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Strategies to enhance anaerobic digestion of biomass waste to produce renewable biofuel

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

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ABSTRACT

Anaerobic digestion is a promising biochemical technology to produce renewable biofuel in the form of biogas, as well as reducing the solid organic wastes. This study explored strategies to enhance the biofuel production through anaerobic digestion of different biomass wastes. Multiple methods including feedstock pretreatment, hybrid process with thermochemical pathway, operation condition optimization, co-digestion, adding additive and metabolic engineering of the microorganism were used to enhance the anaerobic digestion process.

This dissertation comprises four distinct topics organized by chapters based on journal manuscripts: 1) The hybrid process of anaerobic digestion and fast pyrolysis was studied in which the aqueous phase in bio-oil was used as feedstock for anaerobic digestion. Through pretreatment, operation optimization and directed engineering of the anaerobic microorganism, the digestibility of the aqueous phase was significantly improved with higher methane yield; 2) Hybrid process of anaerobic digestion and fast pyrolysis was fulfilled by using biochar as additive in anaerobic digestion. The physiochemical properties of two types of biochar were compared and their function in anaerobic digestion was evaluated. The main properties contributed to the enhanced biomethane yield in the biochar were identified; 3) The proper type of biochar was selected as an effective additive in enhancing anaerobic co-digestion of municipal sludge and food waste. Biochar with high alkalinity relieved the acidification of the digestion process and also had impacts on the microbial structures; 4) Extraction of hydroxycinnamic acids was developed as a pretreatment in anaerobic digestion of corn stover, not only improving the efficiency of the digestion process but also the economics of the whole biorefinery process by extracting high value chemicals. In summary, the results demonstrated in this work provide a

better understanding of the anaerobic digestion and provides many strategies to further develop a more effective and efficient process to produce low-cost, clean and sustainable biofuel.

CHAPTER 1. GENERAL INTRODUCTION

The purpose of this general introduction is to provide background information and summarize relevant literature in the area of anaerobic digestion. Topics discussed and covered include biochemical background of anaerobic digestion, factors affecting anaerobic digestion, enhancement of anaerobic digestion and the hybrid process of pyrolysis and anaerobic digestion. Studies that pertain to these concepts are summarized and major findings and results are discussed. The final section within this chapter provides the research objective and the organization of this dissertation.

1.1 Introduction

Currently, about 88% of the energy demand is from fossil fuels, and the demand is increasing until 2050 up to 50% (Bharathiraja et al., 2018). Meanwhile, the greenhouse gases (GHG) emission is expected to reach new record high to approximately 37Gt in 2035, with carbon dioxide (CO_2) as the main contributor (Ho et al., 2014). The increasing demand of conventional fuels and the effect of GHG emission have led the research efforts into the renewable biofuels. Biofuel is derived from biological raw materials (Biomass) with the final products in the form of solid, liquid and gaseous.

Producing bioenergy from biomass can be achieved through biochemical, thermochemical or hybrid processes. Bioethanol as a major biofuel is often produced from biochemical pathways. It commonly consists of three steps: pretreatment of biomass, enzymatic hydrolysis of treated biomass to reducing sugar and finally fermentation of sugars into bioethanol (Adekunle et al., 2016). However, this process is hindered by several technical and economic barriers, such as recalcitrant structure of lignocellulosic biomass, high cost of

enzymes, and co-fermentation of glucose and xylose (Cheng and Timilsina, 2011). Anaerobic digestion (AD) is another biochemical process to produce biofuel in the form of biogas, containing mainly methane (CH_4) and CO_2. Methane can be transformed into electric energy, heat energy and transportation fuel.

Compared to biochemical technologies, thermochemical conversion can decompose biomass into final products in a much shorter period of time and with less expensive catalysts. There are four common thermochemical conversion technologies: direct combustion, gasification, pyrolysis and solvolysis. Among these technologies, pyrolysis has drawn increased attention and support around the United States. This process can convert biomass into an energy rich liquid-bio-oil, a flammable gas-syngas and a carbon rich solid-biochar (Brown and Brown, 2013).

Hybrid processes refer to those combine biochemical and thermochemical pathways to produce biofuel. For example, fast pyrolysis of biomass can produce sugars-rich bio-oil, which can be further used by fermentation technology to produce bioethanol (Jarboe et al., 2011). However, this hybrid process faced two main challenges: the bio-oil derived sugars were present in the anhydrous form which cannot be directly utilized by most of the microorganisms, and bio-oil is rich in microbial inhibitors.

1.2 Anaerobic digestion

AD is a process in which microorganisms decompose organic materials to produce biogas in the absence of oxygen. Based on the total solid (TS) content, AD can be defined as liquid state AD with TS less than 15%, or solid state AD with TS greater than 15% (Rapport et al., 2008). An AD process typically consists of four stages, hydrolysis, acidogenesis, acetogenesis and

methanogenesis. In the hydrolysis stage, macromolecules such as cellulose, starch, proteins, and lipids are decomposed into monomers such as sugars, amino acids and fatty acids. Those monomers are then converted into C2-C5 based volatile fatty acids (VFAs), alcohols, as well as H_2 and CO_2 in the second acidogenesis stage. In the third acetogenesis stage, VFAs and alcohols are converted into acetate. In the last methanogenesis stage, CH_4 is produced through conversion of acetate to CH_4 and CO_2 (aceticlastic methanogenesis) or reduction of formate or CO_2 to CH_4 (hydrogenotrophic methanogenesis). Hydrolysis in an AD process is commonly the rate-limiting step particularly when feedstock is complex organic substrate. However, when easily digestible organic matters are used as feedstock, methanogenesis becomes the limiting step (Tomei et al., 2009). The biogas produced from an AD process usually contains 60-70% CH_4, 20-30% CO_2 and trace amount ammonia, hydrogen sulfide and hydrogen. The biogas can be combusted to generate heat and/or electricity or upgraded and refined into transportation fuels. Meanwhile, the nutrients (such as nitrogen and phosphorus) rich digestate can be recycled as fertilizers or processed into biochar that can be used as soil amendment (Inyang et al., 2010).

1.2.1 Feedstocks

Historically, AD has mainly been associated with the treatment of animal manure and sewage sludge from aerobic wastewater treatment plants (Steffen et al., 1998). The increasing trends of the feasibility and applicability of biogas production indicate the potential for using various kinds of feedstocks in AD. Generally, the feedstocks can be assigned to the three different sources: agriculture, industry and communities.

Agricultural feedstocks include animal manure (cattle, pig, poultry), energy crops, algal biomass and harvest remains (e.g. corn stover, wheat straw and rice straw). Corn stover, with a 1:1 weight ratio of residue to grain (Langholtz et al., 2016), is the most abundant agricultural

residue in the U.S., with an approximate 80 million dry tons of corn stover are produced each year (Kadam and McMillan, 2003). It has been reported that liquid state AD treatment of corn stover (5% TS) produced a lower CH_4 yield than solid state AD (18% TS) (Brown et al., 2012). Pig slurry is often in low TS (2-5%) due to the pigs are kept in feedlots and the excrements are collected through slots with high amount of liquid (Steffen et al., 1998). Chicken manure usually has higher TS (~20%) and high protein content, which can increase the buffering capacity of the AD process (Sun et al., 2016).

Industrial feedstocks contain more diverse types: food processing, diary, starch industry, pulp and paper, cosmetic industry (Steffen et al., 1998). AD is commonly used for pretreatment of pulp and paper industry for the combined benefit of chemical oxidation demand (COD) removal and energy recovery in Europe (Mussoline et al., 2013). Dairy wastewaters are rich in fats, protein and carbohydrates, while the characteristics depending on the type of systems and the methods of operation. Ammonia produced from the degradation of milk protein was close to the inhibition level of the AD process (Chen et al., 2008). Meat processing wastes are one of the difficult feedstocks in food industry. They contain grease, blood, faeces and recalcitrant organic matter such as straw and hair. Degradation of protein and lipids leads to accumulation of ammonia and long chain fatty acids, which are inhibitors of the anerobic microorganisms (Chen et al., 2008). Thus, co-digestion of meat processing wastes with other feedstocks are recommended to dilute the inhibitory compounds and provide improved C/N ratio.

Communities sourced feedstocks include municipal solid wastes (MSW), sewage sludge, garden waste, food remains. It is estimated the annual production of MSW will reach 2.2 billion tons by 2025 (Hoornweg and Bhada, 2012). AD of the organic fraction of MSW (OFMSW) is driven by the need to reduce waste, generate renewable biogas and also optimize the horticultural

compost-substitute product to improve the economic output of the process. It is estimated 1.3 billion tons per year food waste is produced worldwide (Food and Agriculture Organization and Nations, 2012). In the U.S., food waste accounts for 12% of total municipal solid waste (Zhang et al., 2014). Food waste composition varies widely depending on geographical locations and eating habits of local populations. In general, food wastes containing soluble organic matters can be easily converted in VFAs, which may inhibit the subsequent CH_4 formation if VFAs are over produced. A two-phase AD can successfully overcome this problem (Cho et al., 1995). Among 31 million dry tons per year of yard wastes generated in the U.S., more than 60% were treated through composting, during which energy was wasted as respiration heat (Ge et al., 2016).

1.2.2 Factors affecting anaerobic digestion

Anaerobic bacteria need balanced nutrients such as carbon, nitrogen, phosphorous, and minerals for their growth. Carbon, the primary energy source for cell growth, is usually rich in solid waste. Nitrogen and phosphorous are also essential for anaerobic bacteria for the synthesis of proteins and nucleic acids, respectively. Ammonium is the nitrogen form methanogens can utilize (Takashima et al., 1990) but will inhibit the bacteria growth at high levels. The C/N ratio of the feedstock also plays an important role in digestion process. C/N ratio ranging from 20:1 to 30:1 is recommended with an optimal C/N ratio of 25:1 (Liu et al., 2018). Too low C/N ratios increase the risk of ammonia inhibition, resulting in insufficient utilization of carbon sources; while an excessively high C/N ratio results in an insufficient nitrogen to maintain bacteria growth and biogas production. The demand of phosphorus is usually 15% of that of nitrogen (Speece and McCarty, 1964).

The pH of an AD system also affects the digestion performance. The ideal pH for an AD process is within a narrow range of 6.8-7.2 (Ward et al., 2008). However, different groups of

bacteria in AD have different optimal pH requirements. For example, the optimal pH for acidogens is between 5.5 to 6.5, while methanogens are most active at pH 6.5 to 8.2 with an optimum at pH at 7.0 (Mao et al., 2015). Due to this discrepancy of pH requirement, two-stage AD processes, i.e., separating acidogenesis and methanogenesis into two reactors, are usually used (Ward et al., 2008).

During AD process, pH is affected by many parameters. In AD of OFMSW with liquid digestate recirculation, the pH was low (<6.5) initially due to high VFAs concentration, and then gradually increased to around 8 after VFAs decreased from 12,000 mg/L to 1000 mg/L within one week (Di Maria et al., 2017). The buffer capacity of resisting pH fluctuation in an AD system is often evaluated through alkalinity. For example, in a corn stover AD system with a less alkalinity (1036.4 mg $CaCO_3$/kg), pH dropped from 9 to below 6 rapidly with a declined CH_4 yield (Shi et al., 2014). When the alkalinity of the system was increased (>1700 mg $CaCO_3$/kg) through adjusting feedstock/inoculum (F/I) ratio, pH of the same system was stabilized with only slight decreasing from 9 to 8.4 (Shi et al., 2014). In order to maintain a stable pH during AD process, it is essential to balance VFAs concentration and bicarbonate. In general, reducing organic loading, adding bases or bicarbonates and modifying F/I ratio are used to increase alkalinity in AD systems (Ward et al., 2008).

AD is commonly operated at thermophilic (around 55°C) or mesophilic (around 37°C) conditions. Compared to the mesophilic AD, thermophilic AD has a shorter startup time and hydraulic retention time (HRT) due to accelerated hydrolysis of the feedstock. The methane yield in thermophilic AD is also higher as methanogenic bacteria have an optimal growth at 55°C (Croce et al., 2016). Thermophilic AD can also produce pathogen-free effluent. Pohl et al. (Pohl et al., 2012) compared the performance of wheat straw AD under 37°C and 55°C. The CH_4 yield

from the thermophilic AD was 36.3% higher than that in mesophilic AD, mainly due to a faster disintegration and hydrolysis of the feedstock. Compared to mesophilic AD, however, poor stability and reliability often represent obstacles in the commercialization of thermophilic AD. In general, bacteria in thermophilic conditions are more sensitive to environment changes, exhibiting poor stability and less diversity and richness in bacterial community (Mao et al., 2015). Also, the fast hydrolysis of feedstock in thermophilic processes often result in a rapid VFAs production, causing an imbalance between acidogenesis and methanogenesis. The higher temperature also shifts NH_3/NH_4^+ equilibrium towards the cytotoxic ammonia (Croce et al., 2016). Higher heating energy is also a concern in theomorphic AD (Sheets et al., 2015). Due to those reasons, theomorphic digesters are still not commonly used in commercial AD.

A variety of compounds have been reported inhibitory to AD, causing an adverse shift in microbial population, an instability of the process and decreased CH_4 yield (Chen et al., 2008). The easily digestible feedstock often leads to a rapid hydrolysis and acidification, producing excessive VFAs which inhibit methanogens. For example, in AD of tomato residues, VFAs concertation (12.48 g/L) was much higher than the threshold level (6 g/L), and caused CH_4 production inhibition (Li et al., 2016). Compounds derived from phenolic degradation (such as p-cresol) inhibit acetogenesis, resulting in accumulation of VFAs (Panjičko et al., 2017). The partial pressures of CO_2 and H_2 in AD system also affect the CH_4 production. Increasing CO_2 partial pressure results in an increased dissolved CO_2, which causes acidification in the digestate and inhibition of methanogenesis. An elevated H_2 partial pressure leads to an accumulation of dissolved H_2 which inhibits the degradation of VFAs (Abbassi-Guendouz et al., 2012). In AD of wheat straw, high H_2 partial pressure also led to a strong inhibition on the initial hydrolysis step (Cazier et al., 2015). Since both CO_2 and H_2 are needed to produce acetate/ CH_4, a balanced

CO_2/H_2 pressure in the headspace is essential to prevent inhibition. Ammonia is produced from degradation of nitrogenous compounds (e.g. protein and urea) during AD. A moderate amount of ammonia is essential for bacterial growth and neutralizing VFAs to maintain a stable pH; however, excessive ammonia can inhibit methanogenesis. Ammonia exists as an equilibrium between ammonium ion (NH_4^+) and free ammonia (NH_3) (Chen et al., 2008). Free ammonia can permeate cell membrane and cause proton imbalance and thus is inhibitory to bacterial cells. Animal manures usually contain excessive ammonia, resulting in process inhibition. In AD of chicken manure, for example, the digester was completely inhibited when influent total Kjeldahl nitrogen (TKN) concentration was 8.2 g/L (Bayrakdar et al., 2017). After ammonia was removed from influent, the digester achieved a much higher CH_4 yield.

A certain degree of mixing in AD is necessary to enhance the transfer of organic substrates to bacteria, prevent the sedimentation of denser particles or floating lighter materials, and facilitate the release of gas bubbles trapped in the solid feedstock. In rice straw solid state AD, intermittent mixing with a 5/25 min on/off cycle at 160 rpm resulted in a good mass transfer while saving energy compared to continuous mixing (Zhou et al., 2017). Premixing of feedstock with inoculum is also needed before loading into AD reactor (Li et al., 2018; Zhu et al., 2015). The methods of mixing in AD vary greatly such as liquid (leachate) recirculation, solid mixing using augers, and biogas-circulation (Rapport et al., 2008), among which the leachate recirculation is commonly used. Leachate recirculation as an alternative to mechanical mixing facilitates the nutrients diffusion from substrates to bacterial cells (Fagbohungbe et al., 2015). This practice also reduces the amount of inoculum in AD as the bacteria-containing leachate collected from the reactor can be reapplied to the digestion systems (André et al., 2018).

In addition to mixing, leachate recirculation also promotes AD processes in other aspects. For example, when leachate recirculation was used in the acidogenic reactor of a two-stage hybrid solid-liquid AD system, the extraction of organic matters from the feedstock was facilitated and the pH was buffered (Stabnikova et al., 2008). In the solid-state co-digestion of hay and soybean processing wastes, leachate recirculation accelerated the daily CH_4 yield to peak value through enhancement of VFAs mass transfer from acidogenic to methanogenic pockets (Zhu et al., 2014). However, it should be noted that leachate recirculation may lead to accumulation of high concentration of VFAs and inhibitors compounds, therefore, dilution with freshwater o leachate may be needed (Yang et al., 2015). Also, leachate recirculation rate needs to be carefully controlled to avoid irreversible acidification of the system (Veeken and Hamelers, 2000).

1.2.3 Process operations

Batch and continuous operations are two operation modes commonly used in AD. Compared to continuous operation (Table 1.1), batch operation is easier to maintain to it needs less capital and operating costs with less process control requirements. However, the biogas production in batch AD is variable with the time, and the majority of biogas are produced only at peak production time. It has been reported that in a 55-day batch AD of corn stover, more than 80% of biogas was produced only at 36-day period of methanogenic phase (Liu et al., 2018). Another limitation in batch AD is the requirement of a large amount of inoculum (i.e., low F/I ratio). For example, Capson-Tojo et al. (2017) reported that a batch AD of food waste and cardboard mixture can only produce biogas at a F/I lower than 0.25; the biogas production was completely ceased when F/I ratio was above this ratio due to the overproduction of VFAs. Similar results were obtained for a batch operation of yard trimmings AD process, in which the

highest CH_4 yield (244 L/kg VS) was obtained at the lowest end of the F/I ratio ranging from 0.2 to 2 (Xu et al., 2016). The inoculum sources also significantly affect the batch AD process. Guendouz et al. (2010) studied three successive batches AD of MSW and found the second and third batches inoculated with the residue from the previous batch shortened the lag phase and accelerated reaction, which was due to the adaptation of the bacteria to the digestion system.

Table 1.1. Comparison of batch systems and continuous AD systems

Parameters	Batch systems	Continuous systems
Investment	Low	High
Technical operation	Simple	Complex
Land acreage required	Large	Small
OLR	Low	High
Inoculum	High	Low
Biogas yield	Uneven; low	Even, high
Water consumption	Low	High

Contrary to the batch operation, continuous AD can consistently produce CH_4 at steady state. Organic loading rate (OLR), CH_4 production, and hydraulic retention time (HRT) are three main parameters determining the interaction between microorganisms and substrates, and thus are used in designing and evaluating a continuous AD performance (Fagbohungbe et al., 2015). OLR represents the conversion capacity of an AD system; a maximum OLR level in AD process depends on various parameters such as reactor design, feedstock characteristics, microbial activity, temperature, pH, and toxicity level (Amani et al., 2010). A relatively high OLR is always preferred as it means an improved utilization efficiency and reduced digester size. However, high OLR can lead to overproduction of VFAs and cause an imbalance between acidogens and methanogens. For example, in a batch AD process of rice straw, increasing TS loading from 20% to 24% prolonged lag phase from 15 days to 20 days (Zhou et al., 2017). Similarly, increasing OLR from 2.3 to 9.2 kg VS/m^3 day in semi-continuous AD of food waste

slowed down bacteria's acclimatization to the new environment, resulting in a prolonged adaptation time from 2 days to 31 days (Nguyen et al., 2016). In a co-digestion of chicken manure and poplar leaf, CH_4 yield decreased when OLR increased from 4.0 to 8.0 g VS L^{-1} day^{-1} (Li et al., 2017). In another study, digestion of chicken manure was completely inhibited at an OLR of 3.85 g VS L^{-1} day^{-1} (Bayrakdar et al., 2017).

AD can be operated in a single stage reactor or multiple-stages reactors. In a single-stage system, the multiple steps of conversion of organic substrates into biogas is implemented in one reactor vessel. While in multiple-stage operation, different conversion stages are respectively implemented into different reactor vessels. A two-stage AD is commonly used as multiple-stage operation during which the hydrolysis/acidogenesis is in the first reactor, and the acidogenesis/ methanogenesis is in the second reactor (Fox and Pohland, 1994). Compared to two-stage operation, single-stage reactor is easier to design and build, with less operate cost. However, the OLR in a single-stage digester is often limited in order to avoid VFAs overproduction and rapid pH drop (Rapport et al., 2008). Unlike the single-stage digester, two-stages AD systems can accommodate each stage of AD process (such as acidogenesis and methanogenesis) at their own optimal conditions (pH, temperature, OLR, HRT). Two-stage operation generally have a better performance than the single-stage reactor. For instance, AD of brewery spent grain (BSG) in the single-stage reactor was limited by the inhibitors (such as weak acids, furan derivatives and phenolic substances) generated in the degradation of lignocellulose in BSG. While a two-stage AD system separating hydrolysis and acidogenesis and mostly methanogenesis in a granular biomass reactor was applied, both biogas production and feedstock biodegradation were improved, since granulated biomass has the ability to degrade phenolic compounds (Panjičko et al., 2015).

In some occasions, AD systems with more than two stages (such as three stages) are designed to create different favorable conditions for hydrolyzing bacteria, acidogenic bacteria and methanogens, with each group of bacteria performing a particular role. A three-stage system was used in the co-digestion of food waste and horse manure system, in which the first stage-hydrolysis and second stage-acidogenesis were operated as solid state, while the methanogenesis was operated as a liquid state. This hybrid system increased CH_4 yield by 11.2-22.7% and the abundance of methanogenic archaea by 0.8-1.28 times compared to the single-stage reactor. It should be noted that despite multi-stage AD systems are advantageous in improving AD performance, high capital and operating costs are the main hurdles for industry. As a result, single stage AD is still dominantly used. In Europe, for example, about 90 percent of the installed AD capacity is still from single-stage systems and only about 10 percent is from multi-stage systems (Rapport et al., 2008).

1.3 Fast pyrolysis

Pyrolysis is a thermochemical process conducted at higher than 400 °C in the absence of oxygen. Almost any form of organic material can be introduced into a pyrolysis reactor, including corn and wheat stover, forestry byproducts, urban yard wastes, industrial byproducts, animal manures, and sewage sludge. Low-density biomass (~1.5 GJ/m^3) is converted into a high-energy-density liquid known as bio-oil (~22 GJ/m^3), a high-energy-density solid known as biochar (~18 MJ/kg), and a relatively low-energy-density gas known as syngas (~6 MJ/kg) (Bridgwater et al., 1999). Pyrolysis can be classified into four types: fast pyrolysis, slow pyrolysis, catalytic pyrolysis and autothermal pyrolysis (Laird et al., 2009). Fast pyrolysis is a

rapid process requiring vapor and solid residence times in the order of 2 seconds (Papari and Hawboldt, 2015).

1.3.1 Bio-oil

Bio-oil is the main product in fast pyrolysis, with maximum yields of up to 75%. It arises from depolymerization and fragmentation of cellulose, hemicellulose and lignin in biomass. Bio-oil contains hundreds of compounds with a wide range of molecular weights, including volatile and non-volatile compounds and viscous oligomers (Pollard et al., 2012). Thus, bio-oil exhibits an extremely wide range of boiling points. Researchers at Iowa State University have developed a novel five-stage recovery system with the intention of separately collecting bio-oil with distinct characteristics (Fig.1.1).

The first stage fraction (SF1) contains mainly levoglucosan and other compounds with high dew points. The second stage fraction (SF2) was designed to collect aerosols produced in the pyrolyzer or stage 1. Since both SF1 and SF2 collect high boiling point compounds, they are known as “heavy ends” (Polin et al., 2019). The third stage fraction (SF3) collected compounds with dew points close to phenol. The fourth stage fraction (SF4) was designed to collect bio-oil aerosols at 15 °C. The last stage fraction (SF5) removes water and light oxygenated compounds. SF3, SF4 and SF5 are called bio-oil light ends, consisting of water and other low molecular-weight oxygenated compounds (e.g. furans, phenolic monomers, aldehydes, ketones, etc.) (Polin et al., 2019). SFs can be upgraded individually or combined into desirable products.

