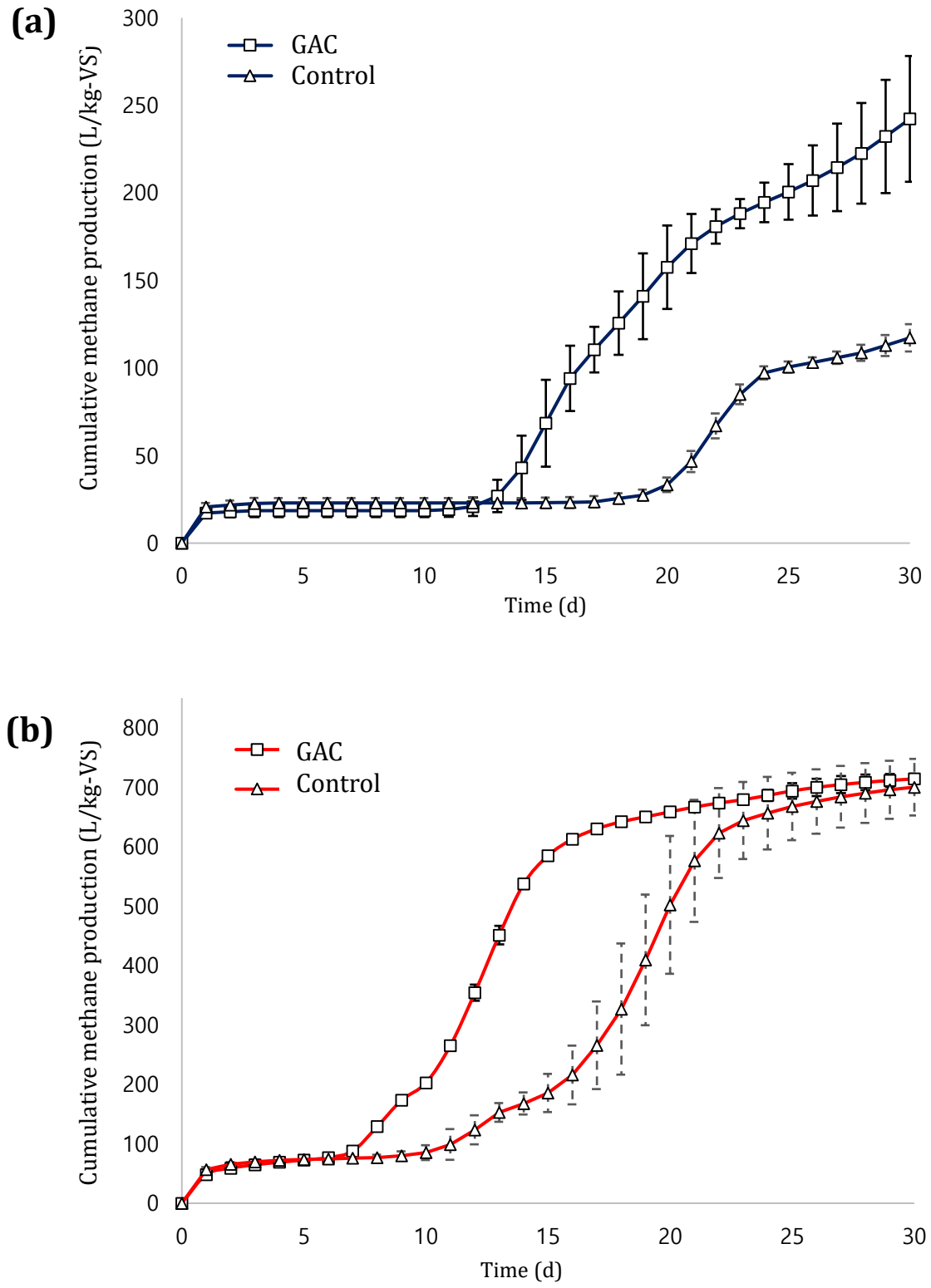

Fig. 4.1. Cumulative methane production under (a) Mesophilic, and (b) Thermophilic conditions.

Furthermore, previous studies have also showed that the optimal F/M was usually higher for thermophilic digestion than that for mesophilic digestion (L. Yang, Xu, Ge, & Li, 2015), which might also explain the different observation under different temperatures in this study. A higher F/M ratio should be studied for thermophilic digestion in the future to further evaluate the effect of conductive materials. As suggested by previous studies, the addition of conductive additives can improve methanogenesis kinetics through (i) establishment of DIET-active communities, (ii) enhancement of MIET kinetics due to reduced interspecies distance for hydrogen transfer, (iii) adsorption of intermediates (i.e. SCVFAs) (Barua & Dhar, 2017; Cheng & Call, 2016; Dang et al., 2016; Lovley, 2017; Park et al., 2018; D. Sasaki et al., 2011; Shrestha, Embree, Zengler, & Wardman, 2014; Zhao, Li, Quan, & Zhang, 2017; Zhao et al., 2016). Thus, it is possible that enhancement from GAC addition was more evident due to the naturally lower reaction rates at the mesophilic temperature in comparison with thermophilic temperature. For thermophilic condition, the increased process kinetics (i.e. rate-limiting hydrolysis step) at elevated temperature might diminish the noticeable effect of GAC addition.

Although the addition of GAC significantly improved the performance of mesophilic AD, economic feasibility would be one of the critical factors for GAC addition in digesters. Based on the current market price of GAC ($300-3,000/tonnes GAC), the approximate cost of GAC loading at 25 g/L will be $7.5-75/$m^3$ of digester (Barua & Dhar, 2017). Considering the cost of biomethane at $0.28/$m^3$ (Dhar et al., 2012), food waste treatment in GAC-amended mesophilic digester presents a potential for extra revenue generation (~$35/tonne food waste, VS basis) due to enhanced biomethane recovery. However, retention/re-utilization of GAC particles in continuous anaerobic bioreactor must be considered to make this approach economically feasible. Hence, further comprehensive economic assessment should be done based on continuous anaerobic digestion studies.

4.2 Accumulation of SCVFAs.

Fig. 4.2 shows the initial and final SCVFA concentrations for the two experimental conditions. Initial total SCVFA concentrations in mesophilic reactors were lower than the thermophilic reactors, despite similar feedstock being used in all reactors. This difference is attributed to the SCVFAs released during the one-week acclimation of mesophilic sludge at thermophilic temperature before the BMP test. Under mesophilic conditions, the accumulation of acetate was slightly higher in the GAC-amended reactor compared to the control (0.22 ± 0.01 g COD/L vs. 0.16 ± 0.00 g COD/L), while propionate and butyrate accumulation was relatively higher in the control reactor (propionate: 0.14 ± 0.00 g COD/L vs. 0.07 ± 0.00 g COD/L; butyrate: 0.12 ± 0.00 g COD/L vs. 0.06 ± 0.01 g COD/L) (see Fig 4.2). Nonetheless, total SCVFA accumulation was relatively higher in the unamended control in comparison with the GAC amended reactors. At thermophilic temperature, both control and GAC-amended reactors resulted in active degradation of various SCVFAs (see Fig. 4.2); final SCVFA concentrations in both reactors were <0.15 g COD/L. Like the mesophilic test, final SCVFA concentrations in the GAC-amended condition was slightly lower than the control. Moreover, minor accumulation of propionate was only observed in control. A similar trend has been observed in previous studies that have indicated that GAC addition in the mesophilic digestion of food waste could lead to lower SCVFA accumulation over the control (Capson-Tojo et al., 2018; Dang et al., 2016; Jang et al., 2018) . These studies also suggested enhanced propionate consumption in the presence of GAC, while overall propionate accumulation in this study was lower at both temperatures, which could be due to the differences in characteristics of food waste used.

The porous structure of GAC could possibly adsorb some SCVFAs or ammonia. However, temperature has been reported to be negatively correlated with adsorption capacity of organic acids onto activated carbon (Freitas, Mendes, & Coelho, 2007). Given the slightly

lower final SCVFAs in GAC-amended reactors under both temperatures and the slightly higher acetate in the GAC-amended reactor under mesophilic temperature, the adsorption ability of GAC might not be the main reason affecting methane production.

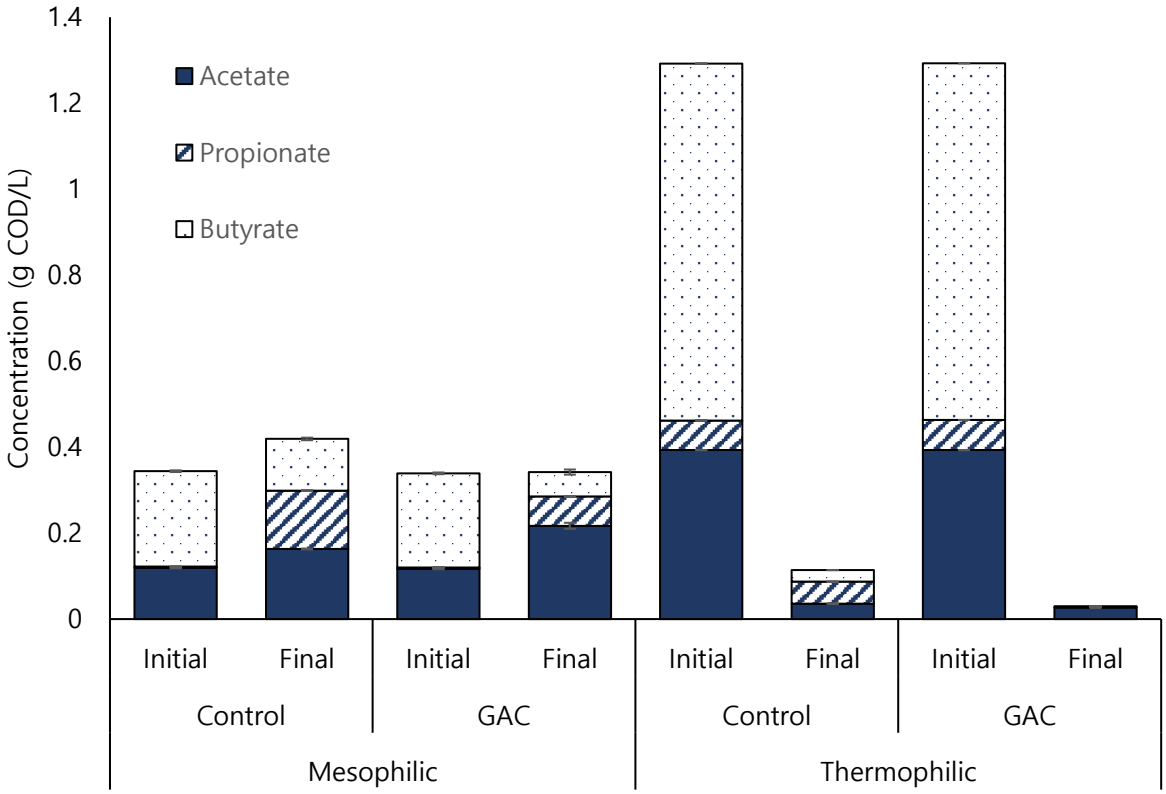

Fig. 4.2. Initial and final concentrations of various SCVFAs.