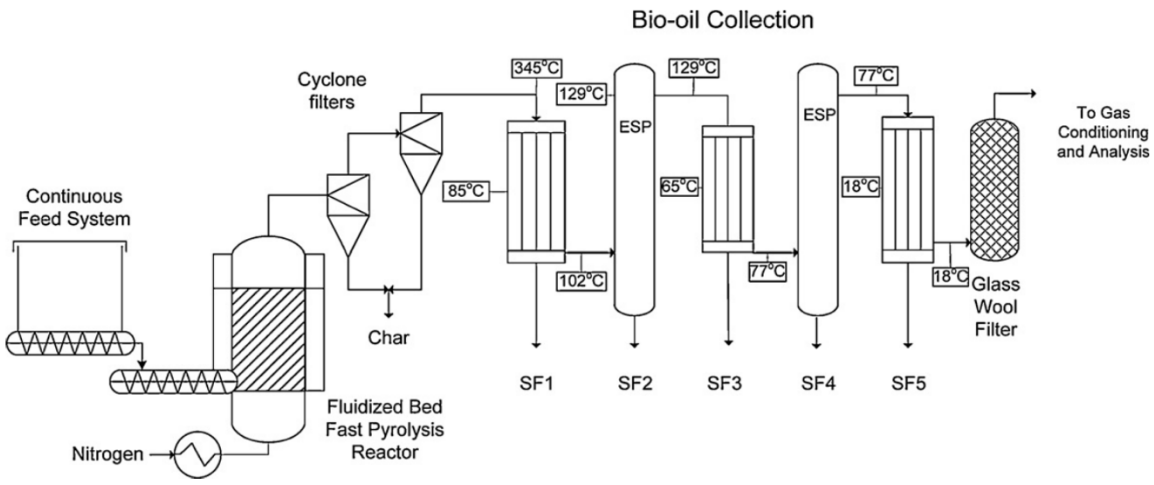

Figure 1.1. Schematic of the pyrolysis process development unit with fractionation of bio-oil recovery system. Source: (Pollard et al., 2012).

1.3.2 Biochar

Biochar is defined as a carbon-rich, fine-grained, porous substance produced by thermal decomposition of biomass under oxygen-limited conditions (Manyà, 2012). It can be produced by several thermochemical processes: slow pyrolysis, fast pyrolysis, flash carbonization and gasification. In the fast pyrolysis, as bio-oil is the main product, biochar accounts for 10-30% by mass in the product yields.

Researches have shown that biochar has advantages in terms of mitigating global warming by sequestering carbon (Laird, 2008) and as an effective in managing soil health and improve productivity (Fowles, 2007). The main property making biochar as an attractive soil amendment is the porous structure, which is responsible for improved water retention and increased soil surface area (Manyà, 2012). Another important factor is that biochar introduces a lot of nutrients into the soil, either increasing the nutrient use efficiency or through physicochemical processes that allow better utilization of soil-inherent or fertilizer-derived nutrients (Manyà, 2012). Moreover, biochar has also been used in wastewater treatment to

absorb contaminants from the antibiotics residues, pesticides, trace metals (Ahmad et al., 2014). The biochar surface is heterogeneous due to the present of carbonised and non-carbonised fractions, in which different sorption mechanisms including chemisorption, physisorption and ion-exchange occur (Masebinu et al., 2019).

More recently, biochar has been applied in anaerobic digestion as additive. Biochar was reported to increase the biomethane production, through acting as support for bacteria colonies, conductor for DIET, and sorbent for indirect inhibitors. A lot of biochar characteristics play an important role in the positive function including specific surface area, porosity, cation exchange capacity, electrical conductivity, alkalinity, aromaticity, Zeta potential (Codignole Luz et al., 2018). However, the mechanism of the facilitating function of biochar in AD is still not fully understood.

1.3.3 Hybrid of pyrolysis and biochemical process

In order to improve the economics of the biorefinery to produce biofuel, pyrolysis is always associated with other biochemical process. Levoglucosan as the main sugar in bio-oil can be converted into glucose via acid hydrolysis or catalysis (Jarboe et al., 2011). After detoxificating of bio-oil by extraction, activated carbon, air stripping and microbial methods, the sugar in bio-oil was fermented with *S. pastorianus* ATCC 2345 to produce ethanol (Wang et al., 2012). The cost of ethanol using an inexpensive fermentation medium in a large-scale plant is around $14/gallon in this study. Besides, the high acetic acid content in bio-oil (especially SF5) can be the carbon source for microalga *Chlamydomonas reinhardtii* fermentation (Liang et al., 2013), although the detoxification step of removing inhibitory compounds such as phenolics, furfural and acetol was essential.

Pyrolysis can be coupled with anaerobic digestion by different ways. The first method is to valorize of the digestate from AD through pyrolysis. Digestate was commonly for land application after AD process, which was with very low value. However, pyrolysis of digestate can produce biochar, aqueous phase, and syngas. It was reported the biochar produced from pyrolysis of digestate was with better performance in the soil than the direct use of digestate (Pecchi and Baratieri, 2019). The main reason was that the phosphorous and potassium content was higher than that in the digestate. Another possible method is to convert the pyrolysis derived aqueous phase into methane through AD. In AD of aqueous phase from the pyrolysis of corn stover, a serious of batch and semi-continuous tests were investigated (Torri and Fabbri, 2014). The AD process was inhibited initially due to the toxicity of the aqueous phase; however, a methane yields up to 65% of the theoretical one was achieved when biochar was added in the digester. Detoxification of the aqueous phase and acclimatization of the bacterial community are the key factors for a further improvement of the AD process. The last method is to use biochar in AD, which can reduce the instability and enhance the biogas production. Shen et al. (2015) found that biochar with high surface area, ash content and concentration of potassium, calcium and magnesium can be used for in-situ biogas upgrading, reaching a biogas with more than 90% methane content. Moreover, the biochar amended digestate was enriched with nutrients, making it more attractive as soil amendment.

1.4 Enhancement of anaerobic digestion

1.4.1 Pretreatment

Among the four main steps, hydrolysis is always the rate-limiting step for most complex feedstocks, due to the formation of inhibitory and excessive VFAs (Ariunbaatar et al., 2014); whereas methanogenesis is the rate-limiting step for easily biodegradable feedstocks. Numerous

researches have been conducted on pretreatment methods to accelerate the hydrolysis step so that the overall biogas yield can be enhanced, or to obtain valuable by-products from this step. Typically, there are three main pretreatment types: physical, chemical and biological methods.

Physical treatment such as milling and grinding reduces the particle size of the feedstock, and thus, provides a greater access for microorganisms. Tian et al. (2017) reported a 29% increase in CH_4 yield in AD of rape straw when the feedstock size was reduced from 2-2.5 cm to 0.5 mm. However, it should be noted that too fine particle size may negatively affect the AD performance. For example, Motte et al.(2015) compared the AD of straw at three particle sizes (0.25 mm, 1 mm and 10 mm) and found that the coarse particles (10 mm) resulted in the highest CH_4 yield followed with the medium size particles (1 mm) and the finest size (0.25 mm). The reason for this phenomenon was due to rapid acidification of the substrate at smaller sizes of particles, which resulted in an overproduction of VFAs and rapid pH drop.

Thermal treatment is a common and effective treatment method for industrial AD (Jain et al., 2015a). In addition to enhancing the reaction rate, thermal treatment can also remove pathogens, improve dewaterability and decrease viscosity of the digestion system. In the AD of steam autoclaved MSW, the digestate passed all the criteria for biosolids land application in the U.S. (Holtman et al., 2017). In the thermal treatment, an appropriate combination of temperature and time is needed as the high energy consumption and requirement of pressurized vessel often offset the overall benefits. Liao et al. (2016) studied the effect of treatment temperature (60, 70 and 80 °C) on the AD of sewage sludge, and found that 70 °C at for 30 min was optimal for AD. Under this condition, biogas yield increased by 11% and HRT reduced from 22 to 15 days. Other physically based treatment was also reported. For example, ultrasound treatment generates both mechanical effects through cavitation and chemical effects through formation of free radicals.

OFMSW treated with low-frequency ultrasound released more soluble organic matters, resulting in a 16% increase in biogas production in AD (Cesaro and Belgiorno, 2013). Microwave treatment is related to structure modification as well as thermal effects, contributing to increased sludge solubility (Ahn et al., 2009), shortened initial lag-phase (Beszédes et al., 2011) and improved CH_4 yield (Jackowiak et al., 2011).

Chemicals such as acids, alkaline or oxidants can facilitate the breaking down of recalcitrant structures of feedstock. The effectiveness of chemical treatment relies on the feedstock characteristics and the reagents used. Feedstock with easily digestible carbohydrates such as starch is not suited for chemical treatment because it will accelerate starch degradation leading to overproduction and accumulation of VFAs (Ariunbaatar et al., 2014). Alkaline treatment is usually carried out at ambient temperature with lime, sodium, potassium, and ammonium hydroxide as agents. The mechanism of alkaline treatment is to remove lignin from lignocellulose, improving the accessibility of the microbes and enzymes to hemicellulose and cellulose (Mosier et al., 2005). Additionally, the presence of residue alkali can neutralize carboxylic acids resulted from lignocellulose degradation in subsequent acidogenesis stage and prevents the pH drop (Zhu et al., 2010). Zhu et al. (2010) reported a 37.0% increase in biogas yield in AD of corn stover treated with 5.0% NaOH compared to that of untreated corn stover. Liew et al. (2011) achieved a 24-fold higher CH_4 yield in AD of fallen leaves treated with 3.5% NaOH. However, excessive alkali loading may inhibit AD due to high pH or ion toxicity (Rinzema et al., 1988). For instance, in AD of corn stover although the lignin degradation of corn stover increased with NaOH loadings from 1.0% to 7.5%, the biogas yield was not improved correspondingly; 7.5% NaOH loading actually inhibited AD due to VFAs accumulation and acidification (Zhu et al., 2010). Compared to alkali-treatment, acid pretreatment is more effective

to break down the recalcitrant lignocellulosic structure and produce reducing sugars (Jain et al., 2015b). However, compound such as furfural and hydroxymethylfurfural (HMF) can be produced during acid treatment which inhibit the AD process (Ariunbaatar et al., 2014). Acid treatment also requires additional bases to neutralize pH before starting AD. Overall, acid treatment is less preferable than alkaline pretreatment in AD.

Ozonation is another chemically based treatment in AD with no chemical residues left in the system. As a strong oxidant, ozone decomposes into radicals and reacts with the soluble and insoluble fractions of the substrates (Braguglia et al., 2012). The optimal ozone dosage for enhancing AD is reported in the range of 0.05 to 0.5 g O_3/g TS (Ariunbaatar et al., 2014). In AD of OFMSW, a 37% increase in biogas yield was achieved with feedstock treated with ozone at 0.16 g O_3/g TS; however, higher ozone dosages (0.4 and 1.2 g O_3/g TS) led to a lower biogas yield, probably due to the formation of intermediate compounds that are difficult to be digested (Cesaro and Belgiorno, 2013). Organic solvent is another chemical used in the treatment of lignocellulose-based feedstock by removing lignin and thus improve degradation of lignocelluloses. For example, in AD of elm, pine and rice straw, treating the feedstock with ethanol prior enhanced CH_4 production by 73%, 84% and 32%, respectively (Mirmohamadsadeghi et al., 2014).

Biological treatment relies on microorganisms and/or enzymes to break down the recalcitrant structure of the feedstock. Enzymes such as peptidase, carbohydrase and lipase (Ariunbaatar et al., 2014) are commonly added to the AD system to speed up the digestion. Microorganisms such as white rot fungi capable of decomposing lignin and altering the linkage between lignin and polysaccharides are commonly used in AD (Wan and Li, 2011). The fungi *Pleurotus ostreatus* and *Trichoderma reesei* were used to decompose rice straw as an effective

way to enhance CH_4 yield in AD of this feedstock (Mustafa et al., 2016). The white-rot fungus *Ceriporiopsis subvermispora* is considered one of the most effective species to degrade lignin while preserving cellulose (Ge et al., 2015). Due to its selective degradation feature, *C. subvermispora* treated AD led to a 20.9% lignin degradation of yard trimming while only 7.4% cellulose degradation, achieving a 154% increase in CH_4 yield in the subsequent AD (Zhao et al., 2014). When *C. subvermispora* was used to treat albizia chips, CH_4 yield increased 3.7-fold compared to the untreated feedstock (Ge et al., 2015).

Composting, an aerobic process facilitated by bacteria and fungi, is another biological pretreatment for AD. Yan et al. (2015) reported that composting rice straw resulted in a decrease of 63.6% TS, while the total carbon did not reduce significantly, proving that composting can effectively improve the biodegradability of rice straw. In order to improve the composting efficiency, pre-aeration is often used to generate enough self-heating to increase the temperature of OFMSW for the start-up of thermophilic AD without external heating (Charles et al., 2009). Composting with pre-aeration can also reduce the excessive organic compounds in feedstock, and thus, reduce the risk of VFAs overproduction and acidification of the following AD process (Charles et al., 2009). However, it should be noted that excessive pre-aeration may cause toxic effect on methanogens by introducing oxygen. For example, Zhou et al. (2017) reported that rice straw aerated for 2 days achieved the highest CH_4 yield, while the CH_4 yield gradually decreased when the aeration times increased from 4 days to 8 days.

1.4.2 Co-digestion

Co-digestion of different feedstock is commonly used to adjust carbon to nitrogen (C/N) ratio of the substrates in AD. Other advantages of co-digestion include improved nutrient profiles, a more balanced microbial community, obtaining a desired moisture content, and

economic advantages by sharing equipment. However, there are several drawbacks of co-digestion such as the extra logistics cost of the different feedstock, pre-mixing requirement, varied policy to control different waste materials, and increased effluent COD (Kangle et al., 2012).

The optimal C/N ratio for an AD process is in the range of 20:1 to 30:1. Most lignocellulose has a higher C/N ratio than 30, therefore, blending lignocellulosic feedstock with animal manures (with a C/N ratio less than 20) is a good approach to balance C/N ratio of SS-AD system. Li et al. (2017) reported co-digestion of poplar leaf (C/N = 35.4) and chicken manure (C/N = 8.09) brought C/N ratio to the optimal range (Table 1.2) and produced 15.28% more CH_4 than digestion of poplar leaf only.

Table 1.2. Co-digestion of different feedstock in AD.

Feedstock	Co-digestion feedstock	Mix ratio	C/N ratio	TS (%)	CH_4 yield (L/kg VS)	CH_4 Increment (%)	Reference
Straw	Pig slurry	1:3 (weight)	41.3	20.7	240.8	N/A	(Wang et al., 2012)
Food waste	Horse manure	1:1 (weight)	20	20	370	N/A	(Zhang et al., 2017)
Poplar leaf	Chicken manure	2:1 (VS)	21.9	22	115.7	15.28	(Li et al., 2017)
Household organic waste	Cow manure	1:1 (weight)	11.1	15	247	10.7	(Khairuddin et al., 2016)
Tomato residues/corn stover	Dairy manure	13:33:54 (weight)	25.1	20	415.4	50-1020	(Li et al., 2016)
Straw	Swine Manure	1:0.23 (weight)	20	27	300	N/A	(Riya et al., 2016)
Food waste	Yard waste	1:9 (VS)	16.9	19.3	120	118	(Brown and Li, 2013)
Food waste	Distiller's grains	1:8 (TS)	22.3	20	159.74	75.73	(Wang et al., 2012)
Spent mushroom substrate	Yard trimmings	1:1 (VS)	74.6	20	194	1500	(Lin et al., 2014)
	Wheat straw	1:1 (VS)	71.9	20	269	2200	
Dog food	Corn stover	1:1 (VS)	32.3	22	304.4	229	(Xu and Li, 2012)
Biological sludge	OFMSW	1:4 (weight)	39.8	38.8	220.6	34	(Nielfa et al., 2015)

In addition to adjusting C/N ratio, co-digestion of different feedstock also provides other benefits such as better nutrients, diverse microorganisms consortium, and stable pH and higher buffering capacity (Li et al., 2017). Khairuddin et al. (2016) reported that the co-digestion of

household organic waste and cow manure, even with a low C/N ratio of 11.1, still increased CH_4 yield by 10.7% compared to digestion of household organic waste only. Similarly, co-digestion of spent mushroom substrate and yard trimmings (with a C/N ratio of 74.6) produced 16-fold higher CH_4 yield than digestion of spent mushroom substrate (Lin et al., 2014). The ratio of the co-digested substrates is important for a successful AD. Li et al. (2016) conducted an AD of tomato residues, corn stover and dairy manure with eight mixing ratios. The authors reported that a mixing ratio of tomato residues, corn stover and dairy manure at 13:33:54 achieved the highest CH_4 yield, while digestion of tomato residues failed due to ammonia inhibition. Similarly, co-digestion of food waste with distiller's grains under four ratios (1:4, 1:6, 1:8, 1:10) showed that a food waste vs distiller's grains ratio at 1:8 resulted in highest CH_4 yield (Wang et al., 2012).

1.4.3 Additives

Various additives have been used to supplement AD system for performance enhancement (Romero-Güiza et al., 2016). Biochar, a charcoal-like product of incomplete combustion of organic materials, has been used as an additive in AD with multiple functions. In the study of chicken manure AD, Liang et al. (2017) found that adding biochar reduced the lag phase by 41%, increased CH_4 production rate by 18%, with reduced H_2S. In another study of AD of sludge amended with biochar, average CH_4 content in biogas was up to 92.3%, corresponding to a CO_2 sequestration by 66.2% (Shen et al., 2016). Biochar addition also enhanced process stability through increasing the alkalinity and alleviated free ammonia inhibition (Shen et al., 2016). Qin et al. (2017) used magnetic biochar (a composite of biochar and magnetic medium) as an additive in sludge AD, and recorded 11.69% increase in CH_4 production. The authors attributed the enhancement as the selective enrichment of functional bacteria and methanogens absorbed on magnetic biochar.

Materials promoting direct interspecies electron transfer (DIET) are also used as additives to accelerate the conversion of organic substrate to CH_4 (Dang et al., 2016). For instance, carbon cloth and granular activated carbon were reported to stimulate CH_4 production in AD of dog food, tolerating high OLR, and recovering from soured digester faster (Dang et al., 2016). Conductive materials were also effective in stimulating the syntrophic conversion of ethanol to CH_4 in up-flow anaerobic sludge blanket reactor (Zhao et al., 2015). The CH_4 production rates increased 30–45% with the addition of conductive materials at each OLR (Zhao et al., 2015).

It should be noted that although various additives have been showed benefits to AD systems, few studies have been done to apply those additives to AD. Further studies are needed to evaluate the technical and economic feasibility of using additive in AD systems.

1.5 Organization of dissertation

The main objective of this research conducted was to enhance the biofuel production in anaerobic digestion through various strategies. This dissertation is organized by chapters with Chapter 1 providing a brief general review of relevant literature, background information, and research objective and organization. Chapters 2-5 present various research projects conducted: Chapter 2 presents anaerobic digestion of aqueous phase from pyrolysis of biomass, Chapter 3 presents comparison of different biochar properties and their function in anaerobic digestion of municipal sludge, Chapter 4 presents the application of biochar in anaerobic digestion of easily-acidified food waste, and Chapter 5 presents the development of hydroxycinnamic acids extraction as a novel pretreatment to enhance anaerobic digestion of corn stover. Chapter 6

provides a high-level conclusion to summarize the results generated as well as recommendation for future work.

1.6 References

Abbassi-Guendouz, A., Brockmann, D., Trably, E., Dumas, C., Delgenès, J.P., Steyer, J.P., Escudié, R., 2012. Total solids content drives high solid anaerobic digestion via mass transfer limitation. Bioresour. Technol. 111, 55–61. https://doi.org/10.1016/j.biortech.2012.01.174

Adekunle, A., Orsat, V., Raghavan, V., 2016. Lignocellulosic bioethanol: A review and design conceptualization study of production from cassava peels. Renew. Sustain. Energy Rev. 64, 518–530. https://doi.org/10.1016/j.rser.2016.06.064

Ahmad, M., Rajapaksha, A.U., Lim, J.E., Zhang, M., Bolan, N., Mohan, D., Vithanage, M., Lee, S.S., Ok, Y.S., 2014. Biochar as a sorbent for contaminant management in soil and water: A review. Chemosphere 99, 19–23. https://doi.org/10.1016/j.chemosphere.2013.10.071

Ahn, J., Gu, S., Hwang, S., 2009. Effect of microwave irradiation on the disintegration and acidogenesis of municipal secondary sludge 153, 145–150. https://doi.org/10.1016/j.cej.2009.06.032

Amani, T., Nosrati, M., Sreekrishnan, T.R., 2010. Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects — a review. Environ. Rev. 18, 255–278. https://doi.org/10.1139/A10-011

André, L., Pauss, A., Ribeiro, T., 2018. Solid anaerobic digestion: State-of-art, scientific and technological hurdles. Bioresour. Technol. 247, 1027–1037. https://doi.org/10.1016/j.biortech.2017.09.003

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., Lens, P.N.L., 2014. Pretreatment methods to enhance anaerobic digestion of organic solid waste. Appl. Energy 123, 143–156. https://doi.org/10.1016/j.apenergy.2014.02.035

Bayrakdar, A., Sürmeli, R.Ö., Çalli, B., 2017. Dry anaerobic digestion of chicken manure coupled with membrane separation of ammonia. Bioresour. Technol. 244, 816–823. https://doi.org/10.1016/j.biortech.2017.08.047

Beszédes, S., László, Z., Horváth, Z.H., Szabó, G., Hodúr, C., 2011. Bioresource Technology Comparison of the effects of microwave irradiation with different intensities on the biodegradability of sludge from the dairy- and meat-industry 102, 814–821. https://doi.org/10.1016/j.biortech.2010.08.121

Bharathiraja, B., Sudharsana, T., Jayamuthunagai, J., Praveenkumar, R., Chozhavendhan, S., Iyyappan, J., 2018. Biogas production – A review on composition, fuel properties, feed

stock and principles of anaerobic digestion. Renew. Sustain. Energy Rev. 90, 570–582. https://doi.org/10.1016/j.rser.2018.03.093

Braguglia, C.M., Gianico, A., Mininni, G., 2012. Comparison between ozone and ultrasound disintegration on sludge anaerobic digestion. J. Environ. Manage. 95, S139–S143. https://doi.org/10.1016/j.jenvman.2010.07.030

Bridgwater, A. V., Meier, D., Radlein, D., 1999. An overview of fast pyrolysis of biomass. Org. Geochem. 30, 1479–1493. https://doi.org/10.1016/S0146-6380(99)00120-5

Brown, R. C., Brown, T.R., 2013. Biorenewable resources: engineering new products from agriculture. John Wiley & Sons.

Brown, D., Li, Y., 2013. Solid state anaerobic co-digestion of yard waste and food waste for biogas production. Bioresour. Technol. 127, 275–280. https://doi.org/10.1016/j.biortech.2012.09.081

Brown, D., Shi, J., Li, Y., 2012. Comparison of solid-state to liquid anaerobic digestion of lignocellulosic feedstocks for biogas production. Bioresour. Technol. 124, 379–386. https://doi.org/10.1016/j.biortech.2012.08.051

Capson-Tojo, G., Trably, E., Rouez, M., Crest, M., Steyer, J.P., Delgenès, J.P., Escudié, R., 2017. Dry anaerobic digestion of food waste and cardboard at different substrate loads, solid contents and co-digestion proportions. Bioresour. Technol. 233, 166–175. https://doi.org/10.1016/j.biortech.2017.02.126

Cazier, E.A., Trably, E., Steyer, J.P., Escudie, R., 2015. Biomass hydrolysis inhibition at high hydrogen partial pressure in solid-state anaerobic digestion. Bioresour. Technol. 190, 106–113. https://doi.org/10.1016/j.biortech.2015.04.055

Cesaro, A., Belgiorno, V., 2013. Sonolysis and ozonation as pretreatment for anaerobic digestion of solid organic waste. Ultrason. Sonochem. 20, 931–936. https://doi.org/10.1016/j.ultsonch.2012.10.017

Charles, W., Walker, L., Cord-Ruwisch, R., 2009. Effect of pre-aeration and inoculum on the start-up of batch thermophilic anaerobic digestion of municipal solid waste. Bioresour. Technol. 100, 2329–2335. https://doi.org/10.1016/j.biortech.2008.11.051

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

Cheng, J.J., Timilsina, G.R., 2011. Status and barriers of advanced biofuel technologies: A review. Renew. Energy 36, 3541–3549. https://doi.org/10.1016/j.renene.2011.04.031

Cho, J.K., Park, S.C., Chang, H.N., 1995. Biochemical methane potential and solid state anaerobic digestion of Korean food wastes. Bioresour. Technol. 52, 245–253. https://doi.org/10.1016/0960-8524(95)00031-9

Codignole Luz, F., Cordiner, S., Manni, A., Mulone, V., Rocco, V., 2018. Biochar characteristics and early applications in anaerobic digestion-a review. J. Environ. Chem. Eng. 6, 2892–2909. https://doi.org/10.1016/j.jece.2018.04.015

Croce, S., Wei, Q., D'Imporzano, G., Dong, R., Adani, F., 2016. Anaerobic digestion of straw and corn stover: The effect of biological process optimization and pre-treatment on total bio-methane yield and energy performance. Biotechnol. Adv. 34, 1289–1304. https://doi.org/10.1016/j.biotechadv.2016.09.004

Dang, Y., Holmes, D.E., Zhao, Z., Woodard, T.L., Zhang, Y., Sun, D., Wang, L.Y., Nevin, K.P., Lovley, D.R., 2016. Enhancing anaerobic digestion of complex organic waste with carbon-based conductive materials. Bioresour. Technol. 220, 516–522. https://doi.org/10.1016/j.biortech.2016.08.114

Di Maria, F., Barratta, M., Bianconi, F., Placidi, P., Passeri, D., 2017. Solid anaerobic digestion batch with liquid digestate recirculation and wet anaerobic digestion of organic waste: Comparison of system performances and identification of microbial guilds. Waste Manag. 59, 172–180. https://doi.org/10.1016/j.wasman.2016.10.039

Fagbohungbe, M.O., Dodd, I.C., Herbert, B.M.J., Li, H., Ricketts, L., Semple, K.T., 2015. High solid anaerobic digestion: Operational challenges and possibilities. Environ. Technol. Innov. 4, 268–284. https://doi.org/10.1016/j.eti.2015.09.003

Food and Agriculture Organization, Nations, of the U., 2012. Towards the future we want: End hunger and make the transition to sustainable agricultural and food systems. Fao 1–42.

Fowles, M., 2007. Black carbon sequestration as an alternative to bioenergy. Biomass and Bioenergy 31, 426–432. https://doi.org/10.1016/j.biombioe.2007.01.012

Fox, P., Pohland, F.G., 1994. Anaerobic treatment applications and fundamentals: Substrate specificity during phase separation. Water Environ. Res. 66, 716–724. https://doi.org/10.2175/WER.66.5.8

Ge, X., Matsumoto, T., Keith, L., Li, Y., 2015. Fungal pretreatment of albizia chips for enhanced biogas production by solid-state anaerobic digestion. Energy and Fuels 29, 200–204. https://doi.org/10.1021/ef501922t

Ge, X., Xu, F., Li, Y., 2016. Solid-state anaerobic digestion of lignocellulosic biomass: Recent progress and perspectives. Bioresour. Technol. 205, 239–249. https://doi.org/10.1016/j.biortech.2016.01.050

Guendouz, J., Buffière, P., Cacho, J., Carrère, M., Delgenes, J.P., 2010. Dry anaerobic digestion in batch mode: Design and operation of a laboratory-scale, completely mixed reactor. Waste Manag. 30, 1768–1771. https://doi.org/10.1016/j.wasman.2009.12.024

Ho, D.P., Ngo, H.H., Guo, W., 2014. A mini review on renewable sources for biofuel. Bioresour. Technol. 169, 742–749. https://doi.org/10.1016/j.biortech.2014.07.022

Holtman, K.M., Bozzi, D. V., Franqui-Villanueva, D., Offeman, R.D., Orts, W.J., 2017. Pilot scale high solids anaerobic digestion of steam autoclaved municipal solid waste (MSW) pulp. Renew. Energy 113, 257–265. https://doi.org/10.1016/j.renene.2017.03.050

Hoornweg, D., Bhada, P., 2012. What a Waste. A Global Review of Solid Waste Management. Urban Dev. Ser. Knowl. Pap. 281, 44 p. https://doi.org/10.1111/febs.13058

Inyang, M., Gao, B., Pullammanappallil, P., Ding, W., Zimmerman, A.R., 2010. Biochar from anaerobically digested sugarcane bagasse. Bioresour. Technol. 101, 8868–8872. https://doi.org/10.1016/j.biortech.2010.06.088

Jackowiak, D., Bassard, D., Pauss, A., Ribeiro, T., 2011. Optimisation of a microwave pretreatment of wheat straw for methane production. Bioresour. Technol. 102, 6750–6756. https://doi.org/10.1016/j.biortech.2011.03.107

Jain, Siddharth, Jain, Shivani, Wolf, I.T., Lee, J., Tong, Y.W., 2015a. A comprehensive review on operating parameters and different pretreatment methodologies for anaerobic digestion of municipal solid waste. Renew. Sustain. Energy Rev. 52, 142–154. https://doi.org/10.1016/j.rser.2015.07.091

Jain, Siddharth, Jain, Shivani, Wolf, I.T., Lee, J., Tong, Y.W., 2015b. A comprehensive review on operating parameters and different pretreatment methodologies for anaerobic digestion of municipal solid waste. Renew. Sustain. Energy Rev. 52, 142–154. https://doi.org/10.1016/j.rser.2015.07.091

Jarboe, L.R., Wen, Z., Choi, D., Brown, R.C., 2011. Hybrid thermochemical processing: Fermentation of pyrolysis-derived bio-oil. Appl. Microbiol. Biotechnol. 91, 1519–1523. https://doi.org/10.1007/s00253-011-3495-9

Kadam, K., McMillan, J., 2003. Availability of corn stover as a sust5ainable feedstock for bioethanol production. Bioresour. Technol. 88, 17–25.

Kangle, K.M., Kore, S. V, Kore, V.S., Kulkarni, G.S., 2012. Recent Trends in Anaerobic Codigestion : A Review. Univers. J. Environ. Res. Technol. 2, 210–219.

Khairuddin, N., Manaf, L.A., Hassan, M.A., Halimoon, N., Wan Ab Karim Ghani, W.A., 2016. High solid anaerobic co-digestion of household organic waste with cow manure for mass and energy recovery. Polish J. Environ. Stud. 25, 1549–1554. https://doi.org/10.15244/pjoes/61882

Laird, D.A., 2008. The charcoal vision: A win-win-win scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water quality. Agron. J. 100, 178–181. https://doi.org/10.2134/agronj2007.0161

Laird, D.A., Brown, R.C., Amonette, J.E., Lehmann, J., 2009. Review of the pyrolysis platform for coproducing bio-oil and biochar. Biofuels, Bioprod. Biorefining 6, 547–562. https://doi.org/10.1002/bbb

Langholtz, M.H., Stokes, B.J., Eaton, L.M., 2016. 2016 BILLION-TON REPORT Advancing Domestic Resources for a Thriving Bioeconomy. Oak Ridge Natl. Lab. 1160, 448–2172. https://doi.org/ORNL/TM-2016/160

Li, W., Lu, C., An, G., Chang, S., 2017. Comparison of Alkali-Buffering Effects and Co-digestion on High-Solid Anaerobic Digestion of Horticultural Waste. Energy & Fuels 31, 10990–10997. https://doi.org/10.1021/acs.energyfuels.7b02269

Li, Y., Yan, F., Li, T., Zhou, Y., Jiang, H., Qian, M., Xu, Q., 2018. High-solid anaerobic digestion of corn straw for methane production and pretreatment of bio-briquette. Bioresour. Technol. 250, 741–749. https://doi.org/10.1016/j.biortech.2017.11.083

Li, Yangyang, Li, Yu, Zhang, D., Li, G., Lu, J., Li, S., 2016. Solid state anaerobic co-digestion of tomato residues with dairy manure and corn stover for biogas production. Bioresour. Technol. 217, 50–55. https://doi.org/10.1016/j.biortech.2016.01.111

Liang, Y., Qiu, L., Guo, X., Pan, J., Lu, W., Ge, Y., 2017. Start-up Performance of Chicken Manure Anaerobic Digesters Amended with Biochar and Operated at Different Temperatures. Nat. Environ. Pollut. Technol.

Liang, Y., Zhao, X., Chi, Z., Rover, M., Johnston, P., Brown, R., Jarboe, L., Wen, Z., 2013. Utilization of acetic acid-rich pyrolytic bio-oil by microalga Chlamydomonas reinhardtii: Reducing bio-oil toxicity and enhancing algal toxicity tolerance. Bioresour. Technol. 133, 500–506. https://doi.org/10.1016/j.biortech.2013.01.134

Liao, X., Li, H., Zhang, Y., Liu, C., Chen, Q., 2016. Accelerated high-solids anaerobic digestion of sewage sludge using low-temperature thermal pretreatment. Int. Biodeterior. Biodegrad. 106, 141–149. https://doi.org/10.1016/j.ibiod.2015.10.023

Liew, L.N., Shi, J., Li, Y., 2011. Enhancing the solid-state anaerobic digestion of fallen leaves through simultaneous alkaline treatment. Bioresour. Technol. 102, 8828–8834. https://doi.org/10.1016/j.biortech.2011.07.005

Lin, Y., Ge, X., Li, Y., 2014. Solid-state anaerobic co-digestion of spent mushroom substrate with yard trimmings and wheat straw for biogas production. Bioresour. Technol. 169, 468–474. https://doi.org/10.1016/j.biortech.2014.07.020

Liu, C.M., Wachemo, A.C., Yuan, H.R., Zou, D.X., Liu, Y.P., Zhang, L., Pang, Y.Z., Li, X.J., 2018. Evaluation of methane yield using acidogenic effluent of NaOH pretreated corn stover in anaerobic digestion. Renew. Energy 116, 224–233. https://doi.org/10.1016/j.renene.2017.07.001

Manyà, J.J., 2012. Pyrolysis for biochar purposes: A review to establish current knowledge gaps and research needs. Environ. Sci. Technol. 46, 7939–7954. https://doi.org/10.1021/es301029g

Mao, C., Feng, Y., Wang, X., Ren, G., 2015. Review on research achievements of biogas from anaerobic digestion. Renew. Sustain. Energy Rev. 45, 540–555. https://doi.org/10.1016/j.rser.2015.02.032

Masebinu, S.O., Akinlabi, E.T., Muzenda, E., Aboyade, A.O., 2019. A review of biochar properties and their roles in mitigating challenges with anaerobic digestion. Renew. Sustain. Energy Rev. 103, 291–307. https://doi.org/10.1016/j.rser.2018.12.048

Mirmohamadsadeghi, S., Karimi, K., Zamani, A., Amiri, H., Horváth, I.S., 2014. Enhanced solid-state biogas production from lignocellulosic biomass by organosolv pretreatment. Biomed Res. Int. 2014. https://doi.org/10.1155/2014/350414

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M., 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol. 96, 673–686. https://doi.org/10.1016/j.biortech.2004.06.025

Motte, J.C., Escudié, R., Hamelin, J., Steyer, J.P., Bernet, N., Delgenes, J.P., Dumas, C., 2015. Substrate milling pretreatment as a key parameter for Solid-State Anaerobic Digestion optimization. Bioresour. Technol. 173, 185–192. https://doi.org/10.1016/j.biortech.2014.09.015

Mussoline, W., Esposito, G., Lens, P., Spagni, A., Giordano, A., 2013. Enhanced methane production from rice straw co-digested with anaerobic sludge from pulp and paper mill treatment process. Bioresour. Technol. 148, 135–143. https://doi.org/10.1016/j.biortech.2013.08.107

Mustafa, A.M., Poulsen, T.G., Sheng, K., 2016. Fungal pretreatment of rice straw with Pleurotus ostreatus and Trichoderma reesei to enhance methane production under solid-state anaerobic digestion. Appl. Energy 180, 661–671. https://doi.org/10.1016/j.apenergy.2016.07.135

Nguyen, D.D., Chang, S.W., Jeong, S.Y., Jeung, J., Kim, S., Guo, W., Ngo, H.H., 2016. Dry thermophilic semi-continuous anaerobic digestion of food waste: Performance evaluation, modified Gompertz model analysis, and energy balance. Energy Convers. Manag. 128, 203–210. https://doi.org/10.1016/j.enconman.2016.09.066

Nielfa, A., Cano, R., Fdz-Polanco, M., 2015. Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. Biotechnol. Reports 5, 14–21. https://doi.org/10.1016/j.btre.2014.10.005

Panjičko, M., Zupančič, G.D., Fanedl, L., Logar, R.M., Tišma, M., Zelić, B., 2017. Biogas production from brewery spent grain as a mono-substrate in a two-stage process composed of solid-state anaerobic digestion and granular biomass reactors. J. Clean. Prod. 166, 519–529. https://doi.org/10.1016/j.jclepro.2017.07.197

Panjičko, M., Zupančič, G.D., Zelić, B., 2015. Anaerobic biodegradation of raw and pre-treated brewery spent grain utilizing solid state anaerobic digestion. Acta Chim. Slov. 62, 818–827. https://doi.org/10.17344/acsi.2015.1534

Papari, S., Hawboldt, K., 2015. A review on the pyrolysis of woody biomass to bio-oil: Focus on kinetic models. Renew. Sustain. Energy Rev. 52, 1580–1595. https://doi.org/10.1016/j.rser.2015.07.191

Pecchi, M., Baratieri, M., 2019. Coupling anaerobic digestion with gasification, pyrolysis or hydrothermal carbonization: A review. Renew. Sustain. Energy Rev. 105, 462–475. https://doi.org/10.1016/j.rser.2019.02.003

Pohl, M., Mumme, J., Heeg, K., Nettmann, E., 2012. Thermo- and mesophilic anaerobic digestion of wheat straw by the upflow anaerobic solid-state (UASS) process. Bioresour. Technol. 124, 321–327. https://doi.org/10.1016/j.biortech.2012.08.063

Polin, J.P., Peterson, C.A., Whitmer, L.E., Smith, R.G., Brown, R.C., 2019. Process intensification of biomass fast pyrolysis through autothermal operation. Appl. Energy 249, 276–285. https://doi.org/10.1016/j.apenergy.2019.04.154

Pollard, A.S., Rover, M.R., Brown, R.C., 2012. Characterization of bio-oil recovered as stage fractions with unique chemical and physical properties. J. Anal. Appl. Pyrolysis 93, 129–138. https://doi.org/10.1016/j.jaap.2011.10.007

Qin, Y., Wang, H., Li, X., Cheng, J.J., Wu, W., 2017. Improving methane yield from organic fraction of municipal solid waste (OFMSW) with magnetic rice-straw biochar. Bioresour. Technol. 245, 1058–1066. https://doi.org/10.1016/j.biortech.2017.09.047

Rapport, J., Zhang, R., Jenkins, B.M., Williams, R.B., 2008. Current Anaerobic Digestion Technologies Used for Treatment of Municipal Organic Solid Waste. Calif. Integr. Waste Manag. Board 90. https://doi.org/10.1016/j.biortech.2006.11.048

Rinzema, A., van Lier, J., Lettinga, G., 1988. Sodium inhibition of acetoclastic methanogens in granular sludge from a UASB reactor. Enzyme Microb. Technol. 10, 24–32. https://doi.org/10.1016/0141-0229(88)90094-4

Riya, S., Suzuki, K., Terada, A., Hosomi, M., Zhou, S., 2016. Influence of C/N Ratio on Performance and Microbial Community Structure of Dry-Thermophilic Anaerobic Co-Digestion of Swine Manure and Rice Straw. J. Med. Bioeng. 5, 11–14. https://doi.org/10.12720/jomb.5.1.11-14

Romero-Güiza, M.S., Vila, J., Mata-Alvarez, J., Chimenos, J.M., Astals, S., 2016. The role of additives on anaerobic digestion: A review. Renew. Sustain. Energy Rev. 58, 1486–1499. https://doi.org/10.1016/j.rser.2015.12.094

Sheets, J.P., Ge, X., Li, Y., 2015. Effect of limited air exposure and comparative performance between thermophilic and mesophilic solid-state anaerobic digestion of switchgrass. Bioresour. Technol. 180, 296–303. https://doi.org/10.1016/j.biortech.2015.01.011

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. J. Clean. Prod. 135, 1054–1064. https://doi.org/10.1016/j.jclepro.2016.06.144

Shen, Y., Linville, J.L., Urgun-Demirtas, M., Schoene, R.P., Snyder, S.W., 2015. Producing pipeline-quality biomethane via anaerobic digestion of sludge amended with corn stover biochar with in-situ CO2 removal. Appl. Energy 158, 300–309. https://doi.org/10.1016/j.apenergy.2015.08.016

Shi, J., Xu, F., Wang, Z., Stiverson, J.A., Yu, Z., Li, Y., 2014. Effects of microbial and non-microbial factors of liquid anaerobic digestion effluent as inoculum on solid-state anaerobic digestion of corn stover. Bioresour. Technol. 157, 188–196. https://doi.org/10.1016/j.biortech.2014.01.089

Speece, R.E., McCarty, P.L., 1964. Nutrient Requirements and Biological Solids Accumulation in Anaerobic Digestion, Advances in Water Pollution Research. PERGAMON PRESS LTD. https://doi.org/10.1016/B978-1-4832-8391-3.50034-8

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. Biochem. Eng. J. 41, 198–201. https://doi.org/10.1016/j.bej.2008.05.008

Steffen, R., Szolar, O., Braun, R., 1998. Feedstock for anaerobic digestion. Inst. Agrobiotechnology Tulin, Univ. Agric. Sci. 1–29.

Sun, C., 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. Bioresour. Technol. 218, 1215–1223. https://doi.org/10.1016/j.biortech.2016.07.082

Takashima, M., Speece, R.E., Parkin, G.F., 1990. Mineral requirements for methane fermentation. Crit. Rev. Environ. Control 19, 465–479. https://doi.org/10.1080/10643389009388378

Tian, J.H., Pourcher, A.M., Bureau, C., Peu, P., 2017. Cellulose accessibility and microbial community in solid state anaerobic digestion of rape straw. Bioresour. Technol. 223, 192–201. https://doi.org/10.1016/j.biortech.2016.10.009

Tomei, M.C., Braguglia, C.M., Cento, G., Mininni, G., 2009. Modeling of Anaerobic digestion of sludge. Crit. Rev. Environ. Sci. Technol. 39, 1003–1051. https://doi.org/10.1080/10643380801977818

Torri, C., Fabbri, D., 2014. Biochar enables anaerobic digestion of aqueous phase from intermediate pyrolysis of biomass. Bioresour. Technol. 172, 335–341. https://doi.org/10.1016/j.biortech.2014.09.021

Veeken, A.H.M., Hamelers, B.V.M., 2000. Effect of substrate-seed mixing and leachate recirculation on solid state digestion of biowaste. Water Sci. Technol. 41, 255–262.

Wan, C., Li, Y., 2011. Effectiveness of microbial pretreatment by Ceriporiopsis subvermispora on different biomass feedstocks. Bioresour. Technol. 102, 7507–7512. https://doi.org/10.1016/j.biortech.2011.05.026

Wang, H., Livingston, D., Srinivasan, R., Li, Q., Steele, P., Yu, F., 2012. Detoxification and fermentation of pyrolytic sugar for ethanol production. Appl. Biochem. Biotechnol. 168, 1568–1583. https://doi.org/10.1007/s12010-012-9879-1

Wang, L.H., Wang, Q., Cai, W., Sun, X., 2012. Influence of mixing proportion on the solid-state anaerobic co-digestion of distiller's grains and food waste. Biosyst. Eng. 112, 130–137. https://doi.org/10.1016/j.biosystemseng.2012.03.006

Ward, A.J., Hobbs, P.J., Holliman, P.J., Jones, D.L., 2008. Optimisation of the anaerobic digestion of agricultural resources. Bioresour. Technol. 99, 7928–7940. https://doi.org/10.1016/j.biortech.2008.02.044

Xu, F., Li, Y., 2012. Solid-state co-digestion of expired dog food and corn stover for methane production. Bioresour. Technol. 118, 219–226. https://doi.org/10.1016/j.biortech.2012.04.102

Xu, F., Wang, F., Lin, L., Li, Y., 2016. Comparison of digestate from solid anaerobic digesters and dewatered effluent from liquid anaerobic digesters as inocula for solid state anaerobic digestion of yard trimmings. Bioresour. Technol. 200, 753–760. https://doi.org/10.1016/j.biortech.2015.10.103

Yan, Z., Song, Z., Li, D., Yuan, Y., Liu, X., Zheng, T., 2015. The effects of initial substrate concentration, C/N ratio, and temperature on solid-state anaerobic digestion from composting rice straw. Bioresour. Technol. 177, 266–273. https://doi.org/10.1016/j.biortech.2014.11.089

Yang, L., Xu, F., Ge, X., Li, Y., 2015. Challenges and strategies for solid-state anaerobic digestion of lignocellulosic biomass. Renew. Sustain. Energy Rev. 44, 824–834. https://doi.org/10.1016/j.rser.2015.01.002

Zhang, C., Su, H., Baeyens, J., Tan, T., 2014. Reviewing the anaerobic digestion of food waste for biogas production. Renew. Sustain. Energy Rev. 38, 383–392. https://doi.org/10.1016/j.rser.2014.05.038

Zhang, J., Loh, K.C., Lee, J., Wang, C.H., Dai, Y., Wah Tong, Y., 2017. Three-stage anaerobic co-digestion of food waste and horse manure. Sci. Rep. 7, 1–10. https://doi.org/10.1038/s41598-017-01408-w

Zhao, J., Zheng, Y., Li, Y., 2014. Fungal pretreatment of yard trimmings for enhancement of methane yield from solid-state anaerobic digestion. Bioresour. Technol. 156, 176–181. https://doi.org/10.1016/j.biortech.2014.01.011

Zhao, Z., Zhang, Y., Woodard, T.L., Nevin, K.P., Lovley, D.R., 2015. Enhancing syntrophic metabolism in up-flow anaerobic sludge blanket reactors with conductive carbon materials. Bioresour. Technol. 191, 140–145. https://doi.org/10.1016/j.biortech.2015.05.007

Zhou, Y., Li, C., Achu, I., Liu, J., 2017. The effects of pre-aeration and inoculation on solid-state anaerobic digestion of rice straw. Bioresour. Technol. 224, 78–86. https://doi.org/10.1016/j.biortech.2016.11.104

Zhu, J., Wan, C., Li, Y., 2010. Enhanced solid-state anaerobic digestion of corn stover by alkaline pretreatment. Bioresour. Technol. 101, 7523–7528. https://doi.org/10.1016/j.biortech.2010.04.060

Zhu, J., Yang, L., Li, Y., 2015. Comparison of premixing methods for solid-state anaerobic digestion of corn stover. Bioresour. Technol. 175, 430–435. https://doi.org/10.1016/j.biortech.2014.10.095

Zhu, J., Zheng, Y., Xu, F., Li, Y., 2014. Solid-state anaerobic co-digestion of hay and soybean processing waste for biogas production. Bioresour. Technol. 154, 240–247. https://doi.org/10.1016/j.biortech.2013.12.045

CHAPTER 2. ANAEROBIC DIGESTION OF AQUEOUS PAHSE FROM PYROLYSIS OF BIOMASS: REDUCING TOXICITY AND IMPROVING MICROBIAL TOLERANCE

Abstract

Among the products of pyrolysis is an aqueous phase (AP), which contains a significant fraction of carbon but is too dilute to make recovery of this organic content cost-effectively. This study was to explore the use of AP for anaerobic digestion. Different treatment methods including overliming, Fenton's reagent oxidation, bleaching and activated carbon adsorption were investigated to reduce toxicity of AP. Overliming treatment increased biogas production up to 32-fold compared to non-treated AP. Enhancing the tolerance of the bacterial and archaeal community to the AP toxicity was also attempted with a directed evolution method, resulting the microbes' tolerance to AP from 5% to 14%. Directed evolution resulted a major bacterial taxon as *Cloacimonetes*, *Firmicutes*, and *Chloroflexi*, while shifted the predominant archaea shifted from acetoclastic to hydrogenotrophic methanogens. Collectively, the results demonstrated that combining feedstock treatment and directed evolution of the microbial community is an effective way for AP anaerobic digestion.