4.3 *Ammonia nitrogen*

During anaerobic digestion, disintegration and hydrolysis of proteins and other particulate materials causes the release of ammonia. Several studies have reported that high TAN levels are one of the leading causes for process disturbance and failure of digesters (Y. Chen et al., 2008b; Yenigün & Demirel, 2013). In particular, high free ammonia (NH_3) concentration is

of particular concern due to its ability to inhibit methanogenesis. Fig. 4.3 shows the initial and final concentrations of ammonium and free ammonia for different experimental conditions.

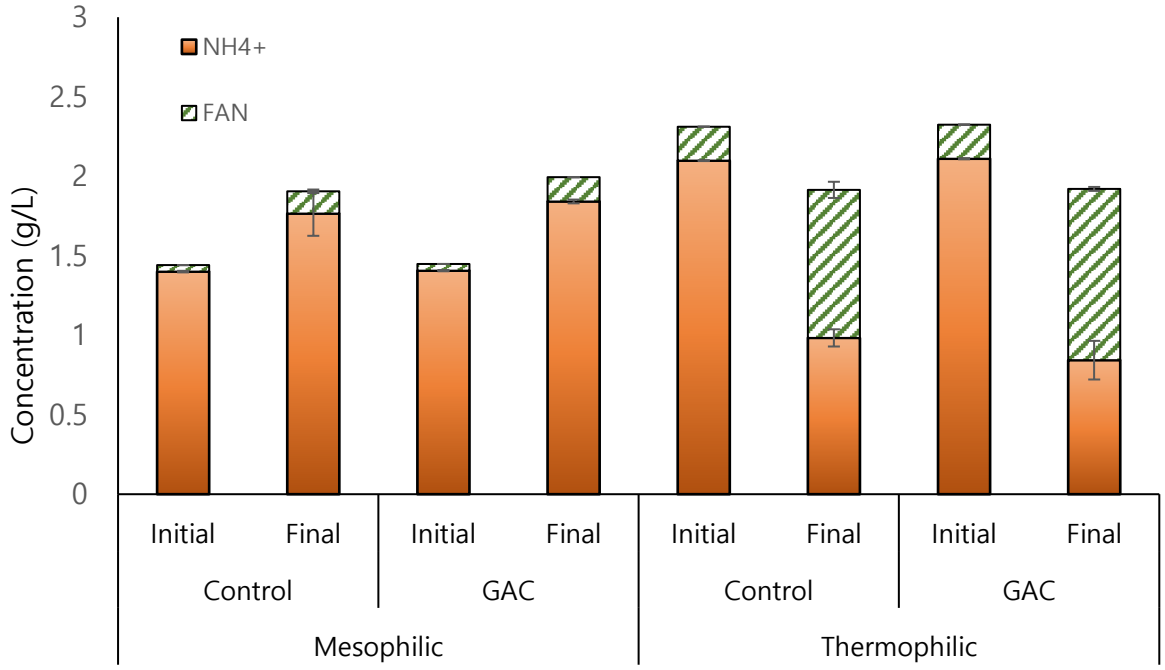

Fig. 4.3. Initial and final concentrations of ammonium ($NH4^+$) and free ammonia nitrogen (FAN).

At mesophilic temperature, the control and GAC-amended reactors had initial TAN concentrations of 1.44 ± 0.01 and 1.45 ± 0.00 g/L, respectively. At the end of the BMP test, TAN concentrations increased by 32 and 37%, respectively. The estimated initial and final FAN concentrations were estimated to be around ~0.04 and ~0.15 g/L for both reactors. Considering mesophilic conditions, this level of FAN is not within inhibitory range (>0.6 g/L) as specified in the literature (Y. Chen et al., 2008b; Rajagopal et al., 2013a; Chen Sun et al., 2016). In comparison with the mesophilic condition, thermophilic reactors showed an opposite trend. Initially, the TAN and FAN concentrations in both control and GAC-amended reactors were ~2.3 g/L and ~0.2 g/L, respectively. After the BMP test, TAN level in both reactors slightly decreased to ~1.9 g/L. As apparent from the final pH of samples from

thermophilic reactors (see Fig. 4.4), relatively alkaline pH could lead to the stripping of ammonium as ammonia gas under thermophilic condition. The final FAN concentrations were calculated at 0.93 and 1.08 g/L for control and GAC-amended reactors, respectively. In addition to pH, FAN/NH_4^+ ratio can be affected by operating temperature (Belmonte et al., 2011; Y. Chen et al., 2008b). Despite high FAN levels in thermophilic bioreactors, methane production was stable throughout the entire batch cycle, which is consistent with previous studies that have suggested that thermophilic microbiome is more resistant to high FAN concentrations (Gallert & Winter, 1997; H. Wang, Fotidis, & Angelidaki, 2015). As discussed later, *Methanosarcina* were dominant methanogens in biomass samples collected from thermophilic bioreactors in this study, which is also in line with the previous reports on their higher tolerance against ammonia (Fotidis, Karakashev, Kotsopoulos, Martzopoulos, & Angelidaki, 2013; Hao et al., 2015).

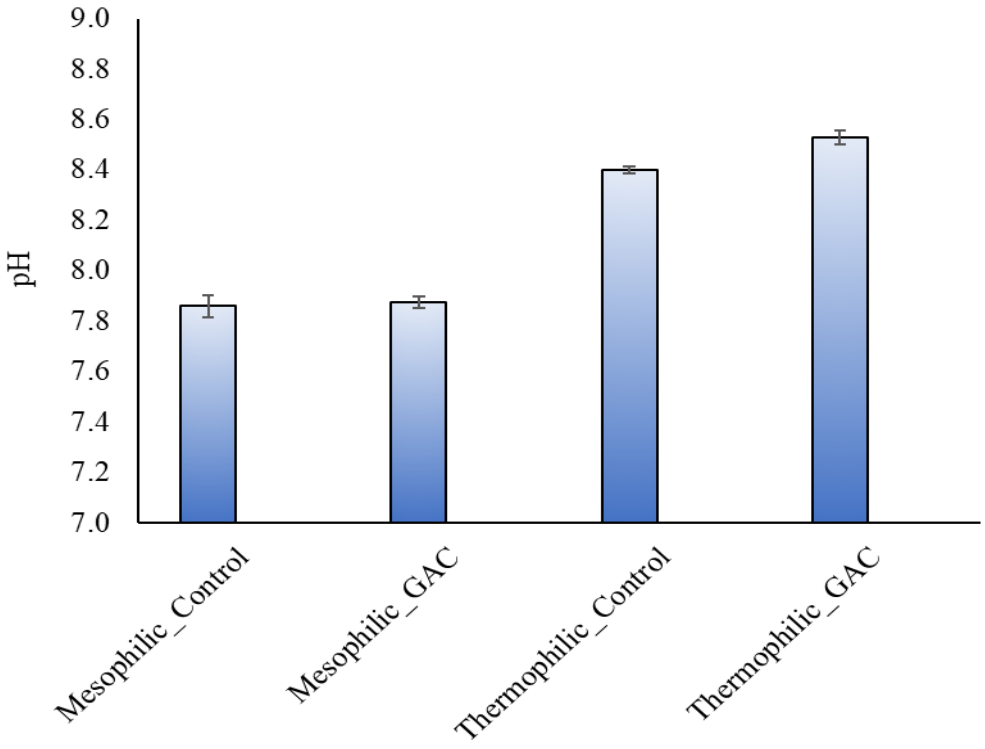

Fig. 4.4. Final pH of samples from different bioreactors.

4.4 *Microbial community diversity and similarity*

Some alpha diversity metrics were calculated to compare the microbial community diversity regarding GAC addition (Table 4.1). For bacterial community, the alpha diversity results showed that GAC addition had a significant (p<0.05) effect under both temperatures. All the richness (OTUs and Chao1) and diversity indices (phylogenetic distance and Shannon index) for bacterial community were significantly higher in GAC amended reactors over control reactors under both temperatures. For instance, Chao1 index increased from 141 to 242–265 with GAC addition in mesophilic digestion. Similarly, the archaeal richness was higher in GAC amended reactors than control under both temperatures. A higher Shannon index was also observed with GAC addition, indicating a higher archaeal diversity due to GAC addition. All the coverage values approaching to 1 indicated that the coverage was sufficient to capture most of the microbial diversity.

These results implied that adding GAC could induce a higher bacterial and archaeal richness and diversity under both mesophilic and thermophilic temperatures. It has been shown in literature that microbial communities with higher richness and diversity could also maintain a more stable system performance and has higher tolerance against environmental changes or organic loading shocks (L. Lin, Yu, & Li, 2017). On the other hand, the microbial richness and diversity were higher in mesophilic reactors than thermophilic reactors, which was consistent with the findings from other studies (Guo et al., 2014; Y.-F. Li et al., 2015). In general, GAC attached biomass showed higher indices of OTUs, Chao1, and phylogenentic distance compared to suspended biomass, except for the archaeal community in thermophilic digestion. Nonetheless, it seems apparent that addition of GAC might induce microbial community toward increased richness and diversity regardless of operating temperature, which could likely enhance system stability.

Table 4.1. Alpha diversity metrics of bacterial and archaeal community in mesophilic and thermophilic anaerobic digestion with/without granular activated carbon.