2.1 Introduction

Lignocellulosic biomass is commonly used as a feedstock for producing renewable fuels and chemicals through a fast pyrolysis process, in which biomass is treated at 350-600 ºC in the absence of oxygen and decomposed into a flammable gas mixture (syngas, 15~20%), a viscous energy rich liquid (bio-oil, 50~70%), and a carbon- and nutrient-rich solid (biochar, 10~30%) (Laird et al., 2009). Bio-oil is an emulsion of lignin-derived oligomers and an aqueous solution of carbohydrate-derived compounds. Phenolic monomers and dimers in bio-oil can be upgraded into drop-in fuels (Mortensen et al., 2011) due to their ideal carbon numbers (C6-C20) and relatively low oxygen content compared to carbohydrate. The pyrolytic sugars such as levoglucosan can be utilized by microorganisms to produce fuels and chemicals (Layton et al., 2011).

However, utilization of bio-oil for fuel production is still facing challenges. The high water content (15-30% wt) in bio-oil reduces its heating value and chemical and thermal stability. The carboxylic acid produced from lignin depolymerization reduces the liquid pH and causes corrosiveness to the vessels and pipework. To address these problems, researchers at Iowa State University have developed a separation system to concentrate carboxylic acids into a distinct aqueous phase (AP) (light fraction) that will be separated from compounds such as phenolic oligomers and sugars (heavy fraction) that can be upgraded into fuel products without the interference from the carboxylic acids (Pollard et al., 2012). The AP produced from this separation system is a dilute solution mainly containing carboxylic acids with smaller amounts of phenolic monomers (Pollard et al., 2012). This AP is corrosive with low heating value and is inevitably produced as a by-product in pyrolysis process, therefore, the disposal of this waste stream needs to be addressed.

Developing an appropriate method to utilize the AP is crucial to achieve a successful pyrolysis-based biomass biorefinery. Acetic acid in the AP has been used as a substrate in fermentation processes. For example, Liang et al. (2013) utilized AP for the growth and lipid production of heterotrophic alga *Chlamydomonas reinhardtii*. Lian et al. (2012) reported the feasibility of yeast fermentation of carboxylic acids in pyrolytic AP. However, these studies on AP utilization are based on pure culture of single species.

Compared to the pure-culture based biological utilizations, anaerobic digestion (AD) is a potentially economical approach to utilize AP by converting organic compounds into methane (CH_4) through a complex bacterial/archaeal consortia. Unlike fermentation of single strain, AD reduces the cost to maintain aseptic conditions. It can also separate gaseous products by outgassing with less expensive equipment. As the decomposition of organic compounds in AD occurs through a sequential acidogenesis and methanogenesis process, acetic acid is an important intermediate serving as substrate for CH_4 production. Therefore, AD can be a simple and feasible way to treat acetic acid-rich AP, increasing the overall energy yield and providing the opportunities for energy recovery from waste.

Recovering energy from pyrolytic liquid by AD has been reported. For example, Andreanna et al. (1990) used swine slurry to co-digest wood pyrolysis liquid by diluting potential inhibitors (mainly phenols and cresol). The digester packed with wood-chips could tolerate up to 10% (v/v) pyrolytic liquid before the process efficiency deteriorated. Torri and Fabbri (2014) added biochar, another biomass pyrolysis co-product, to effectively increased CH_4 yield due to the adsorption of inhibitors, pH buffering, and bacteria attachment provided by the biochar. The enhancement of electron transformation between the microbes may be another benefit of using biochar in the AD of pyrolysis liquid (Yue et al., 2019). Hübner and Mumme

(2015) found that up to 12 g/L initial COD of pyrolysis liquor from pyrolysis of digestate was tolerated in AD and a highest methane yield of 199.1 mL/g COD was achieved. In another study, Seyedi et al. (2019) reported that non-catalyzed AP loading rate higher than 0.5 g COD/L was not sustainable in AD due to toxicity, in which AP was produced from pyrolysis of wastewater biosolids at 800 °C.

Currently, the toxicity of AP is the major hurdle for using this substrate in AD. The AP contains various compounds such as phenolics, furfural, acetol, and hydroxyacetaldehyde that can inhibit microbial cells (Liang et al., 2013; Seuedi et al., 2019). To reduce the toxicity and enhance the digestibility of the AP, various pretreatment methods can be applied. In general, the concept of pretreatment of substrates has been widely used in AD to enhance biogas production. For example, alkaline pretreatment effectively destroyed the complex structure of lignocellulosic biomass and prevented the system from decreasing pH caused by acetogenesis (Zhang et al., 2014). Lin et al. (2009) used NaOH to treat pulp and paper sludge with an 83.5% increment of CH_4 yield. Fenton's reagent, as a non-selective oxidant, decomposed biomass into smaller molecules that were readily available for anaerobic bacteria and thus, biogas formation during AD (Dewil et al., 2007). Activated carbon as an effective absorbent was used to remove heavy metals and organic chemicals (Rengaraj et al., 2002) from wastewater, which benefited the subsequent AD process.

In addition to the substrate pretreatment, developing a robust toxicity-tolerant microbial consortium is another strategy to address the toxicity of substrate such as AP. For example, directed evolution has been successfully used to increase the tolerance of the alga *C. reinhardtii* to the AP toxicity (Liang et al., 2013) and *E. coli* to pyrolytic sugars (Jin et al., 2017) while this approach has not been reported in the AD process with microbial consortium.

The objective of this work was to enhance the anaerobic digestion of AP by applying the aforementioned two strategies, i.e., reducing the toxicity of the substrate and increasing the toxicity tolerance of microbial consortium. Insights gained from this research would provide the foundation for future studies on the effective integration of thermochemical processes and biological conversion to enhance renewable energy production.

2.2 Materials and methods

2.2.1 Preparation of bio-oil and the aqueous phase

The raw bio-oil and AP were prepared as described previously (Pollard et al., 2012). In brief, bio-oil was produced by fast pyrolysis of corn stover in a fluidized bed reactor followed by a five-stage fractionating recovery system. AP was collected as the last stage using water at 18 °C as coolant to collect mostly water and light oxygenated compounds (such as acetic acid). The AP was stored in 1-L Nalgene HDPE bottles at 4 °C. All the samples were mixed by manually shaking prior to use.

2.2.2 Pretreatment of the aqueous phase

The AP was subject to four different pretreatment methods including oxidation treatment with Fenton's reagent, oxidation treatment with bleach, activated carbon adsorption, and overliming treatment with $Ca(OH)_2$ powder. In Fenton experiment, a mixture of 1.5 g/L hydrogen peroxide and 0.75 g/L ferrous sulfate was added to 20mL AP in a 50 mL centrifuge tube. In bleach experiment, NaClO was added to 20mL AP at 5 g/L in a 50 mL centrifuge tube. Both mixed samples were stirred at 100 rpm for 1 hr at room temperature and then stored at 4°C prior to the AD test.

In the treatment of activated carbon adsorption, activated carbon powder was added to AP at a loading of 0.2 g/mL. The mixture was thoroughly mixed in a 50-mL centrifuge tube for

0.5 h using an orbital shaker, and centrifuged at 3,836 g for 4 min. The mixture was then filtered through a 0.45 μm filter. The supernatant was stored at 4°C prior to the AD test. To perform the overliming treatment, AP solution (100 mL) was gradually added with $Ca(OH)_2$ powder (around 11g) to reach the pH of the solution to 10. The mixture was thoroughly stirred during the treatment to dissipate the heat. The solution was cooled to room temperature and centrifuged at 800 g for 5 min to remove precipitants. The supernatant was stored at 4 °C prior to the AD test. Since the dosages of AP in AD were relatively low, the pH of the AP was not adjusted before being added to the AD system.

2.2.3. Inoculum and conditions used for anaerobic digestion

The inoculum for AD was obtained from Water Pollution Control Plant located in Ames, Iowa, USA. The facility runs a two-stage (acidogenesis and methanogenesis) digestion system. The inoculum was obtained from the methanogenesis digester and stored in air-tight bottles at 4°C. The inoculum had a total solid (TS) of 26.62 ± 0.99 g/L and volatile solid (VS) of 19.43 ± 0.83 g/L with pH 7.5. Prior to use, the inoculum was pre-incubated at 37 °C for 7-12 days to reduce the background gas production. The background gas production of the inoculum was around 53 mL/g VS.

Anaerobic digestion of AP was first performed in a batch mode to evaluate the effects of the different pretreatment methods on the biogas production. A digestion system modified from the Hohenheimer Biogas Yield Test (Helffrich and Oechsner, 2003) was used. In brief, a 100-mL syringe barrel was used to hold the digestion inoculum and substrate. The opening end of the barrel was sealed with a rubber stopper. After filled the barrel with digestion materials, the plunger was inserted back to the barrel, and the stopper was removed from the opening end to release the air. The syringe was then re-sealed with the stopper and incubated in a rotatory shaker

with 100 rpm at 37 °C. The biogas was measured on daily basis by reading the graded marker in the syringe and then released for the next cycle of measurement. The system containing inoculum only was used as a control. All the experiments were performed in duplicate.

Continuous AD was then carried out in a 1.5-L New Brunswick BioFlo 110 reactor (Eppendorf, NY, USA) with working volume of 1.2 L. To start the culture, the inoculum and substrate were added to the vessel, and the headspace was flushed with nitrogen for 3 minutes. The reactor was set at 37 °C with 100 rpm agitation. The pH of the slurry was monitored but not controlled. On daily basis, certain amount of the digestion slurry was removed from the reactor, with the same amount of fresh feed (AP and inoculum mixture) being fed to the vessel. Gas production was also measured daily by a water replacement setting. The reactor was operated at a hydraulic retention time (HRT) of 20 days with different loadings of AP.

2.2.4 Directed evolution of anaerobic microbiome

The above results shows that overliming treatment was the most effective method to reduce the AP toxicity. Building on the effectiveness of this treatment method, we further used the directed evolution approach to enhance the strains robustness to tolerate the AP toxicity.

The directed evolution of anaerobic microbial communities was conducted in 150 mL serum bottles with 100 mL working volume. Three replicate bottles were used throughout the directed devolution process. The inoculum without AP addition was used as the control. The culture was initially loaded with 1% overliming-treated AP solution and 99% inoculum (Generation 1, G1). When the cumulative biogas production leveled off, the cumulative AP loading was increased to 4% by exchanged 3 ml of the slurry with the same volume of the fresh overlimed AP. The operation of liquid exchange was repeated resulting in a stepwise increase of cumulative AP loading in the digestion system as 4% (G2), 7% (G3), 10% (G4), and 13% (G5).

The AP loading was then increased at a smaller pace to 13.5% (G6) and 14% (G7). At each AP loading, the slurry in the digestion system was sampled and stored at -80 °C. The samples were freeze dried before microbial community analysis.

2.2.5 Analyses

TS and VS of the digestion liquid were determined based on the standard methods (American Public Health Association, 1989). Chemical oxygen demand (COD) was determined using TNTplus Vial Test Kit with DRB200 Digital Reactor Block (Hach, CO, USA). The element contents were analyzed using TruSpec Micro CHNS analyzer (LECO Corporation, MI, USA). Volatile fatty acid (VFA) concentrations were determined using ion chromatography (IC) (Thermo Fisher Scientific, MA, USA) (Chantarasukon et al., 2008). Gas chromatography/mass spectroscopy (GC/MS) analysis to identify chemical composition of the AP was performed based on the method described previously (Pollard et al., 2012).

Biogas composition was analyzed using a gas chromatography (GC) equipped with a thermal conductivity detector. The HP-PLOT/Q column with 30 m length, 0.320 mm inner diameter, and 0.02 mm film thickness was used. Helium (99.999% purity) was used as the carrier gas with a constant flow rate of 2 mL/min. The temperature of the detector and column was 100 °C and 30 °C, respectively.

2.2.6 DNA extraction, PCR, and high-throughput sequencing

The samples from the directed evolution experiment were characterized for its microbial community using high-throughput sequencing. Total genomic DNA was extracted using the CTAB/SDS method (Sambrook and Russel, 2001). The DNA concentration and purity were verified using agarose gel (1%) electrophoresis. The V4-5 region of the bacterial 16S rRNA was amplified by PCR using the primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R

(5'- CCGTCAATTCCTTTGAGTTT-3'); and the V4-5 region of the archaeal 16S rRNA was amplified using the primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCCTTTGAGTTT-3'). The PCR reactions were conducted in a 30 μL mixture containing 15 μL 2 × Phusion Master Mix, 3 μL each of the primers (6 μM), 10 μL DNA (1 ng/ μL) and completed with 2 μL water. The amplification program was as follows: initial activation at 98°C for 1 min followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and extension at 72°C for 30 s. The PCR extension ended with a final extension at 72°C for 5 min. The PCR products were mixed with 1× loading buffer and detected on 2% agarose gel electrophoresis. Then, the PCR products were purified with Gene JET™ Gel Extraction Kit (Thermo Scientific, USA). The sequencing libraries were generated using Ion Plus Fragment Library Kit 48 rxns (Thermo Scientific, USA) and assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific, USA). The sequencing procedures were conducted on an Ion S5™ XL platform for bacteria and archaea respectively. The raw sequencing data was processed and analyzed following the previous report (Zhou et al., 2018).

2.3 Results and discussion

2.3.1 Comparison of different pretreatment methods for enhancing the AP digestibility

Four pretreatment methods, including overliming, Fenton's reagent oxidation, bleaching, and activated carbon adsorption, were tested to improve the AP digestibility. Table 2.1 shows the characteristics of raw and treated AP solutions. Overliming increased the pH of the AP to alkaline range (pH 10.15) while other treated AP solution did not change pH significantly compared to the raw AP. Compared to the raw AP, all the treatments resulted in a decreased COD indicating the loss of organic compounds. Specifically, bleaching removed 18.9% of COD through oxidation with hypochlorous acid (HClO), activated carbon reduced COD through

absorbing organic components, while overliming precipitated organic compounds. Bleaching also reduced the total VFAs content, while other three methods slightly increased VFAs.

Table 2.1. Characteristics of raw aqueous phase (AP) and AP treated with different methods.

Measurement		Raw AP	Overliming	Fenton's	Bleaching	Activated carbon
pH		2	10.15	2.17	2.97	2.19
COD	g/L	486	413	430	394	427
C	% wt	12.430	12.470	12.690	12.790	13.305
H	% wt	4.753	7.609	5.566	6.559	7.373
N	% wt	0.345	0.170	0.305	0.410	0.565
S	% wt	0.007	0.001	0.030	0.013	0.014
O	% wt	82.465	79.750	81.410	80.228	78.744
C/N		36	73.4	41.6	31.2	23.5
Formate	g/L	4.01	6.17	4.43	3.57	4.27
Acetate	g/L	28.98	31.98	29.79	25.09	29.5
Propionate	g/L	13.33	21.24	15.80	13.22	14.00
Total VFAs	g/L	46.32	59.39	50.01	41.88	47.77

AP treated with different methods were evaluated for their digestibility. The loading of the different AP solutions in the digestion systems was adjusted to either the same volumetric level (3%, v/v) or COD level (14.58 g COD/L, equivalent as 3% raw AP). Figure 2.1 shows the total biogas yield from Biomethane Potential (BMP) tests. Compared to the raw AP, the two oxidation treatments and activated carbon treatments did not improve the biogas yield, while overliming treatment significantly enhanced the biogas production ($p<0.05$).

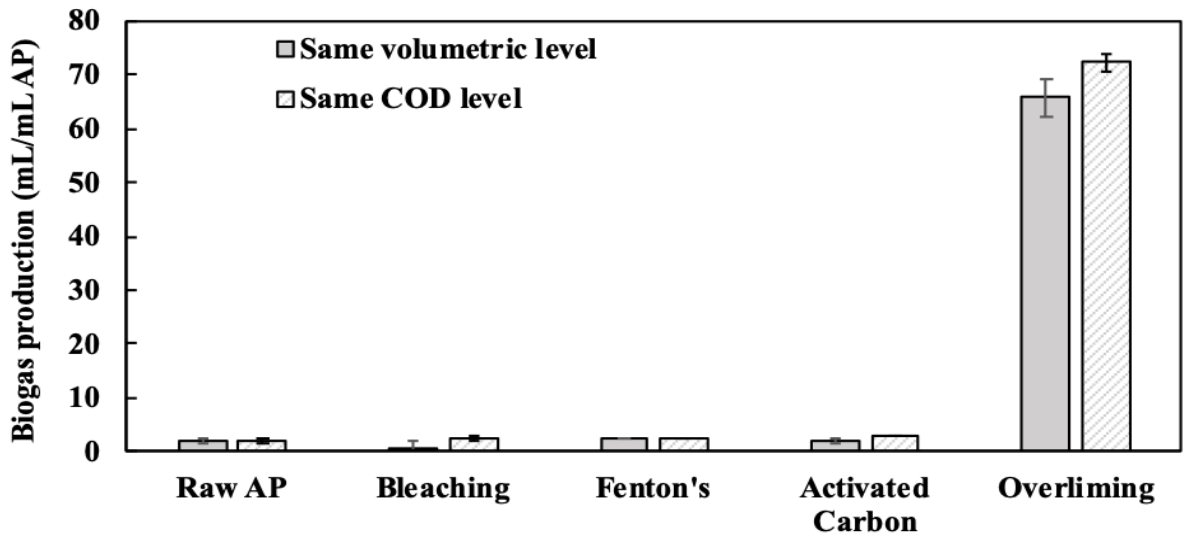

Figure 2.1. Total biogas yield by batch AD of raw AP and AP treated with different methods. The AP loading was based on (1) same volumetric level or (2) same COD level to the system.

The inhibition of the biogas production of the aqueous pyrolysis liquid has been reported previously (Torri and Fabbri, 2014). Here, the compounds in the AP before and after treatments were characterized. As shown in Table 2.2, raw AP contained various organic compounds such as phenol, HMF and creosol. Overliming treatment partially or completely removed a majority of the compounds while oxidation-based Fenton's reagent and bleaching method as well as activated carbon adsorption had limited capabilities of removing those inhibitors. Most compounds presented in Table 2.2 have been proved toxic to microbial cells. For instance, furans (2, 5-dimethoxy furan, and furfural) were reported to inhibit *E. coli* (Wang et al., 2013) and microalgae (Liang et al., 2013). In this work, both 2,5-dimethoxy furan and furfural were completely removed by overliming treatment, while these compounds still existed after treated by oxidation and activated carbon adsorption (Table 2.2). Phenols were another toxic compound group through altering cell membrane permeability, resulting in inactivation of enzymatic systems and cell lysis (Milledge et al., 2018). Table 2.2 shows that phenols were partially

Table 2.2 Compounds identified with GC-MS in raw AP and AP treated with different methods.

Retention time	Compound	Peak area				
		Raw AP	Overliming	Fenton's	Bleaching	Activated carbon
5.493	Ethane, 1,1-dimethoxy-	4686	0	7185	3019	7524
6.655	Trifluoroguanidine	4388	0	6689	6214	6679
10.094	Nitrosodimethylamine	2844	784	3568	3016	3959
13.1	Cyclopentanone	841	0	928	762	795
13.51	1-Hydroxy-2-butanone	3950	550	3709	3776	4386
14.425	Furan, tetrahydro-2,5-dimethoxy-	5882	0	10839	3346	10539
15.913	Furfural	24520	0	26697	19377	22973
18.15	2-Cyclopenten-1-one, 2-methyl-	2567	853	2595	2334	2136
18.602	2-Furanethanol, β-methoxy-(S)-	582	0	1088	950	784
18.757	Ethanone, 1-(2-furanyl)-	1131	0	1106	1200	657
21.401	2-Furancarboxaldehyde, 5-methyl-	1606	0	1580	1362	861
22.522	2(5H)-Furanone	3304	0	2686	1799	2178
23.729	Hexanal dimethyl acetal	1004	0	2293	566	1951
24.313	1,2-Cyclopentanedione, 3-methyl-	2362	0	1195	456	721
26.024	Phenol, 2-methoxy-	8267	1403	10514	3039	4389
29.72	Creosol	3446	864	2545	848	318
32.604	Phenol, 4-ethyl-2-methoxy-	1219	0	920	275	0

removed by overliming, bleach and activated carbon pretreatment; however, Fenton's reagent treatment increased phenols, probably through oxidation reactions. Moreover, hydroxyl radicals introduced by Fenton's reagent have been reported to trigger direct DNA alterations as well as other genotoxic effects to cells, particularly to the activity of methanogens (Singh et al., 2015). Collectively, the above results showed that overliming was the most effective treatment method to remove potential inhibitors and increased the biogas production in AD of AP solution. This treatment was thus further optimized.

2.3.2 Evaluation of AD performance of overlimed aqueous phase at batch and continuous modes

Fig. 2.2 shows that the biogas production at different loadings of overlimed AP in batch test. As shown in Fig. 2.2A, at 1% AP, biogas was produced quickly but slowed down after 5 days, probably due to the exhaustion of substrates. The biogas yield increased when the AP loading increased to 3%, but reduced at 5% AP. When the AP loading was further increased to 10%, biogas production completely ceased. Fig. 2.2B shows that the CH_4 yield reached the highest level at 3% AP loading, while 5% AP resulted in the highest CH_4 content.

Biogas production of the batch AD process were further modelled through Gompertz equation as follows:

$$P = P_{\max} \times \exp\left\{-\exp\left[\frac{R_{\max} \times e}{P_{\max}}(\lambda - t) + 1\right]\right\} \qquad (1)$$

where P is the cumulative biogas production (mL/mL AP), $P_{\max}$ is maximum biogas production potential (mL/mL AP), R_{max} is maximum biogas production rate (mL/mL AP/day), λ is lag phase time (d), and t is digestion time (d). The experimental of biogas production was regressed with the above equation using non-linear regression algorithm in MATLAB R2018a.