Sample		**Bacterial community**				
		OTUs	Chao1	Phylogenetic distance	Shannon	Coverage
Mesophilic	Control	128	141	10.32	3.32	1.00
	GAC_suspended	205	242	11.71	5.00	0.99
	GAC_attached	218	265	13.90	4.63	1.00
Thermophilic	Control	114	135	8.01	2.13	1.00
	GAC_suspended	164	204	10.70	3.83	1.00
	GAC_attached	198	248	13.11	3.66	0.99
Sample		**Archaeal community**				
		OTUs	Chao1	Phylogenetic distance	Shannon	Coverage
Mesophilic	Control	25	26	1.94	1.88	1.00
	GAC_suspended	28	28	1.46	2.09	1.00
	GAC_attached	29	38	1.82	2.08	1.00
Thermophilic	Control	9	12	0.94	0.98	1.00
	GAC_suspended	11	26	1.20	1.05	1.00
	GAC_attached	12	16	1.09	1.09	1.00

Beta diversity was also calculated and principal coordinate analysis (PCoA) was performed to examine the similarity of the microbial communities among different samples (see Fig. 4.5 below). For bacterial community (Fig. 4.5a), mesophilic and thermophilic samples were clearly separated by PC1, which explained about 64% of the total variations. On the other hand, PC2 (23%) separated mesophilic GAC suspended sample from its attached one. Notably, both control samples were distinct from their respective GAC amended samples along both axes. Similarly, for the archaeal community (Fig. 4.5b), mesophilic samples were separated from thermophilic samples by PC2 (44%). Mesophilic GAC attached sample was clearly distinct from other mesophilic samples along PC1 (54%). Together, these results further confirmed that the microbial communities were substantially different under mesophilic and thermophilic conditions. Additionally, GAC addition played an important role in structuring microbial communities, especially bacterial communities, which was also

supported by the alpha diversity results. Furthermore, it is noteworthy that the attached- and suspended-GAC communities were found to be considerably different in mesophilic digestion, but not for thermophilic digestion in this study. However, similarities between attached and suspended microbial communities in GAC amended bioreactors have been previously reported in literature, which could be attributed to disintegration of GAC into tiny particles, making it difficult to differentiate between attached and suspended biomass (Dang et al., 2017). Thus, it is possible that thermophilic temperature might have enhanced disintegration of GAC particles during operation.

4.5 Bacterial and archaeal communities

Fig. 4.6a below shows the relative abundance of bacterial community at phylum level. It shows that microbial communities at phylum level were substantially different under both temperatures. Also, with GAC addition, the shifts of microbial communities behaved differently under both temperatures. In mesophilic digestion, *Bacteroidetes* (63%) was the most dominant phylum in the control, followed by *Firmicutes* (17%), *Actinobacteria* (11%) and *Synergistetes* (7%). With GAC addition, the relative abundances of *Firmicutes* and *Chloreflexi* dramatically increased. The relative abundance of *Firmicutes* was higher in GAC suspended samples than GAC attached samples. Whereas, the *Chloreflexi* was mainly observed in GAC attached samples. The increase of *Chloreflexi* was also previously reported with addition of biochar in mesophilic digestion of synthetic food waste (G. Wang et al., 2018b). Similarly, in thermophilic digestion, the relative abundance of *Firmicutes* increased from 27% to 65–73% in response to GAC addition. However, *Thermotogae* and *Firmicutes* were the only two most dominant phyla in thermophilic digestion regardless of GAC addition, and GAC suspended and attached communities were similar regarding bacterial phylum structure.

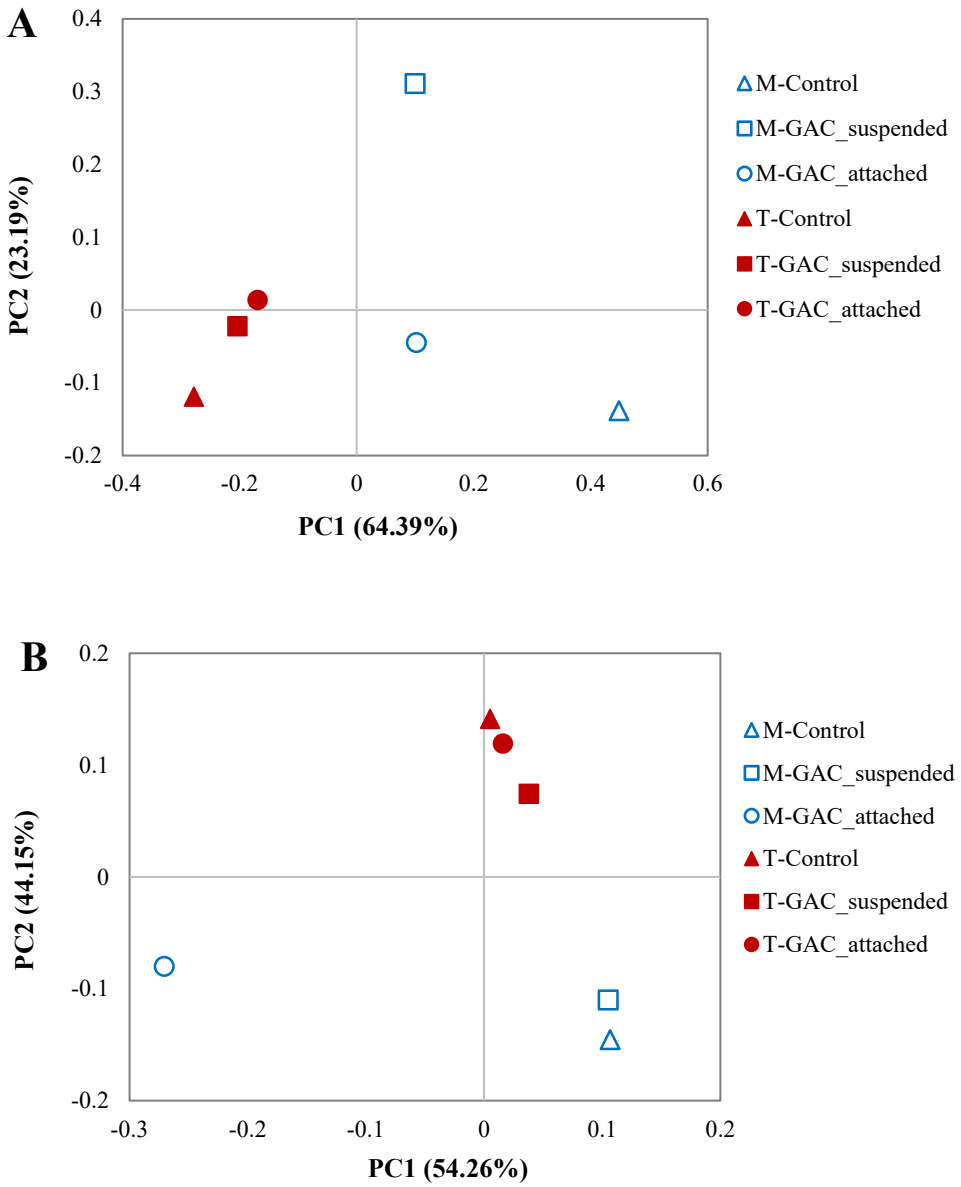

Fig. 4.5. Principal coordinate analysis (PCoA) of (A) bacterial community and (B) archaeal community in mesophilic and thermophilic anaerobic digestion with/without granular activated carbon based on weighted UniFrac distance matrix.

Note: M=Mesophilic, T=Thermophilic.

Fig. 4.6b shows the relative abundance of archaeal community at genus level. In mesophilic digestion, *Methanosphaera* (72%) was the most dominant genus in the control, followed by *Methanobacterium* (23%), and *Methanoculleus* (4%). With GAC addition, *Methanosarcina* was enriched from undetectable to 1.2% in both suspended and attached communities. Additionally, both *Methanobacterium* (46%) and *Methanobrevibacter* (10%) were enriched in GAC suspended biomass, while both *Methanofollis* (56%) and *Methanoculleus* (30%) were enriched on GAC surface. However, acetoclastic *Methanosaeta*, which was generally reported to be dominant under mesophilic conditions (Barua et al., 2018b; R. Lin et al., 2018a), was not identified in this study. In contrast, *Methanosarcina* (38–64%) and *Methanothermobacter* (36–62%) were the two most predominant genera in both thermophilic reactors. This can be attributed to the fact that *Methanosarcina* are resistant to high FAN levels (Fotidis et al., 2013; Hao et al., 2015). Analogous to comparable cumulative methane production, methanogenic communities were also comparable regardless of GAC addition in thermophilic digestion.

The distributions of major bacterial and archaeal genera were further analyzed using a heatmap (Fig. 4.7). Consistent with the PCoA analysis, the clustering of both bacterial and archaeal genera based on Euclidean distance showed that microbial communities were substantially different under mesophilic and thermophilic digestion. Based on their distribution patterns, these bacterial and archaeal genera were clustered into two groups. Group I was more abundant in mesophilic condition, such as *Caloramator*, family *Porphyromonadaceae*, *Methanobacterium*, and *Methanosphaera*. Group II was more abundant in thermophilic digestion, such as candidate genus S1, *Coprothermobacter*, *Methanosarcina*, and *Methanothermobacter*. Clustering of samples also showed that GAC addition greatly shifted microbial communities towards more diverse bacteria and methanogens at the genus level (Fig. 4.7). In mesophilic digestion, the bacterial genera