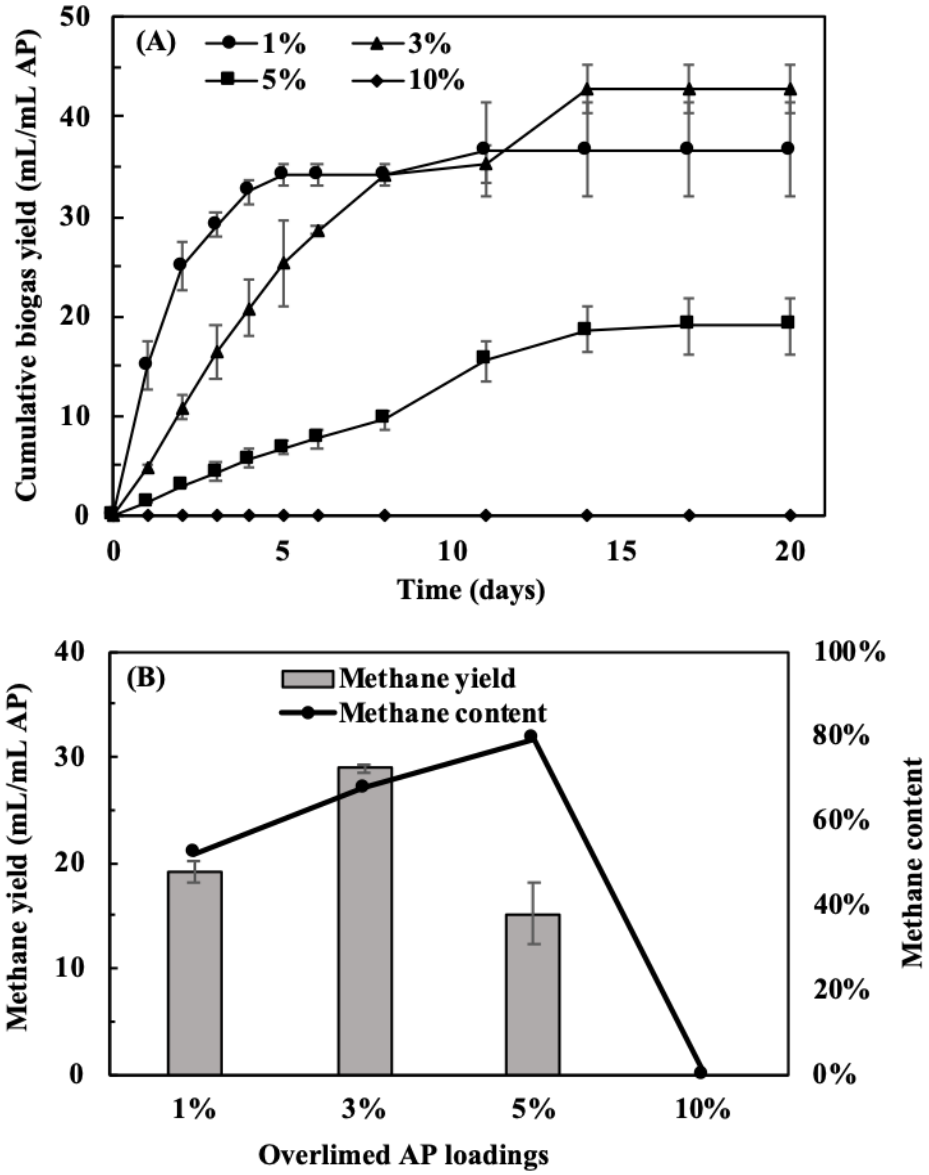

Figure 2.2. Batch (BMP) test of AD of overlimed AP at different volumetric loadings. (**A**) cumulative biogas yield; (**B**) methane yield and methane content.

The Gompertz parameters obtained from regression of the experimental data with Eq.1 are listed in Table 2.3. The results show a good fit to the experimental data ($R^2 > 0.983$). Increasing the AP loading to 5% reduced both P_{max} and R_{max}, while increased the lag phase to 0.78 d. All these results indicated an inhibitory effect of AP at this level.

Table 2.3. Gompertz parameters obtained from regression of experimental data with Eq. 1.

System	P_{max} (mL/mL AP)	R_{max} (mL/mL AP /d)	λ (d)	R^2
1%	35.47	13.01	0	0.9839
3%	42.16	5.16	0.07	0.9878
5%	20.90	1.57	0.78	0.9872

A continuous AD was further studied at an HRT of 20-day with variable AP concentrations in the feed. As shown in Fig. 2.3A, biogas production rate improved from 95.6 to 165 mL/L day^{-1} with increasing the influent AP loading from 6% to 18%. When the loading increased to 36%, the biogas production reduced gradually and ceased after a prolonged period of operation. The biogas yield of the AD system, however, decreased with the AP loading from 6% to 18%. During the continuous AD, the methane content maintained within 50%-65% through all the AP loading range (data not shown). Compared to the batch-based BMP test (Fig. 2.2A), the continuous AD can tolerate higher AP level (Fig. 2.3A). Fig. 2.3B shows pH stayed at alkaline range at all the AP levels, indicating a strong buffering capacity of the system. However, the VFAs increased with the AP. It has been reported that VFAs are inhibitory to methanogens at a concentration exceeding 6 g/L (Siegert and Banks, 2005). In this work, the excessive VFAs (8 g/L) at 36 % AP may be another reason for biogas production inhibition. The VFAs accumulation in the continuous AD may be caused by multiple factors such as overloading of the substrate, low inoculum to substrate ratio, temperature changes and the accumulation of inhibitory compounds (Nguyen et al., 2017). In addition, the AP could introduce extra external VFAs to the system (Table 2.1). Those factors eventually resulted an unbalanced acidogens and methanogens and thus, system failure.

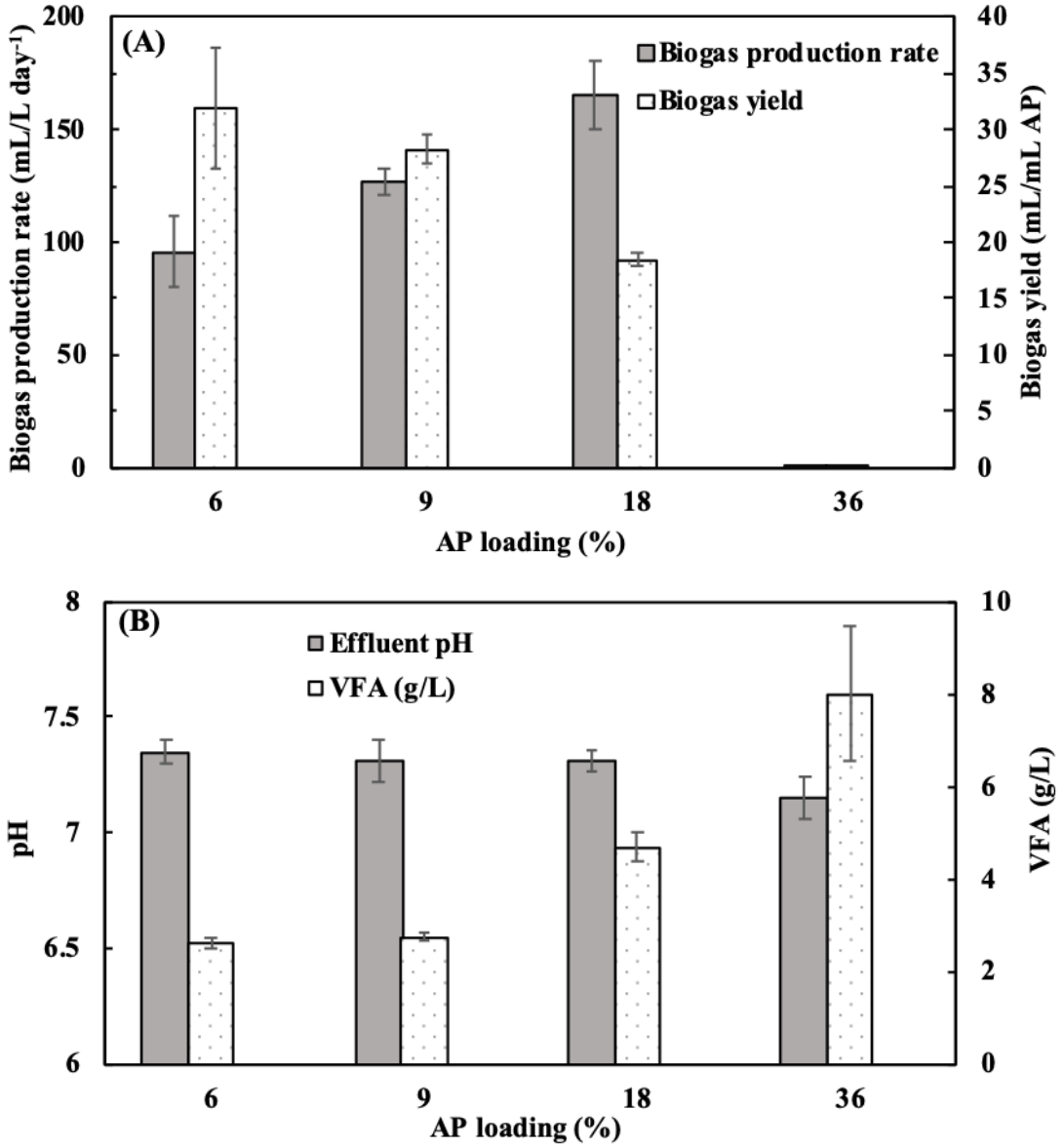

Figure 2.3. Continuous AD (HRT=20-day) of overlimed AP with different volumetric loadings. (**A**) steady state biogas production rate and biogas yield; (**B**) steady stae pH and VFA in the effluent. All data were means of last five consecutive data at steady state.

2.3.3 Enhancing microbe tolerance to the aqueous phase toxicity by directed evolution

Above results showed that although overliming was an effective method to mitigate the toxicity, the overlimed AP still resulted in inhibition in AD at certain levels. Due to the

complexity of the composition, it was extremely challenging to completely remove all the toxic compounds contained in AP. Therefore, another approach was exploited to enhance the AP utilization efficiency by developing a robust microbial community tolerant to the AP toxicity through directed evolution.

Directed evolution has been applied in fermentation processes for different purposes. For example, Liang et al. (2013) used directed evolution to improve the tolerance of microalga *C. sreinhardtii* to the toxicity of acetic acid-rich AP. Dai et al. (2016) identified that *Anaerobrance* and *Methanosarcina* increased the resistance to ammonium stress in a high solid AD after directed evolution. In the directed evolution process, the cells are subjected to non-lethal toxic environment and tend to adapt to it by acquiring mutations in DNA, which results in the tolerance to toxic compound(s). By sub-culturing cells with a gradual increase of toxic compound, strains that acquire these toxic-tolerance traits grow faster than their cohorts and eventually take over the entire population (Nealson and Rye, 2003). However, it should be noted that when directed evolution is applied to a mixed microbial consortium in AD process, it is highly possible that not only the toxicity tolerance ability of specific strain is increased but the amount of functional microbes (more tolerant microbe strains) can also be increased due to the natural selection.

Fig. 2.4 illustrated the process used to stepwise adapt microbial community to the high loading of AP. As shown in Fig. 2.4A, anaerobic digestion was initialized with 1% loading of overlimed AP (G1), with the biogas was produced at the exponential phase, the AP loading was stepwise increased to 4% (G2), 7% (G3) and 10% (G4) with no obvious inhibition, indicating the microbial community gradually adapted to the high AP environment. When the AP increased to 13% (G5), there was still significant amount of biogas produced, but the biogas production rate

started to slow down. Accordingly, the AP loading was still stepwise increased but at a slower pace, i.e., from 13% (G5) to 13.5% (G6) and then 14% (G7). At 14%, the cumulated biogas production almost ceased, suggesting the tolerance of microbial community to the toxic compounds reached to the maximum. Fig. 2.4B shows that the biogas yield at different generations was similar, maintained at around 50-60 mL biogas per mL AP. Throughout all the stage, the methane content of the biogas was around 55%-70% (data not shown).

The above results suggest directed evolution is a superior method to increase the tolerance of the microbial community to the AP toxicity. For example, in the batch AD process in which the AP was not stepwise increased but directly set at the designated level (Fig. 2.2A). We further introduced the concept of IC50 (AP dosage that inhibits biogas yield by 50%) to quantitatively define microbes' tolerance to AP before the after the directed evolution. Based on the results in Fig. 2.2, the IC50 value was determined as 4.8% in the batch AD without directed evolution. When the AD system was treated by directed evolution, the microbe's tolerance to AP was greatly enhanced. Even when the AP loading was increased to 14%, the biogas yield was still not reduced by 50% compared to the initial biogas yield, this indicates the IC50 value was much higher than 14%. Based on this fact, we concluded that the directed evolution significantly increased the microbes' tolerance to AP. As the AD performance is closely linked to the microbial population, the microbial community changes during the directed evolution were further characterized.

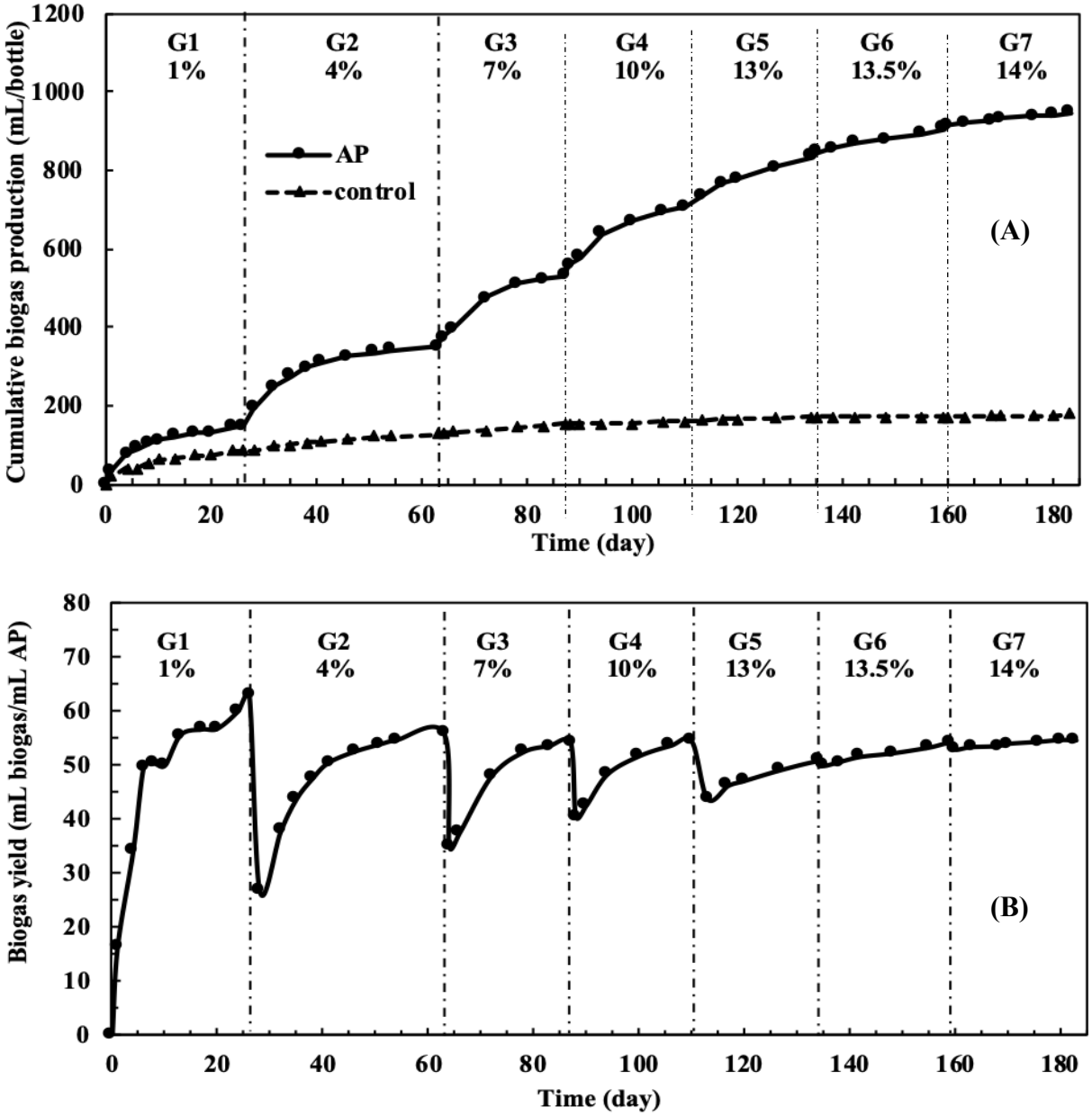

Figure 2.4. Cumulative biogas production of overlimed AP during the directed evolution process. The AP loading was stepwise increased from 1% to 14%. (**A**) Total biogas production per bottle; (**B**) cumulative biogas yield (mL biogas /mL AP). The control in (A) refers to the digestion system containing inoculum only (AP free). Biogas yield in (B) was deducted from the biogas generated from the control.

2.3.4 Characterization of microbial community of the digestion system during directed evolution

2.3.4.1 Diversity and richness of bacterial and archaeal communities

Digestion sludge samples at G1-G7 during directed evolution were subjected to DNA extraction and sequencing of archaeal and bacterial 16S rRNA genes. After removing the low-quality sequences and chimeras, the clean reads for bacteria and archaea ranged between 80,022-90,054 and 53,263-87,134, respectively (data not shown). The coverage of all the samples was above 99.7% (Table 2.4), indicating a high reliability of the data. The alpha diversity of the directed evolution samples was represented as operational taxonomic units (OTUs), Simpson index, Chao1, ACE estimation, and Shannon index (Table 2.4).

For bacterial community, the OTUs of the inoculum was 996, indicating a complex composition of the inoculum. After overlimed AP was added to the AD system, the OTUs of the digested samples were even higher. In addition to OTUs, the Simpson, Chao1, ACE and Shannon index of the digested samples were all higher than the inoculum, indicting a highly diversified bacterial community of those samples. Compared to that of the bacterial community, the OTUs of archaeal community were much lower (Table 2.4). The Simpson and Shannon indexes slightly decreased after directed evolution. Collectively, results in Table 2.4 suggested that the bacterial species were more diverse than archaeal species in AD system. With progression of stepwise increase of the AP loading, the diversity of bacterial community increased, whereas that of the archaeal community reduced.

Table 2.4 Richness and diversity indexes of bacterial and archaeal community at different generations during directed evolution of anaerobic digestion of overlimed AP.

Sample ID	Bacteria						Archaea					
	Coverage[a]	OUTs[b]	Simpson	Chao1	ACE	Shannon	Coverage	OUTs	Simpson	Chao1	ACE	Shannon
Inoculum	99.8%	996	0.95	1022	1044	6.08	99.9%	56	0.89	62	62	3.78
G1	99.7%	1110	0.97	1220	1228	6.65	100.0%	63	0.88	61	61	3.75
G2	99.8%	1101	0.97	1162	1191	6.61	99.9%	56	0.85	53	55	3.41
G3	99.8%	1120	0.97	1195	1163	6.82	99.9%	61	0.87	62	62	3.60
G4	99.7%	1085	0.96	1154	1162	6.47	99.9%	54	0.88	71	72	3.76
G5	99.8%	1058	0.97	1122	1129	6.50	99.9%	65	0.88	46	46	3.61
G6	99.8%	1078	0.97	1126	1126	6.60	99.9%	71	0.75	55	55	2.88
G7	99.8%	1069	0.97	1112	1128	6.61	99.9%	47	0.86	52	53	3.66
Control	99.8%	1067	0.97	1129	1135	6.48	99.9%	53	0.81	54	58	3.09

[a] Calculated by Good's formula.

[b] OTU: operational taxonomic unit.

2.3.4.2 Compositions of bacterial community

Fig.2.5 presented the major bacterial phyla and genera of different generations during directed evolution process. As shown in Fig. 2.5A, more than 13 bacterial phyla were detected and only those with relevant abundance more than 1% were presented. Overall, *Cloacimonetes*, *Firmicutes*, *Chloroflexi*, and *Proteobacteria* were the major phyla. The abundances of each phylum changed with the progression during the directed evolution, indicating their different capabilities of adapting the toxicity of the AP.

Cloacimonetes was the most abundant phylum (22.4% of the bacterial community) in the inoculum. As proteolytic amino-acid degraders, *Cloacimonetes* were commonly found in wastewater treatment plants (Zhang et al., 2016). The addition of the AP decreased the population of *Cloacimonetes* through G1 to G7 generations. However, the population was still higher than that in the control group, indicating the benefit of directed evolution to maintain this phylum. In addition to *Cloacimonetes,* another important phylum is *Bacteroidetes* which plays an important role in degradation of complex polymers such as degrading carbohydrates into monosaccharides (Zhao et al., 2017) and hydrolyzing proteins to VFAs and NH_3 (Liu et al., 2016). Fig. 2.5A shows that *Bacteroidetes* was abundance in inoculum (11.3%) but levelled off at 7.4% during directed evolution, while the control contained 6.9% of this phylum.

On the contrary, several phyla increased with the progression of the directed evolution generations. The abundance of *Firmicutes* was largely enriched, from 5.9% in inoculum to 20.4% in G7 sample. *Firmicutes* is a gram-positive bacterium widely reported in anaerobic digesters. It was one of the two most dominant phyla in solid-state AD of corn stover (Li et al., 2016). *Firmicutes* is capable of secreting a variety of cellulases, lipases, proteases and extracellular enzymes, degrading proteins, fats, cellulose, hemicellulose (Zhao et al., 2017). The

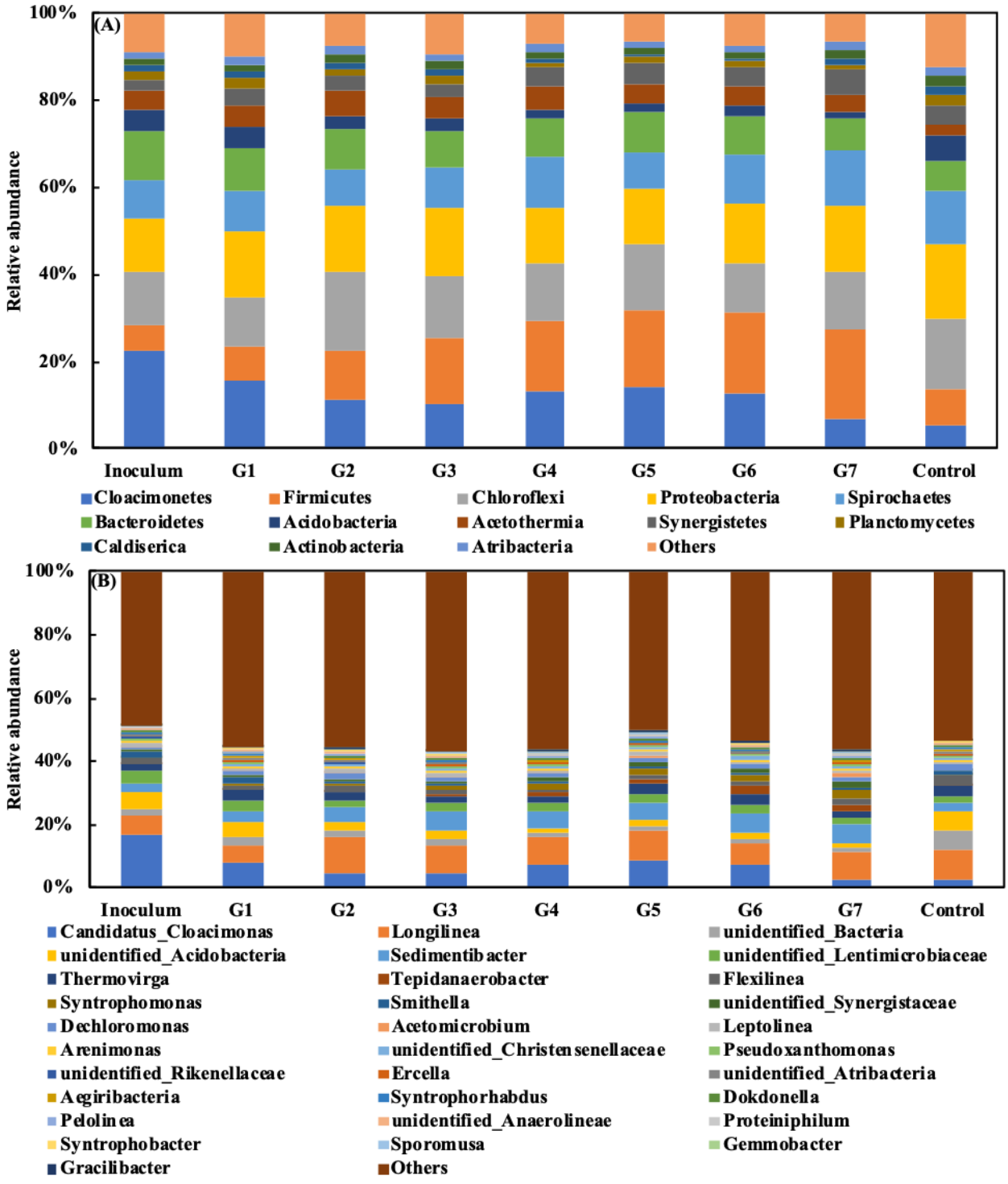

Figure 2.5. Characteristics of bacterial community of the different generations during directed evolution of anaerobci digetion of AP. (**A**) phylum level (relative abundance higher than 1% was presented); (**B**) genus level.

increased abundance of *Firmicutes* in the AP AD may be related to its role in consumption of VFAs, or adaption to high VFAs and low pH in the environment (Li et al., 2016). The abundance of the C*hloroflexi* and *Proteobacteria* during the directed evolution were also increased (12.3% to 13.3% for C*hloroflexi,* and 12.2% to 15.2% for *Proteobacteria*). These two phyla are typical acidogenic bacteria. *Chloroflexi* is also capable of H_2-oxidizing homoacetogenesis (Nobu et al., 2015), this phylum can also use different carbohydrates and amino acids as substrates (Di Maria et al., 2017) and was found in anaerobic digesters as well as other environments such as marine and freshwater sediments (Jabari et al., 2016). *Proteobacteria* involved in the degradation of organic matters (Lin et al., 2017) and was important consumer of glucose and various VFAs (Liu et al., 2016). It was one of the dominant phyla in AD of sewage sludge and played a crucial role in key steps - hydrolysis and acetogenesis (Wang et al., 2013). Fig. 2.5A also shows an increase of the abundance of *Spirochaetes*, a syntrophic acetate-oxidizing bacterium (Lee et al., 2013).