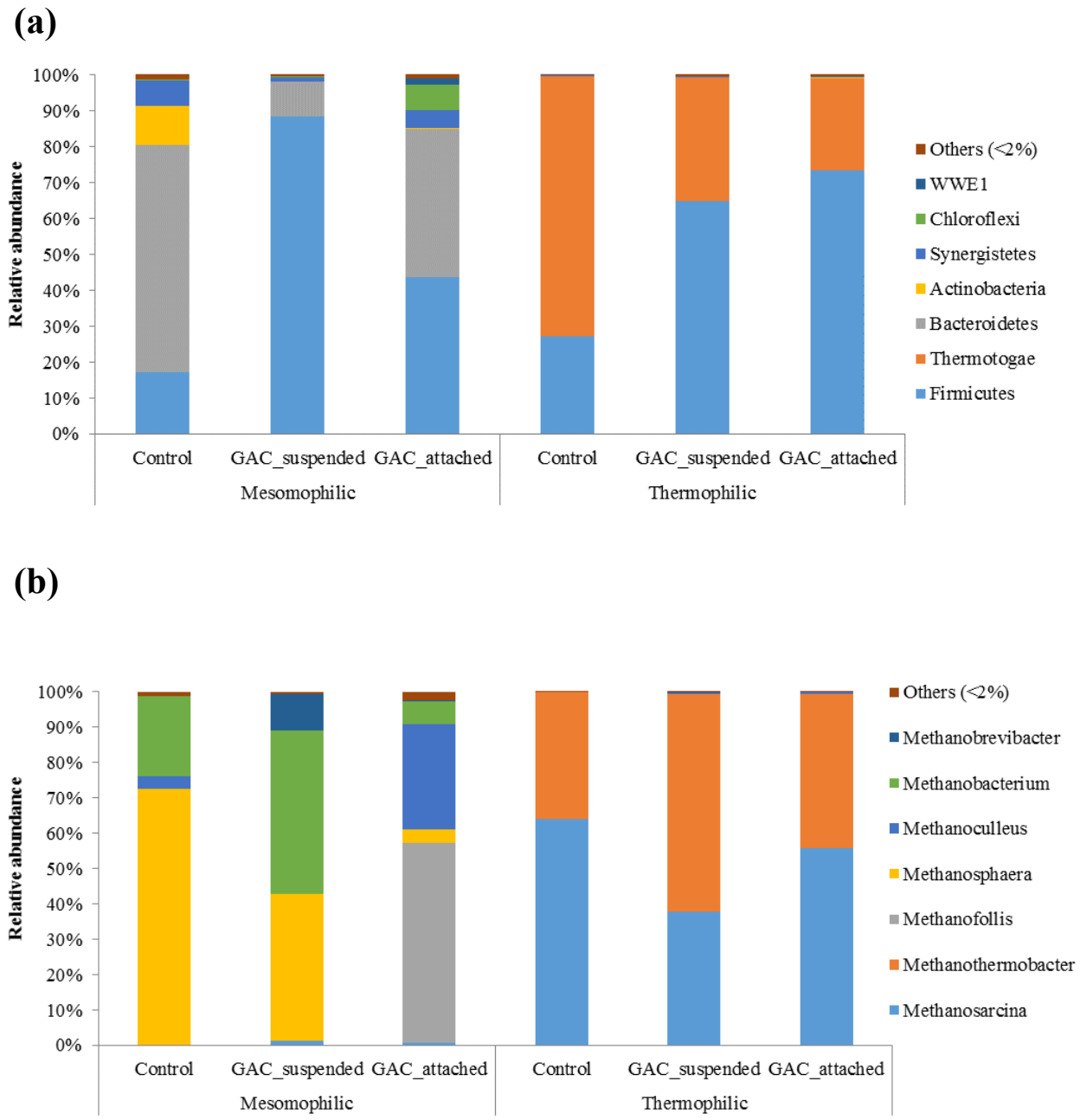

Fig. 4.6. Relative abundance of (A) bacterial community at phylum level and (B) archaeal community at genus level. Note: Sequences that accounted for less than 2% of their population were grouped into “Others”.

Bifidobacterium, *Bulleidia*, *Aminobacterium*, and family *Porphyromonadaceae* were more abundant in the control. These are known as hydrolytic and/or fermentative bacteria, which degrade carbohydrates/amino-acids. With GAC addition, more diverse fermentative bacteria were enriched, including *Caloramator*, *Sporanaerobacter*, T78, *Ethanoligenens*, *Prevotella*, *Clostridium*, and *Syntrophomonas*. The genera *Clostridium* and *Syntrophomonas* contain various well-defined syntrophic hydrogen-producing bacteria, which might explain the relatively lower SCVFA in GAC-amended reactor. Bacteria are known to be less sensitive to pH, SCVFA, ammonia changes in comparison to methanogens (L. Lin, Xu, Ge, & Li, 2018). With GAC addition, microbes were attached to GAC, which may reduce the interspecies distance, thereby enhancing the mass transfer (i.e. acids, H_2) and stimulating more diverse bacteria (Dang et al., 2016, 2017; Zhao, Zhang, et al., 2017). On the other hand, various hydrogenotrophic methanogens including *Methanoculleus*, *Methanofollis*, and *Methanobacterium* were enriched in GAC amended reactors. Additionally, an obvious enrichment of *Methanosarcina* (2 orders of magnitude higher than control) were also observed with GAC addition. *Methanosarcina* is metabolically versatile and can facilitate both the acetoclastic and hydrogenotrophic methanogenesis (Lü et al., 2013).

Several recent studies have reported the electrotrophic activity of *Methanosarcina*, which indicate that they could also be involved in DIET (S. Chen, Rotaru, Shrestha, et al., 2014; S. Chen, Rotaru, Liu, et al., 2014; Rotaru et al., 2014). In thermophilic digestion, the candidate genus S1 (72%) was the most abundant bacterial genus in control. With GAC addition, S1 greatly decreased, while various bacterial genera *Coprothermobacter*, *Thermacetogenium*, family *Clostridiaceae* and order MBA08 increased. *Thermacetogenium* contains well-defined syntrophic acetate-oxidizing bacteria (e.g., *T. phaeum*) (Hattori, Kamagata, Hanada, & Shoun, 2000). S1 might also have the potential to oxidize acetate

considering its close linkage with the genus *Thermotoga* (syntrophic acetate oxidizer) (D. Sasaki et al., 2011). Both family *Clostridiaceae* and order MBA08 are classified within the

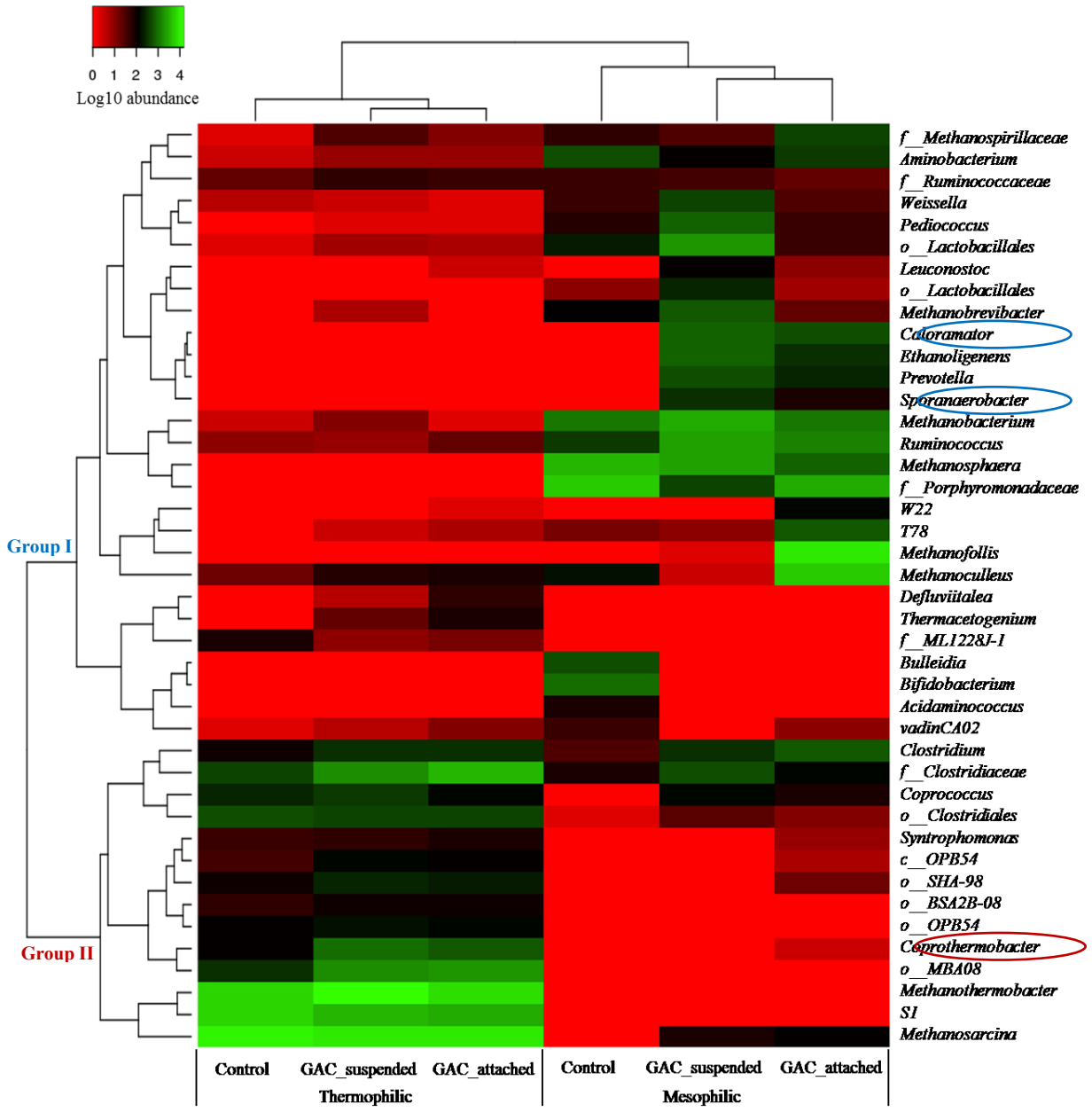

Fig. 4.7. Double hierarchical dendrogram based on log abundance of both bacterial and archaeal genera (each represented ≥0.5% of their population).

class *Clostridia*, which contains many known fermentative bacteria, but their actual metabolic functions are yet to be determined. On the other hand, *Methanosarcina* and hydrogenotrophic *Methanothermobacter* were dominant in both thermophilic reactors. These results further supported that GAC addition induced more diverse bacteria and methanogens at genus level regardless of temperature. Furthermore, GAC stimulated growth of some hydrogenotrophic methanogens and their syntrophic partners that can facilitate syntrophic oxidative methanogenesis partnerships, likely attributed to enhanced methane productivity. This stimulation of more diverse syntrophic acid-oxidizing bacteria might be resulted from the reduced interspecies distance among microbes attached to GAC.

4.6 *Significance of DIET at different temperatures*

The addition of GAC highly enriched various fermentative bacteria capable of facilitating extracellular electron transfer to insoluble electron donors, which might also change their metabolic pathways and syntrophy with methanogens. For instance, *Caloramator*, *Sporanaerobacter*, and T78 have been identified in GAC amended bioreactor at mesophilic temperature. *Caloramator* belongs to the family *Clostridiaceae*, which has been identified as one of the most dominant electroactive microbes in soil (Y. Bin Jiang, Zhong, Han, & Deng, 2016). Yan et al (2017) previously suggested that *Caloramator* and *Methanosaeta*/*Methanosarcina* could promote DIET under thermophilic conditions by utilizing GAC or carbon nanotubes as electron conduits. *Sporanaerobacter* contains known fermentative species (*S. acetigenes*) coupled with the reduction of elemental sulfur, suggesting its potential ability of extracellular electron transfer (Garcia et al., 2002). Dang et al (2016) also proposed DIET from various fermentative bacteria (e.g., *Sporanaerobacter* and *Syntrophomonas* spp.) and methanogens in the presence of conductive additives. T78 belongs to the family *Anaerolinaceae* (phylum *Chloroflexi*), which is a dominant microbe found in the

anode of microbial electrochemical systems, where its relative abundance is comparable to that of *Geobacter* (Cabezas, Pommerenke, Boon, & Friedrich, 2015). Interestingly, *Geobacter* spp. were not detected in this study, which are well-known electroactive bacteria participating in DIET (S. Chen, Rotaru, Shrestha, et al., 2014; S. Chen, Rotaru, Liu, et al., 2014; Rotaru et al., 2014). This lack of *Geobacter* can be explained by the fact that *Geobacter* cannot degrade complex organics (Ullery & Logan, 2015).