Regarding the composition of the genus (Fig. 2.5B), it shows that *Candidatus* Cloacimonas, belonging to the phyla of *Cloacimonetes*, was the most abundant in the inoculum but decreased after directed evolution. *Candidatus* Cloacimonas is a syntrophic bacterium capable of fermenting amino acids and produce H_2 (Alcántara-Hernández et al., 2017), as well as involved in propionate degradation (Ahlert et al., 2016). On the contrary, *Longilinea*, a strictly anaerobic and filamentous bacteria within the phylum Chlorofexi (Chen et al., 2017), was enriched after directed evolution. Since *Longilinea* has shown the ability to degrade the aromatic rings (Zhu et al., 2018), related compounds in the AP (such as phenol, creosol) probably stimulated its growth. As the main genus in Firmicutes, *Sedimentibacter* was involved in carbohydrates catabolism, such as amino acid utilizing and pyruvate metabolism (Ahlert et al.,

2016). The increased abundance of *Sedimentibacter* contributed to the Firmicutes phylum enrichment after directed evolution.

2.3.4.3 Compositions of archaeal community

The archaeal community also demonstrated a dynamic change during directed evolution. As shown in Fig. 2.6A, the inoculum was dominated by *Euryarchaeota* (92.8%). The addition of AP at G1 caused a significant decrease of *Euryarchaeota* while enriched *Bathyarchaeota*, a phylum mainly found in marine sediments involving in carbon cycling (Yu et al., 2018). After 7 generations, the abundance of *Euryarchaeota* recovered and was still predominant in the archaeal community.

As shown in Fig. 2.6B, the dominant genera detected in the inoculum were *Methanosaeta* (58.1%) and *Methanolinea* (25.3%). Zhao et al. (2016) also found these two genera as the main methanogens when the microbial community was acclimatized to butyric acid. *Methanosaeta* is a well-known aceticlastic methanogen, consuming electrons derived from the oxidation of propionate or butyrate to acetate (Wang and Li, 2016). The relatively high amount of propionate (3.1 g/L) in the inoculum probably favored the growth of *Methanosaeta*. However, the addition of overlimed AP through directed evolution decreased the abundance of *Methanosaeta*. While the abundance of *Methanosaeta* declined, *Methanolinea*, on the other hand, increased its abundance with evolving. As a hydrogenotrophic methanogen, *Methanolinea* was gradually adopted to the toxic environment with increased the AP loading. This can be explained by *Methanolinea's* capacity of surviving in phenol-degrading environment (Chen et al., 2018) since phenol is one of the major toxic compounds in the AP. It was reported that methanogens were the most sensitive to phenol compared to other bacteria group in AD (Chapleur et al., 2016), therefore, methanogenesis was the rate limiting process in this study. Another strictly

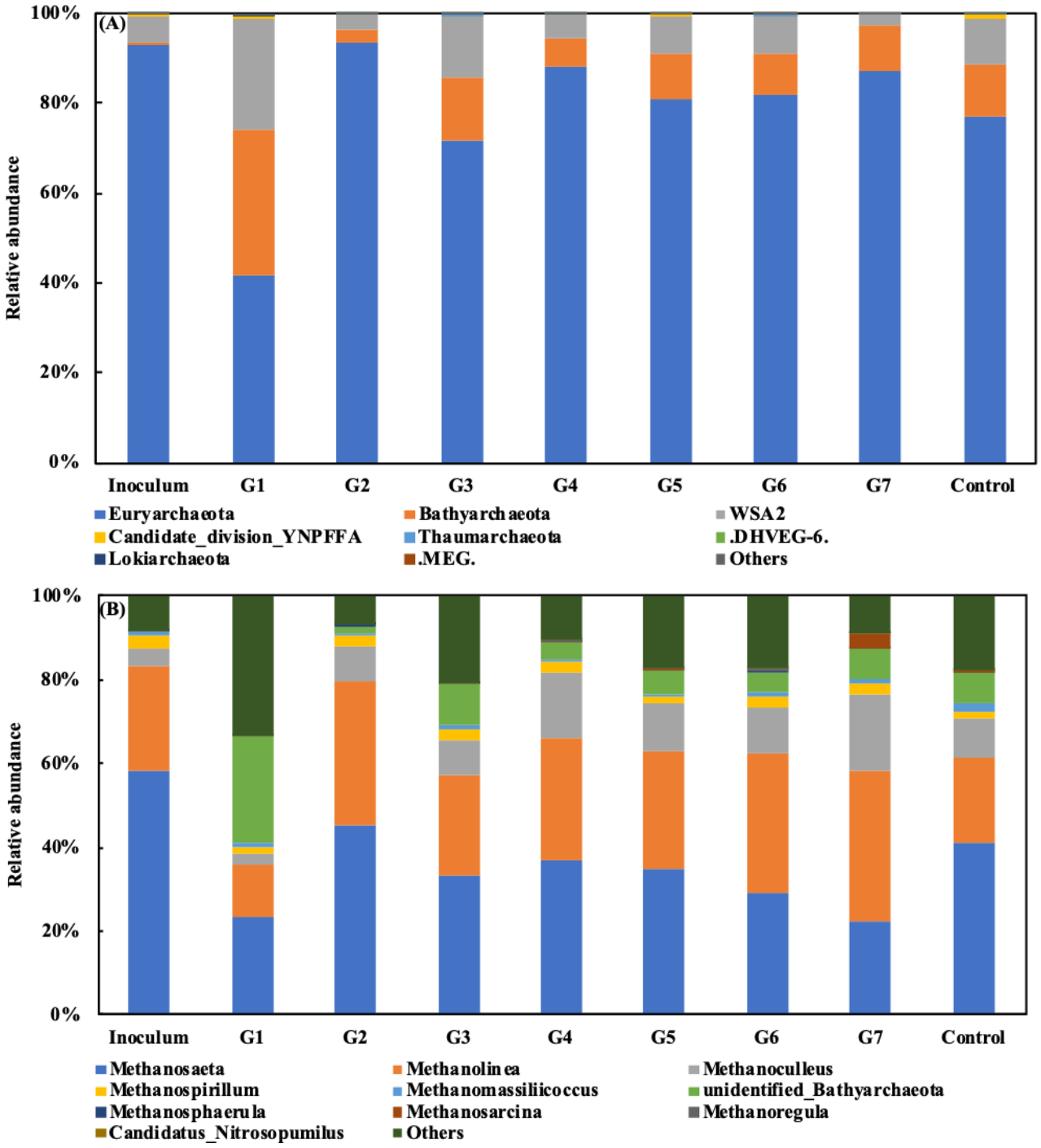

Figure 2.6. Characteristics of archael community of the different generations during directed evolution of anaerobic digetion of AP. (**A**) phylum level; (**B**) genera level. Only top 10 genera were presented.

hydrogenotrophic methanogen *Methanoculleus* was also enriched after evolution, increasing the abundance from 3.9% in inoculum to 17.9% in G7. *Methanoculleus* can utilize H_2/CO_2 and formate as carbon source to produce CH_4 (Demirel and Scherer, 2008), and was also a major contributor of anoxic acetate utilization in swine manure (Barret et al., 2012). In this study, high acetate in the AP favored the growth of *Methanoculleus*. Likewise, the increased abundance of *Bathyarchaeota* was also attributed to high acetate environment since it is a genus involving in acetate fermentation pathway (Zhang et al., 2016).

Methanospirillum, another H_2-utilizing methanogen (Li et al., 2018), existed in all samples but with low abundance, ranging from 1.51% to 3.37%. Since *Methanospirillum* and *Methanolinea* have similar growth conditions (substrates, optimum temperature and pH) (Koo et al., 2017), they might be competing in the digester. That could explain why *Methanolinea* was enriched while *Methanospirillum* was restricted after directed evolution.

Overall, Fig.2.6 shows that compared to the inoculum, the archaeal community in G1 changed significantly, which could be attributed to the initial stress of the AP addition. After directed evolution, the major contributors to methanogenesis shifted from acetoclastic methanogens to hydrogenotrophic methanogens. The factors that control the ratio of acetoclastic to hydrogenotrophic methanogenesis was not clearly understood, although H_2 concentration was thought to be a critical one (Demirel and Scherer, 2008). Further study is needed to elucidate the interactions between acetoclastic and hydrogenotrophic methanogenesis, and their specific functions in synergism.

2.4 Conclusion

This study demonstrated the feasibility of using the AP derived from biomass pyrolysis as AD substrate to product biogas. Overliming treatment was an effective method to reduce the toxicity of the AP by eliminating inhibitory compounds. The batch and continuous AD of overlimed AP can tolerate a loading of 3% and 18% without significant inhibition, respectively. The directed evolution of the AD process further enhanced the tolerance of microbial community to the AP toxicity. The bacterial phylum *Firmicutes* replaced *Cloacimonetes* as the dominant phylum; the hydrogenotrophic methanogens became the predominant genus over acetoclastic methanogens in archaeal community.

2.5 References

Ahlert, S., Zimmermann, R., Ebling, J., König, H., 2016. Analysis of propionate-degrading consortia from agricultural biogas plants. Microbiologyopen 5, 1027–1037. https://doi.org/10.1002/mbo3.386

Alcántara-Hernández, R.J., Taş, N., Carlos-Pinedo, S., Durán-Moreno, A., Falcón, L.I., 2017. Microbial dynamics in anaerobic digestion reactors for treating organic urban residues during the start-up process. Lett. Appl. Microbiol. 64, 438–445. https://doi.org/10.1111/lam.12734

American Public Health Association, A.W.W.A., 1989. Standard Methods for the Examination of Water and Wastewater.

Andreoni, V., Bonfanti, P., Daffonchio, D., Sorlini, C., Villa, M., 1990. Anaerobic digestion of wastes containing pyrolignitic acids. Biol. Wastes 34, 203–214. https://doi.org/10.1016/0269-7483(90)90022-K

Barret, M., Gagnon, N., Morissette, B., Topp, E., Kalmokoff, M., Brooks, S.P.J., Matias, F., Massé, D.I., Masse, L., Talbot, G., 2012. Methanoculleus spp. as a biomarker of methanogenic activity in swine manure storage tanks. FEMS Microbiol. Ecol. 80, 427–440. https://doi.org/10.1111/j.1574-6941.2012.01308.x

Chantarasukon, C., Tukkeeree, S., Rohrer, J., 2008. Determination of organic acids in wastewater using ion-exclusion chromatography and on-line carbonate removal (Application Note 291).

Chapleur, O., Madigou, C., Civade, R., Rodolphe, Y., Mazéas, L., & Bouchez, T., 2016. Increasing concentrations of phenol progressively affect anaerobic digestion of cellulose and associated microbial communities. Biodegradation, 27(1), 15-27.

Chen, C., Liang, J., Yoza, B.A., Li, Q.X., Zhan, Y., Wang, Q., 2017. Evaluation of an up-flow anaerobic sludge bed (UASB) reactor containing diatomite and maifanite for the improved treatment of petroleum wastewater. Bioresour. Technol. 243, 620–627. https://doi.org/10.1016/j.biortech.2017.06.171

Chen, C., Yao, X., Li, Q.X., Wang, Q., Liang, J., Zhang, S., Ming, J., Liu, Z., Deng, J., Yoza, B.A., 2018. Turf soil enhances treatment efficiency and performance of phenolic wastewater in an up-flow anaerobic sludge blanket reactor. Chemosphere 204, 227–234. https://doi.org/10.1016/j.chemosphere.2018.04.040

Dai, X., Yan, H., Li, N., He, J., Ding, Y., Dai, L., Dong, B., 2016. Metabolic adaptation of microbial communities to ammonium stress in a high solid anaerobic digester with dewatered sludge. Sci. Rep. 6, 1–10. https://doi.org/10.1038/srep28193

Demirel, B., Scherer, P., 2008. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: A review. Rev. Environ. Sci. Biotechnol. 7, 173–190. https://doi.org/10.1007/s11157-008-9131-1

Dewil, R., Appels, L., Baeyens, J., Degrève, J., 2007. Peroxidation enhances the biogas production in the anaerobic digestion of biosolids. J. Hazard. Mater. 146, 577–581. https://doi.org/10.1016/j.jhazmat.2007.04.059

Di Maria, F., Barratta, M., Bianconi, F., Placidi, P. and Passeri, D., 2017. Solid anaerobic digestion batch with liquid digestate recirculation and wet anaerobic digestion of organic waste: Comparison of system performances and identification of microbial guilds. Waste Manag. 59, 172-180.

Helffrich, D., Oechsner, H., 2003. Comparison of different laboratory techniques for the digestion of biomass. landtechnik 9, 27–30.

Hübner, T., Mumme, J., 2015. Integration of pyrolysis and anaerobic digestion - Use of aqueous liquor from digestate pyrolysis for biogas production. Bioresour. Technol. 183, 86–92. https://doi.org/10.1016/j.biortech.2015.02.037

Jabari, L., Gannoun, H., Khelifi, E., Cayol, J.L., Godon, J.J., Hamdi, M., Fardeaub, M.L., 2016. Bacterial ecology of abattoir wastewater treated by an anaerobic digestor. Brazilian J. Microbiol. 47, 73–84. https://doi.org/10.1016/j.bjm.2015.11.029

Jin, T., Rover, M.R., Petersen, E.M., Chi, Z., Smith, R.G., Brown, R.C., Wen, Z., Jarboe, L.R., 2017. Damage to the microbial cell membrane during pyrolytic sugar utilization and strategies for increasing resistance. J. Ind. Microbiol. Biotechnol. 44, 1279–1292. https://doi.org/10.1007/s10295-017-1958-4

Koo, T., Shin, S.G., Lee, J., Han, G., Kim, W., Cho, K., Hwang, S., 2017. Identifying methanogen community structures and their correlations with performance parameters in four full-scale anaerobic sludge digesters. Bioresour. Technol. 228, 368–373. https://doi.org/10.1016/j.biortech.2016.12.118

Laird, D.A., Brown, R.C., Amonette, J.E., Lehmann, J., 2009. Review of the pyrolysis platform for coproducing bio-oil and biochar. Biofuels, Bioprod. Biorefining 6, 547–562. https://doi.org/10.1002/bbb

Layton, D.S., Ajjarapu, A., Choi, D.W., Jarboe, L.R., 2011. Engineering ethanologenic Escherichia coli for levoglucosan utilization. Bioresour. Technol. 102, 8318–8322. https://doi.org/10.1016/j.biortech.2011.06.011

Lee, S.H., Park, J.H., Kang, H.J., Lee, Y.H., Lee, T.J., Park, H.D., 2013. Distribution and abundance of Spirochaetes in full-scale anaerobic digesters. Bioresour. Technol. 145, 25–32. https://doi.org/10.1016/j.biortech.2013.02.070

Li, W., Khalid, H., Zhu, Z., Zhang, R., Liu, G., Chen, C., Thorin, E., 2018. Methane production through anaerobic digestion: Participation and digestion characteristics of cellulose, hemicellulose and lignin. Appl. Energy 226, 1219–1228. https://doi.org/10.1016/j.apenergy.2018.05.055

Li, Y.F., Shi, J., Nelson, M.C., Chen, P.H., Graf, J., Li, Y., Yu, Z., 2016. Impact of different ratios of feedstock to liquid anaerobic digestion effluent on the performance and microbiome of solid-state anaerobic digesters digesting corn stover. Bioresour. Technol. 200, 744–752. https://doi.org/10.1016/j.biortech.2015.10.078

Lian, J., Garcia-Perez, M., Coates, R., Wu, H., Chen, S., 2012. Yeast fermentation of carboxylic acids obtained from pyrolytic aqueous phases for lipid production. Bioresour. Technol. 118, 177–186. https://doi.org/10.1016/j.biortech.2012.05.010

Liang, Y., Zhao, X., Chi, Z., Rover, M., Johnston, P., Brown, R., Jarboe, L., Wen, Z., 2013. Utilization of acetic acid-rich pyrolytic bio-oil by microalga Chlamydomonas reinhardtii: Reducing bio-oil toxicity and enhancing algal toxicity tolerance. Bioresour. Technol. 133, 500–506. https://doi.org/10.1016/j.biortech.2013.01.134

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. Bioresour. Technol. 241, 1027–1035. https://doi.org/10.1016/j.biortech.2017.06.011

Lin, Y., Wang, D., Wu, S., Wang, C., 2009. Alkali pretreatment enhances biogas production in the anaerobic digestion of pulp and paper sludge. J. Hazard. Mater. 170, 366–373. https://doi.org/10.1016/j.jhazmat.2009.04.086

Liu, C., Li, H., Zhang, Y., Si, D., Chen, Q., 2016. Evolution of microbial community along with increasing solid concentration during high-solids anaerobic digestion of sewage sludge. Bioresour. Technol. 216, 87–94. https://doi.org/10.1016/j.biortech.2016.05.048

Milledge, J.J., Nielsen, B. V., Harvey, P.J., 2018. The inhibition of anaerobic digestion by model phenolic compounds representative of those from Sargassum muticum. J. Appl. Phycol. 1–8. https://doi.org/10.1007/s10811-018-1512-4

Mortensen, P.M., Grunwaldt, J.D., Jensen, P.A., Knudsen, K.G., Jensen, A.D., 2011. A review of catalytic upgrading of bio-oil to engine fuels. Appl. Catal. A Gen. 407, 1–19. https://doi.org/10.1016/j.apcata.2011.08.046

Nealson, K.H., Rye, R., 2003. Evolution of Metabolism, in: Treatise on Geochemistry. Elsevier, pp. 41–61. https://doi.org/10.1016/B0-08-043751-6/08126-3

Nguyen, D.D., Chang, S.W., Cha, J.H., Jeong, S.Y., Yoon, Y.S., Lee, S.J., Tran, M.C., Ngo, H.H., 2017. Dry semi-continuous anaerobic digestion of food waste in the mesophilic and thermophilic modes: New aspects of sustainable management and energy recovery in South Korea. Energy Convers. Manag. 135, 445–452. https://doi.org/10.1016/j.enconman.2016.12.030

Nobu, M.K., Narihiro, T., Rinke, C., Kamagata, Y., Tringe, S.G., Woyke, T., Liu, W.T., 2015. Microbial dark matter ecogenomics reveals complex synergistic networks in a methanogenic bioreactor. ISME J. 9, 1710–1722. https://doi.org/10.1038/ismej.2014.256

Pollard, A.S., Rover, M.R., Brown, R.C., 2012. Characterization of bio-oil recovered as stage fractions with unique chemical and physical properties. J. Anal. Appl. Pyrolysis 93, 129–138. https://doi.org/10.1016/j.jaap.2011.10.007

Rengaraj, S., Moon, S.H., Sivabalan, R., Arabindoo, B., Murugesan, V., 2002. Agricultural solid waste for the removal of organics: Adsorption of phenol from water and wastewater by palm seed coat activated carbon. Waste Manag. 22, 543–548. https://doi.org/10.1016/S0956-053X(01)00016-2

Sambrook, J., Russel, D.W., 2001. Molecular cloning: A Laboratory manual. Q. Rev. Biol. 76(3), 348–349.