Some previous studies also reported that *Geobacter* spp. were not enriched with conductive additives when digesters are fed with complex fermentable substrates and real organic wastes (Dang et al., 2016, 2017; G. Wang et al., 2018b). Nonetheless, the enrichment of various known electroactive fermentative bacteria (e.g., *Caloramator*, *Sporanaerobacter*, etc.) in GAC amended mesophilic bioreactors suggest that they might participate in DIET using GAC as an electrical connection, thereby enhancing methanogenesis kinetics. Despite trivial differences in methane productivity and archaeal communities, bacterial community structure was also substantially impacted by GAC addition in thermophilic bioreactor. For instance, *Coprothermobacter* were dominant in GAC amended thermophilic bioreactor. *Coprothermobacter* are usually known as H_2-producing strains under thermophilic condition and may also have the function of acetate oxidation in syntrophic relation with methanogens (Lovley, 2008; K. Sasaki et al., 2011). Meanwhile, *Coprothermobacter* spp. were identified in several studies on thermophilic microbial fuel cells and microbial electrolysis cells (Gagliano, Braguglia, Petruccioli, & Rossetti, 2015). Its presence was supposed to be related to its ability to perform extracellular electron transfer. Moreover, the dominance of *Coprothermobacter* has been consistently found in thermophilic methanogenic degradation of simple substrates (e.g., ethanol, acetate, and propionate) in the presence of graphene and magnetite (R. Lin et al., 2018a; Yamada et

al., 2015), suggesting their electroactive potential in the presence of conductive additives. These results clearly showed the difference in microbial communities at both temperatures. Various electroactive genera (e.g., *Caloramator, Sporanaerobacter, Coprothermobacter*) greatly increased in relative abundance in the GAC-amended reactor in comparison to the control bioreactors under both temperatures (7-10% vs. 0-1%). These species have been suggested as major players in the promotion of DIET activities and enhance methanogenesis kinetics (Dang et al., 2016; Yan, Shen, Xiao, Chen, Sun, & Kumar, 2017). Liu et al. (2012) also observed that DIET via conductive GAC was more effective for methane production than DIET via conductive pili due to the higher conductivity of GAC than conductive pili (3000 vs. 2-20 μS/cm). These results suggest that GAC not only act as a medium that reduces the interspecies distance for enhanced mass transfer, but also acts as an electron conduit to promote DIET-based methanogenesis. Overall, the results of this current study indicate that the addition of GAC could stimulate more diverse bacterial communities to possibly promote DIET with enrichment of electroactive bacteria at both temperatures; however, the effects of GAC addition in thermophilic bioreactor was not as significant compared to mesophilic condition, although there were some improvements in methanogenesis rates. It should be noted that microbial community analysis with 16S rRNA gene sequencing does not provide explicit information about the specific roles of the individual microbe or relative contribution of DIET and MIET in overall methanogenesis. Thus, the suggested role of specific microbes herein is reasonable to some degree based on the previous reports on their metabolic functions. Further investigation is required to obtain more insights into the specific metabolic role of these microbes.

Chapter 5

Conclusions and Recommendation

5.1 *Conclusions*

The BMP test showed that the addition of GAC (25 g/L) had significant impact on shaping the microbial communities in comparison with the control. From this study, the following points can be concluded. Perhaps most importantly, GAC increased the richness and diversity indices for bacterial and archaeal communities in anaerobic digestion of food waste under thermophilic and mesophilic temperatures. At both temperatures, GAC promoted the growth of various fermentative bacteria (e.g., *Calorameter*, *Sporanaerobacter*, *Coprothermobacter*, etc.) that could potentially facilitate DIET to methanogens. Furthermore, it was demonstrated that through the addition of conductive materials, the lag phases for methane production could be significantly reduced at both mesophilic and thermophilic temperatures. Nonetheless, regarding methane productivity, the ultimate benefit of GAC addition in thermophilic digestion was not as significant as compared to its mesophilic counterpart. These findings suggest that the temperature-dependent optimization of conductive additives should be considered in future studies on anaerobic digestion of complex organic wastes.

5.2 *Recommendations*

The disparity between mesophilic and thermophilic AD performance in the BMP test warrants further investigations to identify possible causes for sub-optimal performance in the thermophilic regime. One explanation for the diminished performance in T-AD could be due to the F/M ratio used. Due to enhanced process kinetics with increasing temperatures, previous studies have mentioned that thermophilic digestion can tolerate higher optimal F/M ratios. Hence, while the F/M ratio used in this study was sufficient for mesophilic digestion,

the ratio might have not been optimal for the thermophilic test. Therefore, it is recommended that future studies consider using higher F/M ratios in thermophilic digestion when conducting DIET studies to compare mesophilic and thermophilic regimes. Another area that should be further explored is the disintegration of GAC particles (and possibly, other conductive materials) during thermophilic operation, which makes it hard to differentiate between attached and suspended biomass. It is unclear whether disintegrated GAC particles is the cause of similar attached and suspended microbial communities, or whether thermophilic temperatures naturally induces this phenomenon. From the perspective of full-scale implementation, the costs of GAC must be balanced with the revenue generated from enhanced methane yields. The retention/re-utilization of GAC particles in continuous anaerobic bioreactor must be considered to make this approach economically feasible. Hence, it is recommended that economic assessments be considered in future studies.

References

Andriamanohiarisoamanana, F. J., Matsunami, N., Yamashiro, T., Iwasaki, M., Ihara, I., & Umetsu, K. (2017). High-solids anaerobic mono-digestion of riverbank grass under thermophilic conditions. *Journal of Environmental Sciences (China)*, *52*, 29–38. https://doi.org/10.1016/j.jes.2016.05.005

APHA. (1999). *Standard Method for the Examination of Water and Wastewater*. *American Water Works Association and Water Environment Federation*. https://doi.org/10.1089/acm.2013.0216

Babicki, S., Arndt, D., Marcu, A., Liang, Y., Grant, J. R., Maciejewski, A., & Wishart, D. S. (2016). Heatmapper: web-enabled heat mapping for all. *Nucleic Acids Research*, *44*(W1), W147–W153. https://doi.org/10.1093/nar/gkw419

Barua, S., & Dhar, B. R. (2017). Advances towards understanding and engineering direct interspecies electron transfer in anaerobic digestion. *Bioresource Technology*, *244*(July), 698–707. https://doi.org/10.1016/j.biortech.2017.08.023

Barua, S., Zakaria, B. S., & Dhar, B. R. (2018a). Enhanced methanogenic co-degradation of propionate and butyrate by anaerobic microbiome enriched on conductive carbon fibers. *Bioresource Technology*, *266*(April), 259–266. https://doi.org/10.1016/j.biortech.2018.06.053

Barua, S., Zakaria, B. S., & Dhar, B. R. (2018b). Enhanced methanogenic co-degradation of propionate and butyrate by anaerobic microbiome enriched on conductive carbon fibers. *Bioresource Technology*, *266*(June), 259–266. https://doi.org/10.1016/j.biortech.2018.06.053

Bayr, S., Rantanen, M., Kaparaju, P., & Rintala, J. (2012a). Bioresource Technology Mesophilic and thermophilic anaerobic co-digestion of rendering plant and

slaughterhouse wastes. *Bioresource Technology*, *104*, 28–36. https://doi.org/10.1016/j.biortech.2011.09.104

Bayr, S., Rantanen, M., Kaparaju, P., & Rintala, J. (2012b). Mesophilic and thermophilic anaerobic co-digestion of rendering plant and slaughterhouse wastes. *Bioresource Technology*, *104*, 28–36. https://doi.org/10.1016/j.biortech.2011.09.104

Belmonte, M., Hsieh, C. F., Figueroa, C., Campos, J. L., & Vidal, G. (2011). Effect of free ammonia nitrogen on the methanogenic activity of swine wastewater. *Electronic Journal of Biotechnology*, *14*(3). https://doi.org/10.2225/vol14-issue3-fulltext-11

Braguglia, C. M., Gallipoli, A., Gianico, A., & Pagliaccia, P. (2018). Anaerobic bioconversion of food waste into energy: A critical review. *Bioresource Technology*, *248*, 37–56. https://doi.org/10.1016/j.biortech.2017.06.145

Cabezas, A., Pommerenke, B., Boon, N., & Friedrich, M. W. (2015). Geobacter, Anaeromyxobacter and Anaerolineae populations are enriched on anodes of root exudate-driven microbial fuel cells in rice field soil. *Environmental Microbiology Reports*, *7*(3), 489–497. https://doi.org/10.1111/1758-2229.12277

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335–336. https://doi.org/10.1038/nmeth.f.303

Capson-Tojo, G., Moscoviz, R., Ruiz, D., Santa-Catalina, G., Trably, E., Rouez, M., … Escudié, R. (2018). Addition of granular activated carbon and trace elements to favor volatile fatty acid consumption during anaerobic digestion of food waste. *Bioresource Technology*, *260*(March), 157–168. https://doi.org/10.1016/j.biortech.2018.03.097

Chen, J. L., Ortiz, R., Steele, T. W. J., & Stuckey, D. C. (2014). Toxicants inhibiting anaerobic digestion: A review. *Biotechnology Advances*, *32*(8), 1523–1534. https://doi.org/10.1016/j.biotechadv.2014.10.005

Chen, S., Rotaru, A.-E., Shrestha, P. M., Malvankar, N. S., Liu, F., Fan, W., … Lovley, D. R. (2014). Promoting Interspecies Electron Transfer with Biochar. *Scientific Reports*, *4*(1), 5019. https://doi.org/10.1038/srep05019

Chen, S., Rotaru, A. E., Liu, F., Philips, J., Woodard, T. L., Nevin, K. P., & Lovley, D. R. (2014). Carbon cloth stimulates direct interspecies electron transfer in syntrophic co-cultures. *Bioresource Technology*, *173*, 82–86. https://doi.org/10.1016/j.biortech.2014.09.009

Chen, Y., Cheng, J. J., & Creamer, K. S. (2008a). Inhibition of anaerobic digestion process : A review, *99*, 4044–4064. https://doi.org/10.1016/j.biortech.2007.01.057

Chen, Y., Cheng, J. J., & Creamer, K. S. (2008b). Inhibition of anaerobic digestion process: A review. *Bioresource Technology*, *99*(10), 4044–4064. https://doi.org/10.1016/j.biortech.2007.01.057

Cheng, Q., & Call, D. F. (2016). Hardwiring microbes: Via direct interspecies electron transfer: Mechanisms and applications. *Environmental Science: Processes and Impacts*, *18*(8), 968–980. https://doi.org/10.1039/c6em00219f

Dang, Y., Holmes, D. E., Zhao, Z., Woodard, T. L., Zhang, Y., Sun, D., … Lovley, D. R. (2016). Enhancing anaerobic digestion of complex organic waste with carbon-based conductive materials. *Bioresource Technology*, *220*, 516–522. https://doi.org/10.1016/j.biortech.2016.08.114

Dang, Y., Sun, D., Woodard, T. L., Wang, L., Nevin, K. P., & Holmes, D. E. (2017). Stimulation of the anaerobic digestion of the dry organic fraction of municipal solid waste (OFMSW) with carbon-based conductive materials. *Bioresource Technology*, *238*, 30–38. https://doi.org/10.1016/j.biortech.2017.04.021

Dhar, B. R., Nakhla, G., & Ray, M. B. (2012). Techno-economic evaluation of ultrasound and thermal pretreatments for enhanced anaerobic digestion of municipal waste activated sludge. *Waste Management*, *32*(3), 542–549.

https://doi.org/10.1016/j.wasman.2011.10.007

Duan, N., Dong, B., Wu, B., & Dai, X. (2012). High-solid anaerobic digestion of sewage sludge under mesophilic conditions: Feasibility study. *Bioresource Technology*, *104*, 150–156. https://doi.org/10.1016/j.biortech.2011.10.090

Facchin, V., Cavinato, C., Fatone, F., Pavan, P., Cecchi, F., & Bolzonella, D. (2013). Effect of trace element supplementation on the mesophilic anaerobic digestion of foodwaste in batch trials: The influence of inoculum origin. *Biochemical Engineering Journal*, *70*, 71–77. https://doi.org/10.1016/j.bej.2012.10.004

Fotidis, I. A., Karakashev, D., Kotsopoulos, T. A., Martzopoulos, G. G., & Angelidaki, I. (2013). Effect of ammonium and acetate on methanogenic pathway and methanogenic community composition. *FEMS Microbiology Ecology*, *83*(1), 38–48. https://doi.org/10.1111/j.1574-6941.2012.01456.x

Franke-whittle, I. H., Walter, A., Ebner, C., & Insam, H. (2014). Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities. *Waste Management*, *34*(11), 2080–2089. https://doi.org/10.1016/j.wasman.2014.07.020

Franke-Whittle, I. H., Walter, A., Ebner, C., & Insam, H. (2014). Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities. *Waste Management*, *34*(11), 2080–2089. https://doi.org/10.1016/j.wasman.2014.07.020

Freitas, A. F., Mendes, M. F., & Coelho, G. L. V. (2007). Thermodynamic study of fatty acids adsorption on different adsorbents, *39*, 1027–1037. https://doi.org/10.1016/j.jct.2006.12.016

Gagliano, M. C., Braguglia, C. M., Petruccioli, M., & Rossetti, S. (2015). Ecology and biotechnological potential of the thermophilic fermentative Coprothermobacter spp .,

(February), 1–12. https://doi.org/10.1093/femsec/fiv018

Gallert, C., & Winter, J. (1997). Mesophilic and thermophilic anaerobic digestion of source-sorted organic waste: effect of ammonia on glucose degradation and methane production. *Applied Microbiology and Biotechnology*, *48*, 405–410.

Gao, S., Zhao, M., Chen, Y., Yu, M., & Ruan, W. (2015). Tolerance response to in situ ammonia stress in a pilot-scale anaerobic digestion reactor for alleviating ammonia inhibition. *Bioresource Technology*, *198*, 372–379. https://doi.org/10.1016/j.biortech.2015.09.044

Garcia, J.-L., Macarie, H., Cayol, J.-L., Fardeau, M.-L., Thomas, P., Patel, B. K. C., … Ollivier, B. (2002). Sporanaerobacter acetigenes gen. nov., sp. nov., a novel acetogenic, facultatively sulfur-reducing bacterium. *International Journal of Systematic and Evolutionary Microbiology*, *52*(4), 1217–1223. https://doi.org/10.1099/00207713-52-4-1217

Guo, X., Wang, C., Sun, F., Zhu, W., & Wu, W. (2014). A comparison of microbial characteristics between the thermophilic and mesophilic anaerobic digesters exposed to elevated food waste loadings. *Bioresource Technology*, *152*, 420–428. https://doi.org/10.1016/j.biortech.2013.11.012

Hao, L., Lü, F., Mazéas, L., Desmond-Le Quéméner, E., Madigou, C., Guenne, A., … He, P. (2015). Stable isotope probing of acetate fed anaerobic batch incubations shows a partial resistance of acetoclastic methanogenesis catalyzed by Methanosarcina to sudden increase of ammonia level. *Water Research*, *69*(0), 90–99. https://doi.org/10.1016/j.watres.2014.11.010

Hattori, S., Kamagata, Y., Hanada, S., & Shoun, H. (2000). Thermacetogenium phaeum gen. nov., sp. nov., a strictly anaerobic, thermophilic, syntrophic acetate-oxidizing bacterium. *International Journal of Systematic and Evolutionary Microbiology*, *50*(4), 1601–1609. https://doi.org/10.1099/00207713-50-4-1601

Hidaka, T., Wang, F., Togari, T., Uchida, T., & Suzuki, Y. (2013a). Bioresource Technology Comparative performance of mesophilic and thermophilic anaerobic digestion for high-solid sewage sludge. *Bioresource Technology*, *149*, 177–183. https://doi.org/10.1016/j.biortech.2013.09.033

Hidaka, T., Wang, F., Togari, T., Uchida, T., & Suzuki, Y. (2013b). Comparative performance of mesophilic and thermophilic anaerobic digestion for high-solid sewage sludge. *Bioresource Technology*, *149*, 177–183. https://doi.org/10.1016/j.biortech.2013.09.033

Jang, H. M., Choi, Y. K., & Kan, E. (2018). Effects of dairy manure-derived biochar on psychrophilic, mesophilic and thermophilic anaerobic digestions of dairy manure. *Bioresource Technology*, *250*(November 2017), 927–931. https://doi.org/10.1016/j.biortech.2017.11.074

Jiang, Y. Bin, Zhong, W. H., Han, C., & Deng, H. (2016). Characterization of electricity generated by soil in microbial fuel cells and the isolation of soil source exoelectrogenic bacteria. *Frontiers in Microbiology*, *7*(NOV), 1–10. https://doi.org/10.3389/fmicb.2016.01776

Jiang, Jianguo, Zhang, Y., Li, K., Wang, Q., Gong, C., & Li, M. (2013a). Bioresource Technology Volatile fatty acids production from food waste : Effects of pH , temperature , and organic loading rate. *Bioresource Technology*, *143*, 525–530. https://doi.org/10.1016/j.biortech.2013.06.025

Jiang, Jianguo, Zhang, Y., Li, K., Wang, Q., Gong, C., & Li, M. (2013b). Volatile fatty acids production from food waste: Effects of pH, temperature, and organic loading rate. *Bioresource Technology*, *143*, 525–530. https://doi.org/10.1016/j.biortech.2013.06.025

Jiang, Junfeng, Li, L., Cui, M., Zhang, F., Liu, Y., Liu, Y., … Guo, Y. (2018). Anaerobic digestion of kitchen waste: The effects of source, concentration, and temperature. *Biochemical Engineering Journal*. https://doi.org/10.1016/j.bej.2018.04.004

Karthikeyan, O. P., & Visvanathan, C. (2012). Bioresource Technology Effect of C / N ratio and ammonia-N accumulation in a pilot-scale thermophilic dry anaerobic digester. *Bioresource Technology*, *113*, 294–302. https://doi.org/10.1016/j.biortech.2012.02.028

Karthikeyan, O. P., Visvanathan, C., Zeshan, Karthikeyan, O. P., & Visvanathan, C. (2012). Effect of C/N ratio and ammonia-N accumulation in a pilot-scale thermophilic dry anaerobic digester. *Bioresource Technology*, *113*, 294–302. https://doi.org/10.1016/j.biortech.2012.02.028

Kayhanian, M. (1994). Performance of a High-Solids Anaerobic Digestion Process Under Various Ammonia Concentrations, 349–352.

Kim, M., Liu, C., Noh, J. W., Yang, Y., Oh, S., Shimizu, K., … Zhang, Z. (2013). Hydrogen and methane production from untreated rice straw and raw sewage sludge under thermophilic anaerobic conditions. *International Journal of Hydrogen Energy*, *38*(21), 8648–8656. https://doi.org/10.1016/j.ijhydene.2013.04.079

Labatut, R. A., Angenent, L. T., & Scott, N. R. (2014). Conventional mesophilic vs. thermophilic anaerobic digestion: Atrade-off between performance and stability? *Water Research*, *53*, 249–258. https://doi.org/10.1016/j.watres.2014.01.035

Lauterböck, B., Ortner, M., Haider, R., & Fuchs, W. (2012). Counteracting ammonia inhibition in anaerobic digestion by removal with a hollow fiber membrane contactor. *Water Research*, *46*(15), 4861–4869. https://doi.org/10.1016/j.watres.2012.05.022

Li, Q., Xu, M., Wang, G., Chen, R., Qiao, W., & Wang, X. (2018). Biochar assisted thermophilic co-digestion of food waste and waste activated sludge under high feedstock to seed sludge ratio in batch experiment. *Bioresource Technology*, *249*(October 2017), 1009–1016. https://doi.org/10.1016/j.biortech.2017.11.002

Li, X., Guo, S., Peng, Y., He, Y., Wang, S., Li, L., & Zhao, M. (2018). Anaerobic digestion using ultrasound as pretreatment approach: Changes in waste activated sludge, anaerobic

digestion performances and digestive microbial populations. *Biochemical Engineering Journal*, *139*, 139–145. https://doi.org/10.1016/j.bej.2017.11.009

Li, Y.-F., Nelson, M. C., Chen, P.-H., Graf, J., Li, Y., & Yu, Z. (2015). Comparison of the microbial communities in solid-state anaerobic digestion (SS-AD) reactors operated at mesophilic and thermophilic temperatures. *Applied Microbiology and Biotechnology*, *99*(2), 969–980. https://doi.org/10.1007/s00253-014-6036-5

Lin, L., Xu, F., Ge, X., & Li, Y. (2018). Improving the sustainability of organic waste management practices in the food-energy-water nexus: A comparative review of anaerobic digestion and composting. *Renewable and Sustainable Energy Reviews*, *89*(February), 151–167. https://doi.org/10.1016/j.rser.2018.03.025

Lin, L., Yu, Z., & Li, Y. (2017). Sequential batch thermophilic solid-state anaerobic digestion of lignocellulosic biomass via recirculating digestate as inoculum – Part II: Microbial diversity and succession. *Bioresource Technology*, *241*, 1027–1035. https://doi.org/10.1016/j.biortech.2017.06.011