Seyedi, S., Venkiteshwaran, K., Zitomer, D., 2019. Toxicity of Various Pyrolysis Liquids From Biosolids on Methane Production Yield 7, 1–12. https://doi.org/10.3389/fenrg.2019.00005

Siegert, I., Banks, C., 2005. The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. Process Biochem. 40, 3412–3418. https://doi.org/10.1016/j.procbio.2005.01.025

Singh, R., Dong, H., Liu, D., Zhao, L., Marts, A.R., Farquhar, E., Tierney, D.L., Almquist, C.B., Briggs, B.R., 2015. Reduction of hexavalent chromium by the thermophilic methanogen Methanothermobacter thermautotrophicus. Geochim. Cosmochim. Acta 148, 442–456. https://doi.org/10.1016/j.gca.2014.10.012

Torri, C., Fabbri, D., 2014. Biochar enables anaerobic digestion of aqueous phase from intermediate pyrolysis of biomass. Bioresour. Technol. 172, 335–341. https://doi.org/10.1016/j.biortech.2014.09.021

Wang, J., Li, Y., 2016. Synergistic pretreatment of waste activated sludge using CaO_2 in combination with microwave irradiation to enhance methane production during anaerobic digestion. Appl. Energy 183, 1123–1132. https://doi.org/10.1016/j.apenergy.2016.09.042

Wang, W., Xie, L., Luo, G., Zhou, Q., Angelidaki, I., 2013. Performance and microbial community analysis of the anaerobic reactor with coke oven gas biomethanation and in situ biogas upgrading. Bioresour. Technol. 146, 234–239. https://doi.org/10.1016/j.biortech.2013.07.049

Wang, X., Yomano, L.P., Lee, J.Y., York, S.W., Zheng, H., Mullinnix, M.T., Shanmugam, K.T., Ingram, L.O., 2013. Engineering furfural tolerance in *Escherichia coli* improves the fermentation of lignocellulosic sugars into renewable chemicals. Proc. Natl. Acad. Sci. 110, 4021–4026. https://doi.org/10.1073/pnas.1217958110

Yu, T., Wu, W., Liang, W., Lever, M.A., Hinrichs, K.-U., Wang, F., 2018. Growth of sedimentary Bathyarchaeota on lignin as an energy source. Proc. Natl. Acad. Sci. U. S. A. 115, 6022–6027. https://doi.org/10.1073/pnas.1718854115

Yue, X., Arena, U., Chen, D., Lei, K., Dai, X., 2019. Anaerobic digestion disposal of sewage sludge pyrolysis liquid in cow dung matrix and the enhancing effect of sewage sludge char. J. Clean. Prod. 235, 801–811. https://doi.org/10.1016/j.jclepro.2019.07.033

Zhang, W., Ding, W., Yang, B., Tian, R., Gu, S., Luo, H., Qian, P.Y., 2016. Genomic and transcriptomic evidence for carbohydrate consumption among microorganisms in a cold seep brine pool. Front. Microbiol. 7, 1–11. https://doi.org/10.3389/fmicb.2016.01825

Zhang, Z., Li, W., Zhang, G., Xu, G., 2014. Impact of pretreatment on solid state anaerobic digestion of yard waste for biogas production. World J. Microbiol. Biotechnol. 30, 547–554. https://doi.org/10.1007/s11274-013-1473-3

Zhao, X., Liu, J., Liu, J., Yang, F., Zhu, W., Yuan, X., Hu, Y., Cui, Z., Wang, X., 2017. Effect of ensiling and silage additives on biogas production and microbial community dynamics during anaerobic digestion of switchgrass. Bioresour. Technol. 241, 349–359. https://doi.org/10.1016/j.biortech.2017.03.183

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 Res. 102, 475–484. https://doi.org/10.1016/j.watres.2016.07.005

Zhou, H., Sheng, Y., Zhao, X., Gross, M., Wen, Z., 2018. Treatment of acidic sulfate-containing wastewater using revolving algae biofilm reactors: Sulfur removal performance and microbial community characterization. Bioresour. Technol. 264, 24–34. https://doi.org/10.1016/j.biortech.2018.05.051

Zhu, Y., Xu, J., Cao, X., Cheng, Y., 2018. Characterization of functional microbial communities involved in different transformation stages in a full-scale printing and dyeing wastewater treatment plant. Biochem. Eng. J. 137, 162–171. https://doi.org/10.1016/j.bej.2018.05.026

CHAPTER 3. EFFECT OF BIOMASS ACID PRETREATMENT ON BIOCHAR PROPERTIES AND ITS FUNCTION ON ANAEROBIC DIGESTION OF MUNICIPAL SLUDGE

Abstract

Fast pyrolysis of biomass pretreated with mineral acid produces high quality of bio-oil, however, biochar produced from this process has not been characterized and its performance as an additive to anaerobic digestion (AD) is unknown. This study reports the effects of physicochemical properties of two distinct biochars on AD of municipal sludge: one was produced from pyrolysis of raw corn stover (BC-1); the other produced from sulfuric acid pretreatment of the same corn stover (BC-2). BC-1 had higher carbon content, alkalinity, specific surface area but lower ash and sulfur than BC-2. Both biochars contained volatile fatty acids and residual sugars serving as substrates for anaerobic bacteria to enhance biogas/methane production. When the biochars were added to the AD, their effects on biogas production showed contrary trends. Addition of BC-1 resulted in higher methane yield and content, while BC-2 reduced methane yield and content. The strong buffering capacity of BC-1 was a major factor for its beneficial effect, while the high sulfur content of BC-2 was inhibitory to anaerobic bacteria. Collectively, the results indicated that the effects of biochar on AD depends on biochar properties, and selection of appropriate biochar was important in facilitating higher biogas production and maintaining a stable process.

3.1 Introduction

Sewage sludge is the semi-solid residue resulted from municipal wastewater treatment processes (Raheem et al., 2018). As a potential source of secondary environmental pollution, sludge can cause human health problems if not disposed properly. Anaerobic digestion (AD) is an effective technology to treat the sludge by reducing the solid content and recovering biogas as an energy source. Biogas as a renewable fuel generates cellulosic (D3) and advanced fuel (D5) renewable identification numbers (RINs) (Shen et al., 2018). Additionally, digestate from AD can be used as fertilizer as it contains high amounts of nutrients such as nitrogen and phosphorus.

Various studies have been conducted to enhance yield and quality (methane content) of biogas produced from AD of sludge. For example, thermal pretreatment has been used to enhance sludge digestibility and dewaterability (Takashima et al., 2018). Co-digestion of sludge with other organic wastes such as food wastes or paper pulp rejects resulted in higher methane yield and greater chemical oxygen demand (COD) removal (Xie et al., 2017). Various additives in AD have also been studied to enhance biogas production through facilitating microbial growth, biofilm formation, sulfur removal and CO_2 sequestration (Arif et al., 2018a). For instance, activated carbon was used in AD as a supporting material to facilitate bacteria attachment and shorten the sludge granulation time (Arif et al., 2018b).

In recent years, biochar as an additive to enhance AD performance has drawn great interests. As a carbon rich material derived from pyrolysis of biomass, biochar is well known as soil amendment to improve the plant growth (Sanchez-Monedero et al., 2018). When applied to AD, biochar facilitates biofilm formation due to its porous structure (Mumme et al., 2014) and promotes direct interspecies electron transfer (DIET) of functional microbes due to its redox-active property (Wang et al., 2018). Oxidized biochar serves as electron acceptor to facilitate

organic compound degradation, while reduced biochar can be used as electron donor for nitrate and magnetite reduction (Yuan et al., 2018). Biochar addition also increases alkalinity and mitigates ammonia inhibition in AD system (Shen et al., 2015). Fagbohungbea et al. (2016) reported that biochar absorbed toxic limonene compounds and reduced the lag phase with enhanced methane production in AD of citrus peel waste.

While the beneficial effects of biochar on AD have been widely reported, some negative impacts of biochar on AD of different feedstocks were also observed. For example, Qin et al (2017) found that magnetic biochar (fabricated with $FeCl_3$) decreased the methane production from organic fraction of municipal solid waste (OFMSW) by 38% due to the competition with iron oxide for electron. In a co-digestion system containing food waste and activated sludge, biochar addition reduced the methane yield (Li et al., 2018). Shen et al. (2016) also reported that higher loading of biochar caused inhibition in biogas and methane production in sludge AD.

The above results exhibit different effects of biochar on the AD systems due to differences in biochar properties, which arises from various factors including composition and structure of the biomass, pretreatments, pyrolysis conditions and post pyrolysis treatment. For instance, the specific surface area of biochar is strongly influenced by the type of biomass while pH of biochar is mainly determined by the pyrolysis temperature (Li et al., 2019). The increasing interest in applying biochar in AD creates a need for comprehensive understanding of biochar physicochemical properties and their implication on AD performance (Ghidotti et al., 2017).

Iowa State University researchers have performed a significant study on fast pyrolysis of lignocellulosic biomass for bio-oil as drop-in fuels (Laird et al., 2009). In particular, mineral acid was used to treat feedstock to passivate catalytic activity of alkali and alkaline earth metals (AAEMs) so that the quantity and quality of bio-oil can be improved (Dalluge et al., 2014).

However, biochar produced from fast pyrolysis of this acid pretreated feedstock has not been characterized and its performance as an additive to AD is unknown. The aim of this study is to explore the effect of physicochemical properties of biochar on anaerobic digestion (AD) of municipal sludge. Two distinct biochars were employed: one was produced from pyrolysis of raw corn stover while the other was from sulfuric acid pretreated corn stover.

3.2 Materials and methods

3.2.1 Biochar preparation.

Two distinct biochars were produced from fast pyrolysis of raw and pretreated corn stover. The corn stover was dried and ground with a hammer mill to reduce the particle size to less than 1/8-in through screening. The particles were then directly used for fast pyrolysis or pretreated with sulfuric acid prior to fast pyrolysis. To perform the pretreatment, 1kg diluted sulfuric acid (3.5 wt %) was mixed with 1 kg corn stover particles. The mixture was incubated for 24h at room temperature (~25°C) and then dried at 105°C. Fast pyrolysis was conducted in a continuous fluidized bed reactor at 500°C (Polin et al., 2019). Biochar produced from the raw corn stover was designated as Biochar #1 (BC-1), while biochar produced from the acid-pretreated corn stover particles was designated as Biochar #2 (BC-2).

3.2.2 Characterization of biochar.

The elemental composition (C, H, O, N, S) of biochar was determined with a TruSpec Micro CHNS analyzer (LECO Corporation, MI, USA). The content of moisture, ash, volatile matter and fixed carbon were analyzed using the standard ASTM method (D3172-89). To determine volatile fatty acids (VFAs) and sugar contents in biochar, 1 g raw biochar materials were first mixed biochar with 20 mL DI water in a flask. The mixture was incubated in an orbital shaker (100 rpm) at room temperature overnight and then filtered through 0.45 μm filters to

collect the supernatant. The rinse of the solid was repeated three times and the collected supernatant was combined for determination of VFAs and sugar concentration with an ion chromatograph (IC) (Thermo Fisher Scientific, MA, USA) based on a standard method (Chantarasukon et al., 2008) The VFA and sugar contents in biochar were calculated based on the concentrations of VFA and sugar in the liquid phase and the mass of the original biochar used.

Scanning electron micrographs of biochar were produced with a Field Emission Scanning Electron Microscope (SEM) (SU4800 Hitachi, Japan) under 1,000 to 5000× magnifications. Specific surface area (SSA) of the biochar (area/unit mass of biochar) was determined using the Ethylene Glycol Monoethyl Ether (EGME) method (Cerato and Lutenegger, 2002). Briefly, 0.5 g oven-dried (105°C) biochar was added to a pre-weighted aluminum pan; prescribed amount of EGME (4 mL for BC-1 and 2 mL for BC-2) was added to completely cover the biochar particles. The pans were placed in a vacuum oven under at least 650 mm Hg of vacuum. The mass of EGME absorbed per gram of biochar was recorded at 24th, 48th and 72nd hour until the weight of the mixture did not vary more than 0.001 g. SSA was calculated as:

$$SSA = \frac{W_a}{0.000286 \times W_b} \qquad (1)$$

where W_a (g) is the mass of EGME absorbed by the biochar and W_b (g) is the mass of biochar. The constant 0.000286 is the mass of EGME required to form a monomolecular layer per square meter of surface area (g/m^2).

To perform the acid titration of biochar, 0.5 g biochar was mixed with 20 mL DI water in a 125-mL flask, the mixture was vigorously stirred with a magnetic stirrer at room temperature. The sample was then manually titrated by adding 0.1 M HCl at 0.4 mL/min to bring the pH to 2.0 (end point). The pH data was recorded every 15 seconds.

To determine the content of trace elements (Al, Ca, Fe, Mg, P, Na and K) in biochar, approximately 500 mg biochar was mixed with nitric acid (10 mL) and digested at 180°C with a Multiwave 3000 Anton Paar Microwave system (Graz, Australia) based on EPA 3051A method (USEPA, 2007). Then the concentrations of the trace element were analyzed using an inductively coupled plasma-optical emission spectrometer (ICP-OES) using EPA 200.7 Method (EPA., 1994). To measure the trace elements in biochar leachate, 2 mL leachate was mixed with nitric acid (0.2 mL) and water (7.8 mL), the mixture was then subject to ICP-OES analysis.

3.2.3 Anaerobic digestion setup.

Anaerobic bacterial inoculum and activated sludge were obtained from an anaerobic digester at Water Pollution Control Facility at Muscatine, IA. The materials were stored in air-tight bottles at 4 °C prior to use. Total solids (TS) and volatile solids (VS) of the inoculum were 7.28 g/L and 3.88 g/L, respectively; TS and VS of the sludge were 35.52 g/L and 26.19 g/L, respectively. Anaerobic digestion was performed through Biochemical Methane Potential (BMP) test in 125 mL serum bottles with 80 mL working volumes. The bottles were incubated in a shaker at 37 °C with 100 rpm. Each test condition was conducted in three replicates.

The first AD experiment was to evaluate the effect of two types of raw biochar materials on AD. Each serum bottle contained 60 mL inoculum and 10 mL sludge. Each of the two types of biochar (BC-1 and BC-2) was added to the bottle at low (16 g/L), medium (32 g/L) and high (66.6 g/L) loadings, respectively. The AD system without biochar was treated as the control. After biochar addition, deionized (DI) water was added to the bottle to make up the working volume to 80 mL.

The second AD experiment was to study the effect of sulfur in the biochar. Each serum bottle contained 60 mL inoculum and 10 mL sludge. The bottles were then added with 66.6 g/L

of BC-1, 66.6 g/L of BC-2, and 66.6 g/L of BC-1 with 6 g/L sodium sulfate, respectively. The biochar-free AD was the control. DI water was added to make up the working volume to 80 mL.

The third AD experiment was to evaluate the effect of the organic compounds contained in biochar on AD. First, raw BC1 and BC-2 were respectively mixed with DI water (1:10 w/w) in a flask and incubated in an orbital shaker (100 rpm) at room temperature for 24 hr. The mixture was then filtered through 0.45 μm filters to separate the mixture into rinsed biochar and supernatant (leachate). The rinsing/separation operations were repeated three times to ensure the water-soluble organic compounds was completely transferred from biochar solid into the leachate. The washed biochar solid was dried at 105 °C. The leachate and washed biochar were then respectively evaluated for their effects on AD. Serum bottle loaded with the 20 mL inoculum was added with either leachate (0.666 L/L) or washed biochar (66.6 g/L). DI water was used to make up the working volume to 80 mL.

3.2.4 Analysis

Total solids (TS) and volatile solids (VS) were measured based on the standard methods (APHA et al., 2005) Total alkalinity was measured by Hach test kit (Method 8203) (CO, USA). Biogas was measured in a water replacement setting. Biogas composition was analyzed by a gas chromatography (GC) equipped with a thermal conductivity detector. The HP-PLOT/Q column has 30 m long, 0.320 mm inner diameter with a 0.02 mm film thickness. Helium (99.999% purity) was used as the carrier gas with a constant flow rate of 2 mL/min. The temperature of the detector and column was 100 °C and 30 °C, respectively.

3.2.5 Modeling of AD process with biochar.

Cumulative methane production was modelled with the Gompertz equation (Li et al., 2014):

$$P = P_{\max} \times \exp\left\{-\exp\left[\frac{R_{\max} \times e}{P_{\max}}(\lambda - t) + 1\right]\right\} \qquad (2)$$

where P is the cumulative methane production (mL/g VS), $P_{\max}$ is the maximum methane potential (mL/g VS), R_{max} is the maximum methane production rate (mL/g d^{-1} VS), e is Euler's Number (2.718), λ is the lag phase time (d), and t is the digestion time (d). Gompertz parameters were regressed from experimental data using non-linear regression algorithm in MATLAB R2019a software.

3.3 Results and discussion

3.3.1 Modeling of AD process with biochar.

The physiochemical properties of the two types of biochar were characterized. As shown in Table 3.1, BC-1 had a higher moisture content but lower ash content than BC-2. The high ash content in BC-2 was due to the reaction of sulfuric acid with naturally occurring AAEM in corn stover to produce sulfates. BC-1 had higher fixed carbon than BC-2, which is a parameter inversely correlated with ash content (Crombie et al., 2013). The trend of carbon content between BC-1 and BC-2 was consistent with the fixed carbon, as the acid pretreatment of feedstock increased bio-oil yield resulting in less carbon content in BC-2 (Oudenhoven et al., 2015). The volatile matter, the content of the elements H, N, and O between BC-1 and BC-2 were in a similar range. However, BC-2 contained much higher S than BC-1 arising from the formation of sulfate during sulfuric acid pretreatment. From the elemental analysis, H:C and O:C ratios were calculated, which are commonly used to evaluate the degree of carbonization in biochars (Kookana et al., 2011). In general, a H:C ratio less than 0.7 commonly resulted in a stable volatile organic compounds (VOCs) at ambient temperatures (Ghidotti et al., 2017). The

low H:C and O:C ratios reported in in this work (Table 3.1) indicate the two biochar materials have a high degree of aromaticity and chemical stability with minimal VOCs emission.

Table 3.1 also shows that the specific surface area of BC-1 is nearly two-fold greater than for BC-2. It has shown previously that biochar with high surface area is beneficial for biofilm formation (Sunyoto et al., 2016) and CO_2 sequestration (Shen et al., 2016) in AD. The large specific surface area also provides sheltered spaces for microbes to attach while avoiding direct exposure to acids or potential metabolic inhibitors (Li et al., 2018).

Sugars and VFAs are major organic compounds found in biochar. Sugars are suitable substrates for acidogens, while formic acid and acetic acid in VFAs are substrates for methanogens. As shown in Table 3.1, the two biochars contained considerable amount of sugars particularly levoglucosan, an anhydrosugar produced from fast pyrolysis of lignocellulosic biomass (Li et al., 2017). BC2 had a higher sugar content than BC1. On the other hand, VFAs in BC-1 was 6.7-fold higher than that in BC-2. VFAs were known to mainly derived from deacetylation of the hemicellulose (Mohan et al., 2006). The trend of increased sugar and reduced VFAs in BC-2 relative to BC1 was similar to those compounds in the bio-oil derived from different feedstock (Dalluge et al., 2014). Table 3.1 also shows that the most abundant inorganic constituent in the biochars was potassium with smaller amounts of calcium and magnesium (Table 3.1). Corn stover is well known to contain large amounts of potassium content due to K-rich fertilizers are commonly used for corn cultivation, which reports to the biochar during pyrolysis (Wang et al., 2013).

The buffering capacities of BC-1 and BC-2 were compared through titration. As shown in Figure 3.1, both BC1 and BC2 were initially alkaline, while BC-1 had higher initial pH than BC-2. The pH of BC-2 decreased more rapidly than BC-1 with acid addition. In total, BC-1

consumed more HCl (44.70 mL/g biochar) than BC-2 (30.30 mL/g biochar) to reach pH 2, indicating BC-1 had a higher buffering capacity than BC-2, as expected from the pretreatment with acid. Since pH fluctuation in AD is expected as compositions are undergoing degradation and metabolism, high buffering capacity during AD is thus desired to resist pH fluctuation caused by VFAs production and accumulation (Zhou and Wen, 2019).

Table 3.1. Physiochemical properties of two types of biochar produced from fast pyrolysis of corn stover

Parameter (Unit)	**BC-1[a]**	**BC-2[a]**
Moisture (wt%)	6.10	4.15
Ash (wt%)	20.42	33.92
Fixed carbon (wt%)	44.21	33.44
Volatile matter (wt%)	29.27	28.56
C (wt%)	59.45	42.71
H (wt%)	2.74	1.71
N (wt%)	1.27	1.25
O (wt%)	16.05	18.31
S (wt%)	0.07	2.10
H:C	0.046	0.040
O:C	0.270	0.429
Specific surface area (m^2/g) [b]	216.08	109.10
Sugars (mg/g biochar)	4.705	5.631
Levoglucosan	4.395	5.452
Cellobiosan	0.262	0.129
Glucose	0.048	0.050
Total VFAs (mg/g biochar)	27.512	4.093
Formate	0.495	0.019
Acetate	3.698	0.010
Propionate	1.018	0.009
Isobutyrate	22.301	4.055
Elements (mg/g biochar)		
Al	3.48	3.78
Ca	12.04	18.99
Fe	3.04	2.92
Mg	8.24	9.02
P	1.79	1.64
Na	0.50	0.29
K	21.71	25.13

[a] BC-1: Biochar produced from fast pyrolysis of raw corn stover; BC-2: Biochar produced from fast pyrolysis of acid pretreated corn stover.

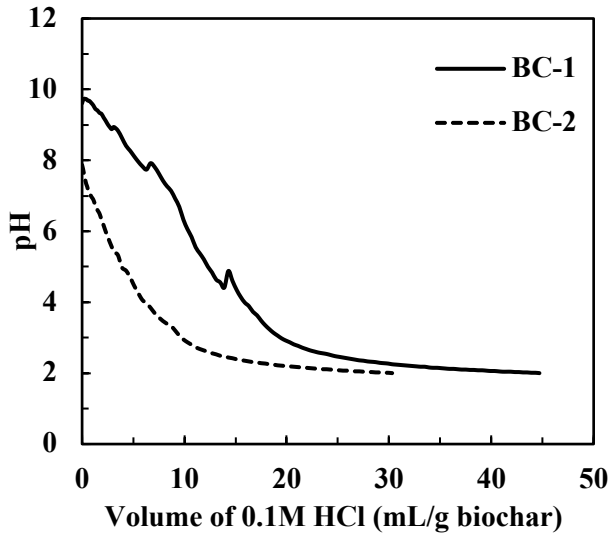

Figure 3.1. Titration of two types of biochar with 0.1 M HCl.

The SEM images show that both BC-1 and BC-2 had irregular structures and coarse surfaces (Figure 3.2). BC-1 retained more intact structure than BC-2, which shows evidence of disintegration to small particles from the acid pretreatment. The honeycomb-like structure reported by others (Shen et al., 2016; Romero-Güiza et al., 2017) was not observed in this study, which is assumed to arise from differences feedstock type and pyrolysis conditions.

3.3.2. Comparison of AD performance for the two types of biochar.

As shown in Figure 3.3A, addition of BC-1 to the digestion system resulted in significantly higher biogas than the control for which no biochar was added (p<0.01); the increment of the biogas production is positively related with biochar loadings. When BC-2 was added to the AD system, however, only the highest loading resulted in significant (p<0.05) biogas enhancement. The medium- and low-loading of BC-2 did not significant affect biogas production (p>0.05) (Figure 3.3B). The trend of methane production with BC-1 addition was similar to that of biogas production (Figure 3.3C), while addition of BC-2 did not significantly enhance methane yield for any of the three loading levels (Figure 3.3D). The biochars also

produced different profiles for methane content. As shown in Figure 3.3E, upon addition of untreated biochar (BC-1) methane yields was initially higher before leveling off to a level similar to the control. The increased methane content observed for BC-1 addition may be due to the removal of CO_2 from the biogas via its reaction with the alkaline minerals in BC-1 to form carbonates (Shen et al., 2016). Another possible explanation is the promotion of direct interspecies electron transfer (DIET) (Wang et al., 2018), which would be manifested by the enhanced conversion of CO_2 to CH_4 by aceticlastic methanogens such as *M. harundinacea*. In contrast, the methane content for biochar from corn stover pretreatment (BC-2) was significantly lower (p<0.05) than for the control throughout the cultivation period (Figure 3.3F).

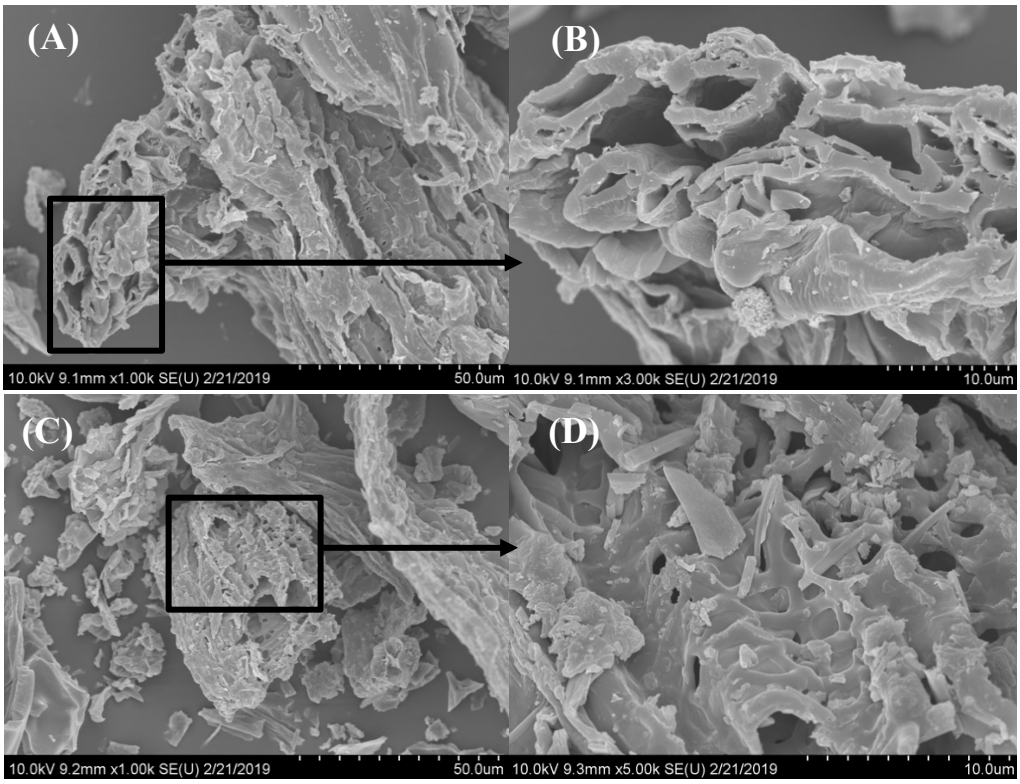

Figure 3.2. Scanning Electron Microscope (SEM) images of two types of biochar used in this work. (A) BC-1 at 1000× magnification; (B) BC-1 at3000× magnification; (**C**) BC-2 at 1000× magnification; (**D**) BC-2 at 5000× magnification.