Lin, R., Cheng, J., Ding, L., & Murphy, J. D. (2018a). Improved efficiency of anaerobic digestion through direct interspecies electron transfer at mesophilic and thermophilic temperature ranges. *Chemical Engineering Journal*, *350*(May), 681–691. https://doi.org/10.1016/j.cej.2018.05.173

Lin, R., Cheng, J., Ding, L., & Murphy, J. D. (2018b). Improved efficiency of anaerobic digestion through direct interspecies electron transfer at mesophilic and thermophilic temperature ranges. *Chemical Engineering Journal*, *350*(March), 681–691. https://doi.org/10.1016/j.cej.2018.05.173

Lin, R., Cheng, J., Zhang, J., Zhou, J., Cen, K., & Murphy, J. D. (2017). Boosting biomethane yield and production rate with graphene: The potential of direct interspecies electron transfer in anaerobic digestion. *Bioresource Technology*, *239*, 345–352.

https://doi.org/10.1016/j.biortech.2017.05.017

Liu, F., Rotaru, A. E., Shrestha, P. M., Malvankar, N. S., Nevin, K. P., & Lovley, D. R. (2012). Promoting direct interspecies electron transfer with activated carbon. *Energy and Environmental Science*, *5*(10), 8982–8989. https://doi.org/10.1039/c2ee22459c

Lovley, D. R. (2008). The microbe electric: conversion of organic matter to electricity. *Current Opinion in Biotechnology*, *19*(6), 564–571. https://doi.org/10.1016/j.copbio.2008.10.005

Lovley, D. R. (2017). Syntrophy Goes Electric: Direct Interspecies Electron Transfer. *Annual Review of Microbiology*, *71*(1), annurev-micro-030117-020420. https://doi.org/10.1146/annurev-micro-030117-020420

Lü, F., Hao, L., Guan, D., Qi, Y., Shao, L., & He, P. (2013). Synergetic stress of acids and ammonium on the shift in the methanogenic pathways during thermophilic anaerobic digestion of organics. *Water Research*, *47*(7), 2297–2306. https://doi.org/10.1016/j.watres.2013.01.049

Mace, S. (2009). Ammonia influence in anaerobic digestion of OFMSW Ammonia influence in anaerobic digestion of OFMSW, (October 2014). https://doi.org/10.2166/wst.2009.100

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. Journal*, *17*(1), pp--10. https://doi.org/10.14806/ej.17.1.200

Min, H., Kim, M., Hyub, J., & Moon, J. (2015). Reactor performance and methanogenic archaea species in thermophilic anaerobic co-digestion of waste activated sludge mixed with food wastewater. *Chemical Engineering Journal*, *276*, 20–28. https://doi.org/10.1016/j.cej.2015.04.072

Morita, M., Malvankar, N. S., Franks, A. E., Summers, Z. M., Giloteaux, L., Rotaru, A. E., … Lovleya, D. R. (2011). Potential for direct interspecies electron transfer in methanogenic wastewater digester aggregates. *MBio*, *2*(4), 5–7. https://doi.org/10.1128/mBio.00159-11

Nakakubo, R., Nielsen, A. M., & Matsuda, J. (2008). Ammonia Inhibition of Methanogenesis

and Identification of Process Indicators during Anaerobic Digestion, *25*(10). https://doi.org/10.1089/ees.2007.0282

Nakashimada, Y., & Ohshima, Y. (2008). Ammonia – methane two-stage anaerobic digestion of dehydrated waste-activated sludge, 1061–1069. https://doi.org/10.1007/s00253-008-1501-7

Park, J. H. J. H., Park, J. H. J. H., Je Seong, H., Sul, W. J., Jin, K. H., & Park, H. D. (2018). Metagenomic insight into methanogenic reactors promoting direct interspecies electron transfer via granular activated carbon. *Bioresource Technology*, *259*(December 2017), 414–422. https://doi.org/10.1016/j.biortech.2018.03.050

Rajagopal, R., Massé, D. I., & Singh, G. (2013a). A critical review on inhibition of anaerobic digestion process by excess ammonia. *Bioresource Technology*, *143*, 632–641. https://doi.org/10.1016/j.biortech.2013.06.030

Rajagopal, R., Massé, D. I., & Singh, G. (2013b). Bioresource Technology A critical review on inhibition of anaerobic digestion process by excess ammonia. *Bioresource Technology*, *143*, 632–641. https://doi.org/10.1016/j.biortech.2013.06.030

Rideout, J. R., He, Y., Navas-Molina, J. a, Walters, W. A., Ursell, L. K., Gibbons, S. M., … Caporaso, J. G. (2014). Subsampled open-reference clustering creates consistent, comprehensive OTU definitions and scales to billions of sequences. *PeerJ*, *2*, e545. https://doi.org/10.7717/peerj.545

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ*, *4*, e2584. https://doi.org/10.7717/peerj.2584

Rotaru, A. E., Shrestha, P. M., Liu, F., Markovaite, B., Chen, S., Nevin, K. P., & Lovley, D. R. (2014). Direct interspecies electron transfer between Geobacter metallireducens and Methanosarcina barkeri. *Applied and Environmental Microbiology*, *80*(15), 4599–4605. https://doi.org/10.1128/AEM.00895-14

Sajeena Beevi, B., Madhu, G., & Sahoo, D. K. (2015). Performance and kinetic study of semi-dry thermophilic anaerobic digestion of organic fraction of municipal solid waste. *Waste Management*, *36*, 93–97. https://doi.org/10.1016/j.wasman.2014.09.024

Sasaki, D., Hori, T., Haruta, S., Ueno, Y., Ishii, M., & Igarashi, Y. (2011). Methanogenic pathway and community structure in a thermophilic anaerobic digestion process of organic solid waste. *Journal of Bioscience and Bioengineering*, *111*(1), 41–46. https://doi.org/10.1016/j.jbiosc.2010.08.011

Sasaki, K., Morita, M., Sasaki, D., Nagaoka, J., Matsumoto, N., Ohmura, N., & Shinozaki, H. (2011). Syntrophic degradation of proteinaceous materials by the thermophilic strains Coprothermobacter proteolyticus and Methanothermobacter thermautotrophicus. *Journal of Bioscience and Bioengineering*, *112*(5), 469–472. https://doi.org/10.1016/j.jbiosc.2011.07.003

Shen, Y., Linville, J. L., Ignacio-de Leon, P. A. A., Schoene, R. P., & Urgun-Demirtas, M. (2016). Towards a sustainable paradigm of waste-to-energy process: Enhanced anaerobic digestion of sludge with woody biochar. *Journal of Cleaner Production*, *135*, 1054–1064. https://doi.org/10.1016/j.jclepro.2016.06.144

Shi, J., Wang, Z., Stiverson, J. A., Yu, Z., & Li, Y. (2013). Reactor performance and microbial community dynamics during solid-state anaerobic digestion of corn stover at mesophilic and thermophilic conditions. *Bioresource Technology*, *136*, 574–581. https://doi.org/10.1016/j.biortech.2013.02.073

Shi, X., Lin, J., Zuo, J., Li, P., Li, X., & Guo, X. (2017). Effects of free ammonia on volatile fatty acid accumulation and process performance in the anaerobic digestion of two typical bio-wastes. *Journal of Environmental Sciences (China)*, *55*, 49–57. https://doi.org/10.1016/j.jes.2016.07.006

Shrestha, D., Embree, M., Zengler, K., & Wardman, C. (2014). Environmental Science A new

model for electron fl ow during anaerobic digestion : direct interspecies electron transfer to Methanosaeta for the reduction of carbon dioxide to methane †, 408–415. https://doi.org/10.1039/c3ee42189a

Siles, J. A., Brekelmans, J., Martín, M. A., Chica, A. F., & Martín, A. (2010). Impact of ammonia and sulphate concentration on thermophilic anaerobic digestion. *Bioresource Technology*, *101*(23), 9040–9048. https://doi.org/10.1016/j.biortech.2010.06.163

Sprott, G. D., Shaw, K. M., & Jarrell, K. F. (1984). Ammonia/potassium exchange in methanogenic bacteria. *Journal of Biological Chemistry*, *259*(20), 12602–12608. https://doi.org/10.1016/j.biombioe.2012.06.041

Stabnikova, O., Liu, X. Y., & Wang, J. Y. (2008). Anaerobic digestion of food waste in a hybrid anaerobic solid-liquid system with leachate recirculation in an acidogenic reactor. *Biochemical Engineering Journal*, *41*(2), 198–201. https://doi.org/10.1016/j.bej.2008.05.008

Stams, A. J. M., & Plugge, C. M. (2009). Electron transfer in syntrophic communities of anaerobic bacteria and archaea, *7*(auguST). https://doi.org/10.1038/nrmicro2166

Sun, Caiyu, Liu, F., Song, Z., Wang, J., Li, Y., Pan, Y., … Li, L. (2019). Bioresource Technology Feasibility of dry anaerobic digestion of beer lees for methane production and biochar enhanced performance at mesophilic and thermophilic temperature. *Bioresource Technology*, *276*(December 2018), 65–73. https://doi.org/10.1016/j.biortech.2018.12.105

Sun, Chen, Cao, W., Banks, C. J., Heaven, S., & Liu, R. (2016). Biogas production from undiluted chicken manure and maize silage: A study of ammonia inhibition in high solids anaerobic digestion. *Bioresource Technology*, *218*, 1215–1223. https://doi.org/10.1016/j.biortech.2016.07.082

Sung, S., & Liu, T. (2003). Ammonia inhibition on thermophilic anaerobic digestion. *Chemosphere*, *53*(1), 43–52. https://doi.org/10.1016/S0045-6535(03)00434-X

Tchobanoglous, G., Theisen, H., & Vigil, S. A. (1993). *Integrated solid waste management: engineering principles and management issues* (2nd ed.). McGraw-Hill.

Ullery, M. L., & Logan, B. E. (2015). Anode acclimation methods and their impact on microbial electrolysis cells treating fermentation effluent. *International Journal of Hydrogen Energy*, *40*(21), 6782–6791. https://doi.org/https://doi.org/10.1016/j.ijhydene.2015.03.101

Vavilin, V. A., Qu, X., Mazéas, L., Lemunier, M., Duquennoi, C., He, P., & Bouchez, T. (2008). Methanosarcina as the dominant aceticlastic methanogens during mesophilic anaerobic digestion of putrescible waste. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *94*(4), 593–605. https://doi.org/10.1007/s10482-008-9279-2

Wang, G., Li, Q., Gao, X., & Wang, X. C. (2018a). Sawdust-Derived Biochar Much Mitigates VFAs Accumulation and Improves Microbial Activities To Enhance Methane Production in Thermophilic Anaerobic Digestion. *ACS Sustainable Chemistry & Engineering*, *7*, 2141–2150. research-article. https://doi.org/10.1021/acssuschemeng.8b04789

Wang, G., Li, Q., Gao, X., & Wang, X. C. (2018b). Synergetic promotion of syntrophic methane production from anaerobic digestion of complex organic wastes by biochar: Performance and associated mechanisms. *Bioresource Technology*, *250*(December 2017), 812–820. https://doi.org/10.1016/j.biortech.2017.12.004

Wang, H., Fotidis, I. A., & Angelidaki, I. (2015). Ammonia effect on hydrogenotrophic methanogens and syntrophic acetate-oxidizing bacteria. *FEMS Microbiology Ecology*, *91*(11), 1–8. https://doi.org/10.1093/femsec/fiv130

Wang, H., Zhang, Y., & Angelidaki, I. (2016a). Ammonia inhibition on hydrogen enriched anaerobic digestion of manure under mesophilic and thermophilic conditions. *Water Research*, *105*, 314–319. https://doi.org/10.1016/j.watres.2016.09.006

Wang, H., Zhang, Y., & Angelidaki, I. (2016b). Ammonia inhibition on hydrogen enriched

anaerobic digestion of manure under mesophilic and thermophilic conditions. *Water Research*, *105*, 314–319. https://doi.org/10.1016/j.watres.2016.09.006

Wang, T., Zhang, D., Dai, L., Dong, B., & Dai, X. (2018). Magnetite Triggering Enhanced Direct Interspecies Electron Transfer: A Scavenger for the Blockage of Electron Transfer in Anaerobic Digestion of High-Solids Sewage Sludge. *Environmental Science and Technology*, *52*(12), 7160–7169. https://doi.org/10.1021/acs.est.8b00891

Wang, Y., Zhang, Y., Wang, J., & Meng, L. (2009). Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. *Biomass and Bioenergy*, *33*(5), 848–853. https://doi.org/10.1016/j.biombioe.2009.01.007

Wang, Z., Xu, F., & Li, Y. (2013). Effects of total ammonia nitrogen concentration on solid-state anaerobic digestion of corn stover. *Bioresource Technology*, *144*, 281–287. https://doi.org/10.1016/j.biortech.2013.06.106

Wijekoon, K. C., Visvanathan, C., & Abeynayaka, A. (2011). Effect of organic loading rate on VFA production, organic matter removal and microbial activity of a two-stage thermophilic anaerobic membrane bioreactor. *Bioresource Technology*, *102*(9), 5353–5360. https://doi.org/10.1016/j.biortech.2010.12.081

Xu, S., Han, R., Zhang, Y., He, C., & Liu, H. (2018). Differentiated stimulating effects of activated carbon on methanogenic degradation of acetate, propionate and butyrate. *Waste Management*, *76*, 394–403. https://doi.org/10.1016/j.wasman.2018.03.037

Yamada, C., Kato, S., Ueno, Y., Ishii, M., & Igarashi, Y. (2015). Conductive iron oxides accelerate thermophilic methanogenesis from acetate and propionate. *Journal of Bioscience and Bioengineering*, *119*(6), 678–682. https://doi.org/10.1016/j.jbiosc.2014.11.001

Yan, W., Shen, N., Xiao, Y., Chen, Y., Sun, F., Kumar Tyagi, V., & Zhou, Y. (2017). The role of conductive materials in the start-up period of thermophilic anaerobic system. *Bioresource*

Technology, *239*, 336–344. https://doi.org/10.1016/j.biortech.2017.05.046

Yan, W., Shen, N., Xiao, Y., Chen, Y., Sun, F., & Kumar, V. (2017). Bioresource Technology The role of conductive materials in the start-up period of thermophilic anaerobic system. *Bioresource Technology*, *239*, 336–344. https://doi.org/10.1016/j.biortech.2017.05.046

Yang, L., Xu, F., Ge, X., & Li, Y. (2015). Challenges and strategies for solid-state anaerobic digestion of lignocellulosic biomass. *Renewable and Sustainable Energy Reviews*, *44*, 824–834. https://doi.org/10.1016/j.rser.2015.01.002

Yang, Y., Zhang, Y., Li, Z., Zhao, Z., Quan, X., & Zhao, Z. (2017a). Adding granular activated carbon into anaerobic sludge digestion to promote methane production and sludge decomposition. *Journal of Cleaner Production*, *149*, 1101–1108. https://doi.org/10.1016/j.jclepro.2017.02.156

Yang, Y., Zhang, Y., Li, Z., Zhao, Z. Z., Quan, X., & Zhao, Z. Z. (2017b). Adding granular activated carbon into anaerobic sludge digestion to promote methane production and sludge decomposition. *Journal of Cleaner Production*, *149*, 1101–1108. https://doi.org/10.1016/j.jclepro.2017.02.156

Yenigün, O., & Demirel, B. (2013). Ammonia inhibition in anaerobic digestion: A review. *Process Biochemistry*, *48*(5–6), 901–911. https://doi.org/10.1016/j.procbio.2013.04.012

Yuan, H., & Zhu, N. (2016a). Progress in inhibition mechanisms and process control of intermediates and by-products in sewage sludge anaerobic digestion. *Renewable and Sustainable Energy Reviews*, *58*, 429–438. https://doi.org/10.1016/j.rser.2015.12.261

Yuan, H., & Zhu, N. (2016b). Progress in inhibition mechanisms and process control of intermediates and by-products in sewage sludge anaerobic digestion. *Renewable and Sustainable Energy Reviews*, *58*, 429–438. https://doi.org/10.1016/j.rser.2015.12.261

Zhang, Q., Hu, J., & Lee, D. J. (2016). Biogas from anaerobic digestion processes: Research updates. *Renewable Energy*, *98*, 108–119. https://doi.org/10.1016/j.renene.2016.02.029

Zhao, Z., Li, Y., Quan, X., & Zhang, Y. (2017). New Application of Ethanol-Type Fermentation: Stimulating Methanogenic Communities with Ethanol to Perform Direct Interspecies Electron Transfer. https://doi.org/10.1021/acssuschemeng.7b02581

Zhao, Z., Zhang, Y., Li, Y., Dang, Y., Zhu, T., & Quan, X. (2017). Potentially shifting from interspecies hydrogen transfer to direct interspecies electron transfer for syntrophic metabolism to resist acidic impact with conductive carbon cloth. *Chemical Engineering Journal*, *313*, 10–18. https://doi.org/10.1016/j.cej.2016.11.149

Zhao, Z., Zhang, Y., Yu, Q., Dang, Y., Li, Y., & Quan, X. (2016). Communities stimulated with ethanol to perform direct interspecies electron transfer for syntrophic metabolism of propionate and butyrate. *Water Research*, *102*, 475–484. https://doi.org/10.1016/j.watres.2016.07.005

Appendix

Fig. S.1. Overhead view of BMP setup (bioreactors connected to CO_2 adsorption bottle with gas-bags).

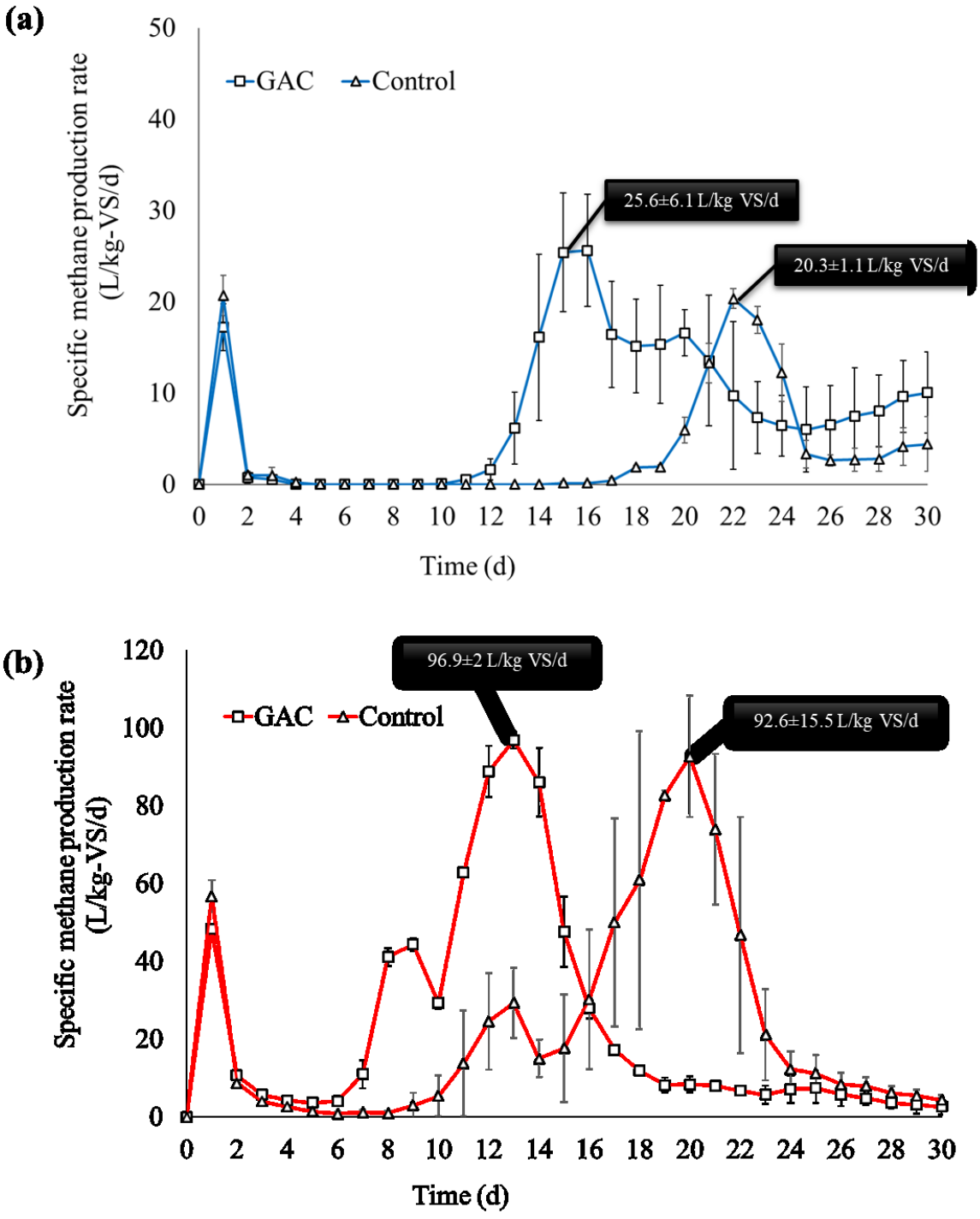

Fig. S.2. Specific methane production rate under (a) mesophilic, and (b) thermophilic conditions.