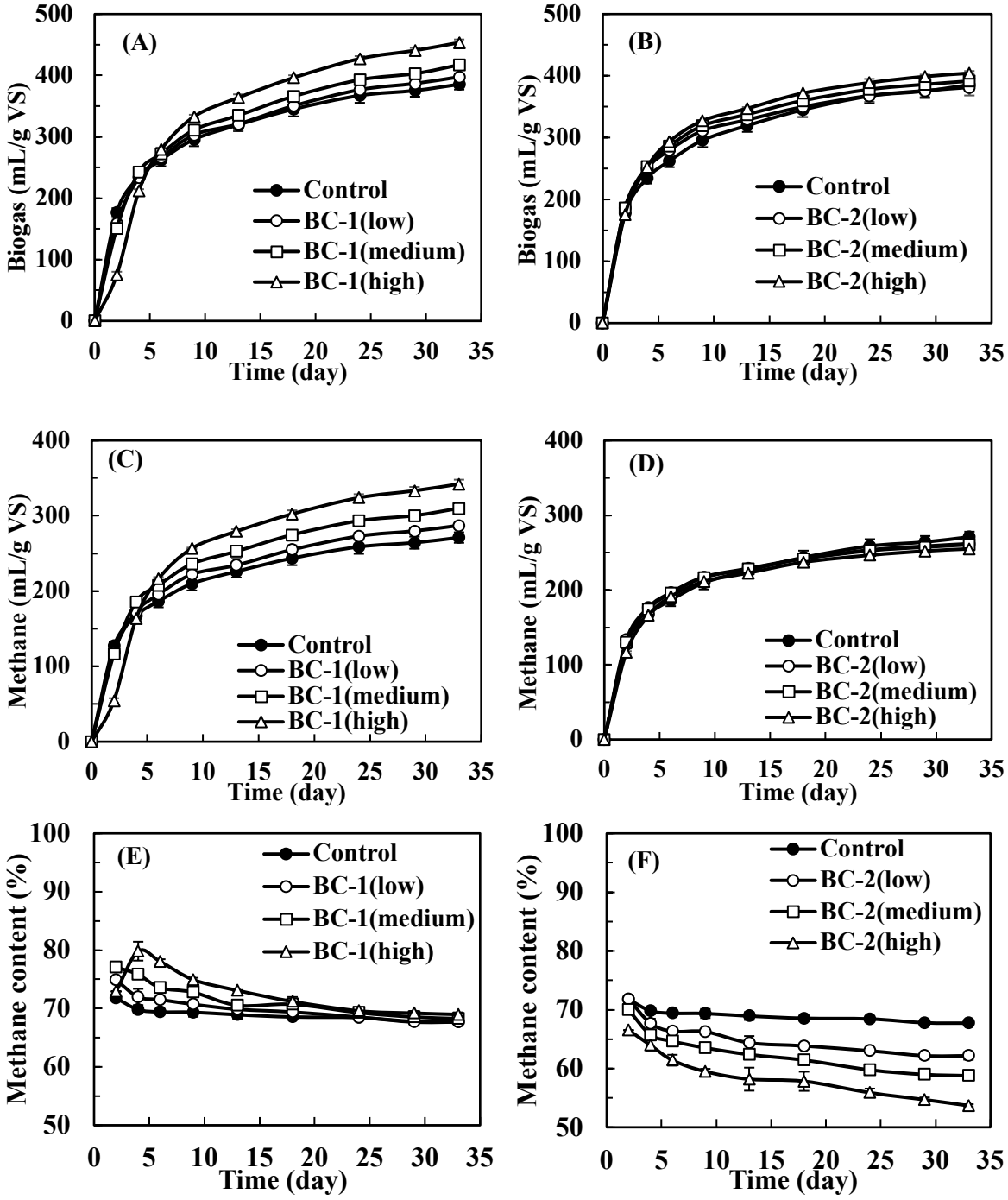

Figure 3.3. Cumulative biogas production of AD added with BC-1 (**A**) and BC-2 (**B**); cumulative methane production of AD added with BC-1 (**C**) and BC-2 (**D**); methane content of the AD added with BC-1 (**E**) and BC-2 (**F**). Low: biochar dosage at 16 g/L; Medium: biochar dosage at 32 g/L; High: biochar dosage at 66.6 g/L.

Methane production kinetics were further correlated to the Gompertz equation (Eq. 2) to quantify the effect of the biochar on the AD process. As shown in Table 3.2, the Gompertz model well predicted methane production with correlation coefficients (R^2) greater than 0.92. The lag phase (λ) of most experimental conditions was nearly 0, while BC-1 with high loading demonstrated a relatively longer lag phase. The reason may be that adding BC-1 to the AD system caused a higher initial pH (since BC-1 was more alkaline than BC-2), which may stress the anaerobic bacteria. Compared to the control, BC-1 increased the maximum methane potential (P_{max}), while BC-2 slightly reduced it. Table 3.2 also shows that the maximum methane production rate (R_{max}) of BC-1 was comparable to that of the control while BC-2 increased R_{max} by 6-20%. As R_{max} was affected by the initial conditions of the digesters, the longer lag phase for the BC-1 AD trials led to reduced R_{max}. The increase in methane production rate through biochar addition was also reported by Li et al. (2018), who explained this phenomenon as a result of low efficiency of electron exchange between syntrophic partners with H_2 as the electron carrier.

Table 3.2. Kinetic parameters of methane production by the Gompertz Modeling.

Treatment		**Actual yield (mL/g VS)**	***P*$_{max}$ (mL/g VS)**	***R*$_{max}$ (mL/g VS d^{-1})**	***λ* *(d)***	**R^2**
Control		271.0	259.1	40.4	3.11×10^{-6}	0.9237
BC-1	Low	287.1	274.8	39.6	1.51×10^{-7}	0.9373
	Medium	309.9	296.7	40.8	5.57×10^{-7}	0.9432
	High	342.1	332.7	34.7	0.018	0.9781
BC-2	Low	262.0	251.5	48.5	1.09×10^{-5}	0.9386
	Medium	260.5	250.6	47.2	1.87×10^{-6}	0.9428
	High	255.0	246.1	42.9	1.88×10^{-6}	0.9539

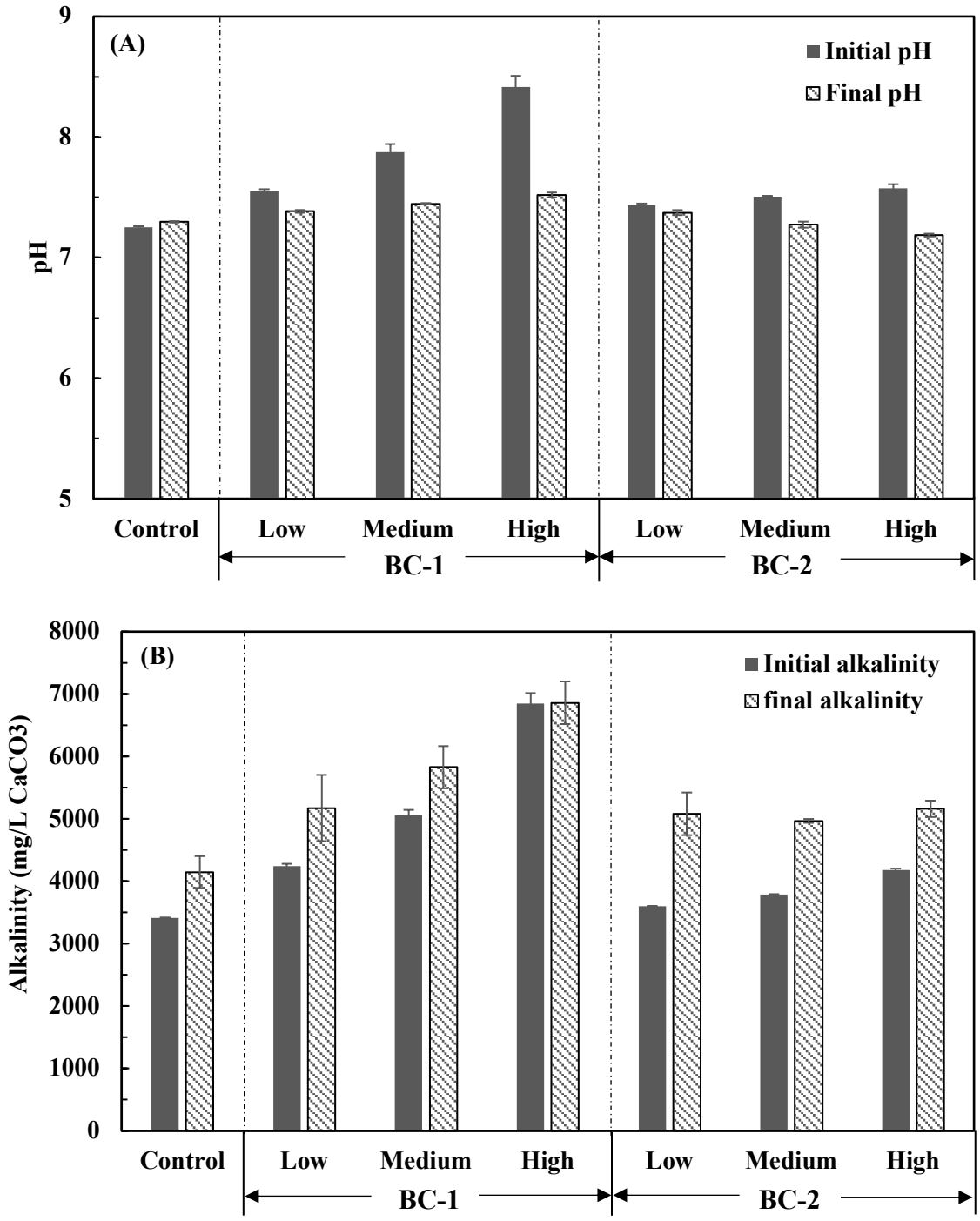

Figure 3.4. Characteristics of pH (**A**) and alkalinity (**B**) of the digestion system before and after AD added with different biochar at different dosages. Low: biochar dosage at 16 g/L; Medium: biochar dosage at 32 g/L; High: biochar dosage at 66.6 g/L.

The pH and alkalinity of digestion system were also characterized as a response of biochar addition. As shown in Figure 3.4A, BC-1 increased the initial pH of the digestion system compared to the control. In particular, the initial pH reached to 8.42 at the highest BC-1 loading which is consistent with the observed increased lag phase for biogas and methane production (Figures 3.3A & 3.3C). The final pH values for AD with BC-1 addition were in the range of 7.38-7.52, slightly higher than for the control (pH 7.30). When BC-2 was added to the digester, the initial increase in pH was not significant compared to the control, while the final pH was reduced. Figure 4B shows the alkalinity of the digestion systems. Similar to the trend of pH, BC-1 addition significantly ($p<0.05$) increased initial alkalinity, while BC-2 did not result in a significant increase in initial alkalinity ($p>0.05$). Final alkalinity of digestate for both BC-1 and BC-2 tests were higher than initial alkalinity, although alkalinity enhancement in the presence of BC-1 was more pronounced than for BC-2 (Figure 3.4B). Shen et al. (2015) reported that the increase in alkalinity at the end of AD was due to cation release and ammonium formation. The above alkalinity effect has also been reported by other research (Pan et al., 2019) Different digesters with various types of feedstock and operation conditions might require different alkalinity. For example, Martin-Gonzalez et al. (2013) reported that to achieve stable reactor performance in AD of municipal solid wastes, total alkalinity should be maintained at 13,000-15,000 mg/L $CaCO_3$. It was considered as one major reason for enhanced AD with biochar addition as it relieved the inhibition of methanogens by the acids that were produced in the hydrolysis and acidogenesis steps. Meanwhile, high calcium and magnesium which contributed to the high alkalinity can also provide essential elements for some methanogens to grow (Li et al., 2019).

3.3.3. Effect of sulfur content of biochar on AD performance.

The previous results clearly show differences in AD performance for the two biochars. Although differences in alkalinity could potentially explain these results, sulfur content was also significantly different (Table 3.1). Therefore, a further experiment was conducted to test whether sulfur might affect biogas/methane production for AD in the presence of biochar.

Three equal mass loadings of biochar were prepared: BC-1, BC-2 and BC-1 with sodium sulfate added to achieve sulfur content comparable to BC-1 (referred to as BC-1+sulfate). As shown in Figure 3.5, BC-1 improved both biogas and methane production while BC-2 reduced biogas and methane production relative to the control, in agreement with previous results shown in Figure 3.3. For BC-1+sulfate, both biogas and methane were comparable or lower than for BC-2. Initially, biogas and methane production were comparable for BC-1 and BC-1+sulfate but BC-1 performed significantly better than BC 1+sulfate after 8 days. At the end of this batch test, BC-1 resulted in significantly higher methane production (p<0.05) while BC-2 and BC-1+sulfur had a similar and significantly lower methane production (p<0.05) compared to the control.

The above results indicate an inhibitory effect of sulfur on AD in these trials. It has been reported that sulfate oxidation usually takes place in AD by sulfate-reducing bacteria (SRB), producing undesirable hydrogen sulfide (Madden et al., 2014), which causes unpleasant odor and inhibits methanogens (Khanal and Huang, 2006). Sulfate-reducing bacteria also compete with methanogens for substrates, thus reducing biogas production (Yang et al., 2015). The total sulfur content of biochar from biomass without pretreatment is solely dependent on the sulfur content of the feedstock, while the thermochemical conversion conditions such as temperature determines the sulfur speciation. For example, Cheah et al. (2014) reported that biochar produced under pyrolysis at 500-600°C conditions contained sulfate as the major sulfur compound (77-

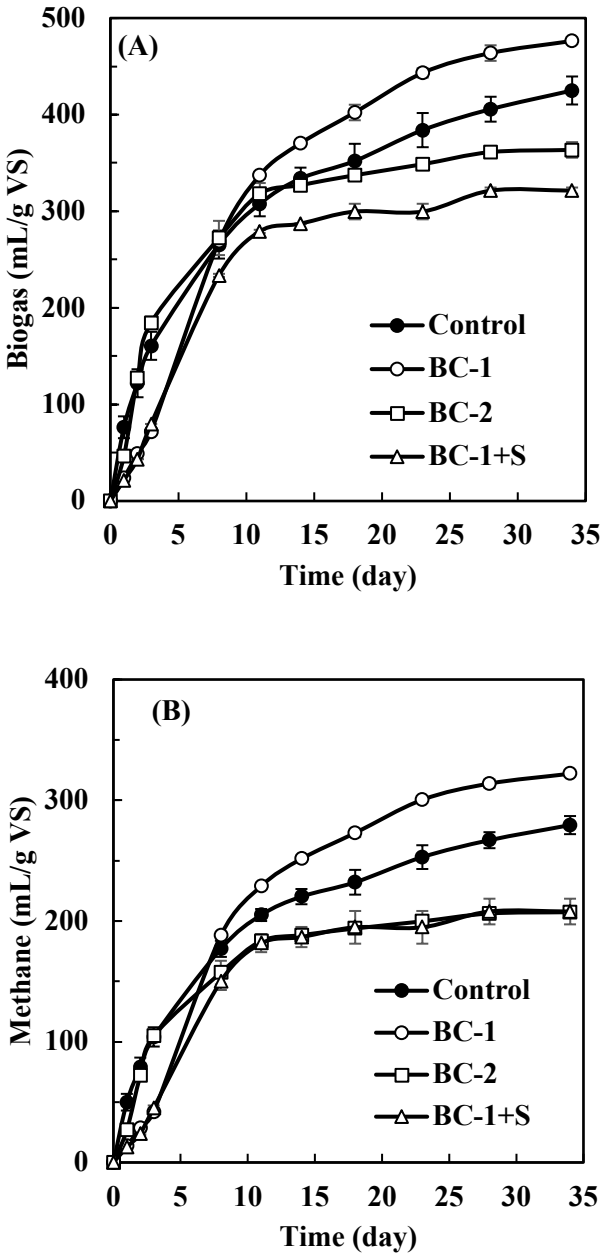

Figure 3.5. Cumulative biogas (**A**) and methane (**B**) production of AD system added with BC-1, BC-2, and BC-1 augmented with extra sulfur (BC-1+sulfur). The biochar loading was 66.6 g/L.

100%) with minor organosulfur and sulfide. In this work, the majority sulfur in biochar from pretreatment (BC-2) was due to the sulfate formed between the reaction of sulfuric acid used in the pretreatment with alkali and alkaline earth metals in the biomass.

3.3.4. Effect of biochar organic and inorganic compounds on AD.

Fast pyrolysis of lignocellulosic biomass often produces biochar with vaporized organic compounds condensed on the surface and/or trapped inside the porous structure of the biochar (Smith et al., 2016). While most of the organic compounds are water soluble and could cause pollution, when being applied as soil amendment (Ghidotti et al., 2017), the effects of those organic compounds on AD have been rarely reported. To investigate the role of the organic compounds accumulated in BC-1 and BC-2, the raw biochar materials were rinsed with DI water to separate the organic compounds from the solid. The effects of the organic compounds-containing leachate and washed biochar solid on AD were studied.

Figure 3.6 compared the concentrations of sugars and VFAs in the leachates from washing the two biochars. Both leachates contained comparable amounts of sugar (Figure 3.6A), mainly levoglucosan, an anhydrosugar produced during fast pyrolysis of lignocellulosic biomass (Li et al., 2017). Figure 3.6B shows that VFA content of Leachate-1 was 6.7 times higher than for Leachate-2. The most abundant VFA was isobutyrate. VFAs are important intermediates in AD, ultimately serving as substrates in biogas production.

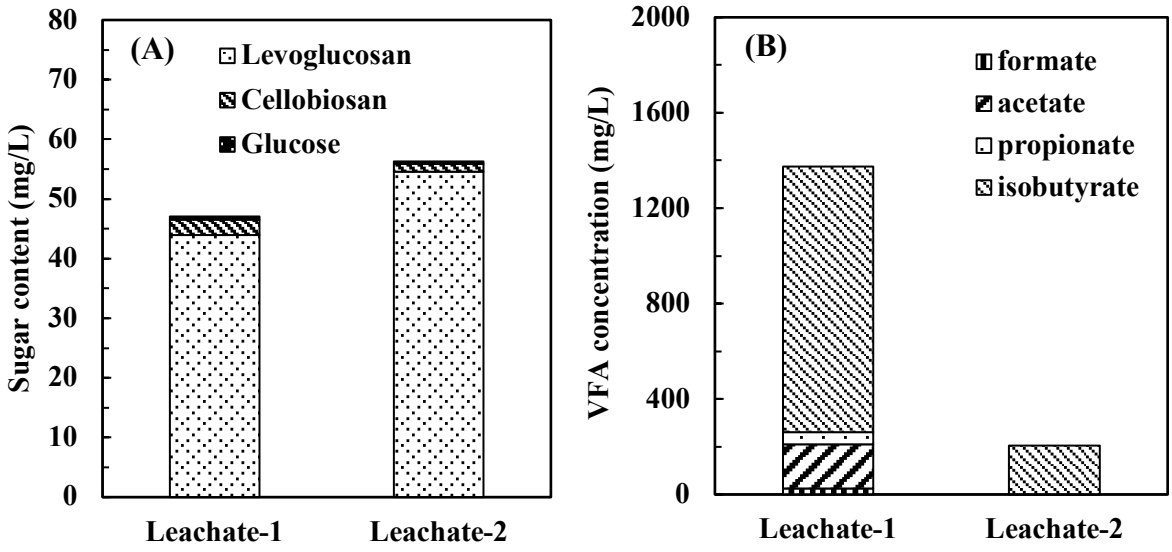

Figure 3.6. Sugars (**A**) and VFA (**B**) concentration in leachates derived from biochar washing. Biochar to water ration was 1:20 (w/w).

In addition to the sugars and VFAs, the inorganic compounds in biochar may also play a role in AD. Table 3.3 shows the distribution of inorganic elements in the leachate and the washed biochar solid. Aluminum and iron in biochar were insoluble and these two elements were not found in the leachates. Phosphorus was only slightly soluble as most phosphorus was retained in the solid biochar. Table 3 also shows Leachate-2 contained a higher portion of those elements than Leachate-1, suggesting acid pretreatment increased solubility of calcium, magnesium, sodium and potassium. It was thought that acid pretreatment of feedstock reduced insoluble $CaCO_3$ and $MgCO_3$ in ash, which are major contributors to the alkalinity of biochar (Chen et al., 2015). Calcium and magnesium were also thought to play an important role in capturing CO_2 in natural weathering process (Mun and Cho, 2013), which could improve the methane purity in digesters. In BC-1, more than half of sodium was insoluble and left in washed biochar; while acid pretreatment increased the solubility of Na that 98.7% were presented in leachate. Most of the potassium salts were leached out from BC-1 and BC-2, respectively.

Table 3.3 Distribution of inorganic elements in washed biochar and leachate.

Elements	Raw BC-1		Raw BC-2	
	Washed BC-1	Leachate-1	Washed BC-2	Leachate-2
Aluminum (Al)	100.0%	0.0%	100.0%	0.0%
Calcium (Ca)	93.3%	6.7%	42.2%	57.8%
Iron (Fe)	100.0%	0.0%	99.9%	0.1%
Magnesium (Mg)	82.9%	17.1%	27.6%	72.4%
Phosphorus (P)	90.8%	9.2%	89.3%	10.7%
Sodium (Na)	59.4%	40.6%	1.3%	98.7%
Potassium (K)	26.0%	74.0%	17.5%	82.5%
Sulfur (S)	ND*	ND*	47.3%	52.7%

*ND: not determined.

The leachates and washed biochar were independently added to AD to evaluate their effects on biogas production. As shown in Figure 3.7A, Leachate-1 produced more biogas than Leachate-2, due to the higher organic matters contained in BC-1 and Leachate-1 (Table 3.1 & Figure 3.6). Although leachate may contain various soluble compounds such as benzene, phenols, and polycyclic aromatic hydrocarbons that are inhibitory to microorganisms (Ghidotti et al., 2017; Smith et al., 2016), the results in Figure 3.7A clearly showed that the soluble organic compounds in biochar have an overall beneficial effect on biogas production. It was also found that Washed BC-1 enhanced biogas production while Washed BC-2 did not produce any biogas. Figure 3.7B shows that the methane production of the different types of leachate and washed biochar had a similar trend with total biogas production. With the exception of Washed BC-2, the methane content for the other three materials ranged from 68% - 76% (Figure 3.7C). The possible reason that Washed BC-2 resulted in no methane production may be due to its high sulfur content (52.7% of the sulfur from raw BC-2) (Tables 3.1 & 3.3), which greatly inhibited the biogas production. Although Leacate-2 also contained sulfur, the beneficial effect of organic

compounds to biogas/methane production may offset and outcompete the inhibition caused by sulfur. However, at later stage (after 23 days), the net biogas and methane production from Leachate-2 was slightly decreased (Figures 3.7A & 3.7B), probably due to the severance of sulfur inhibition.

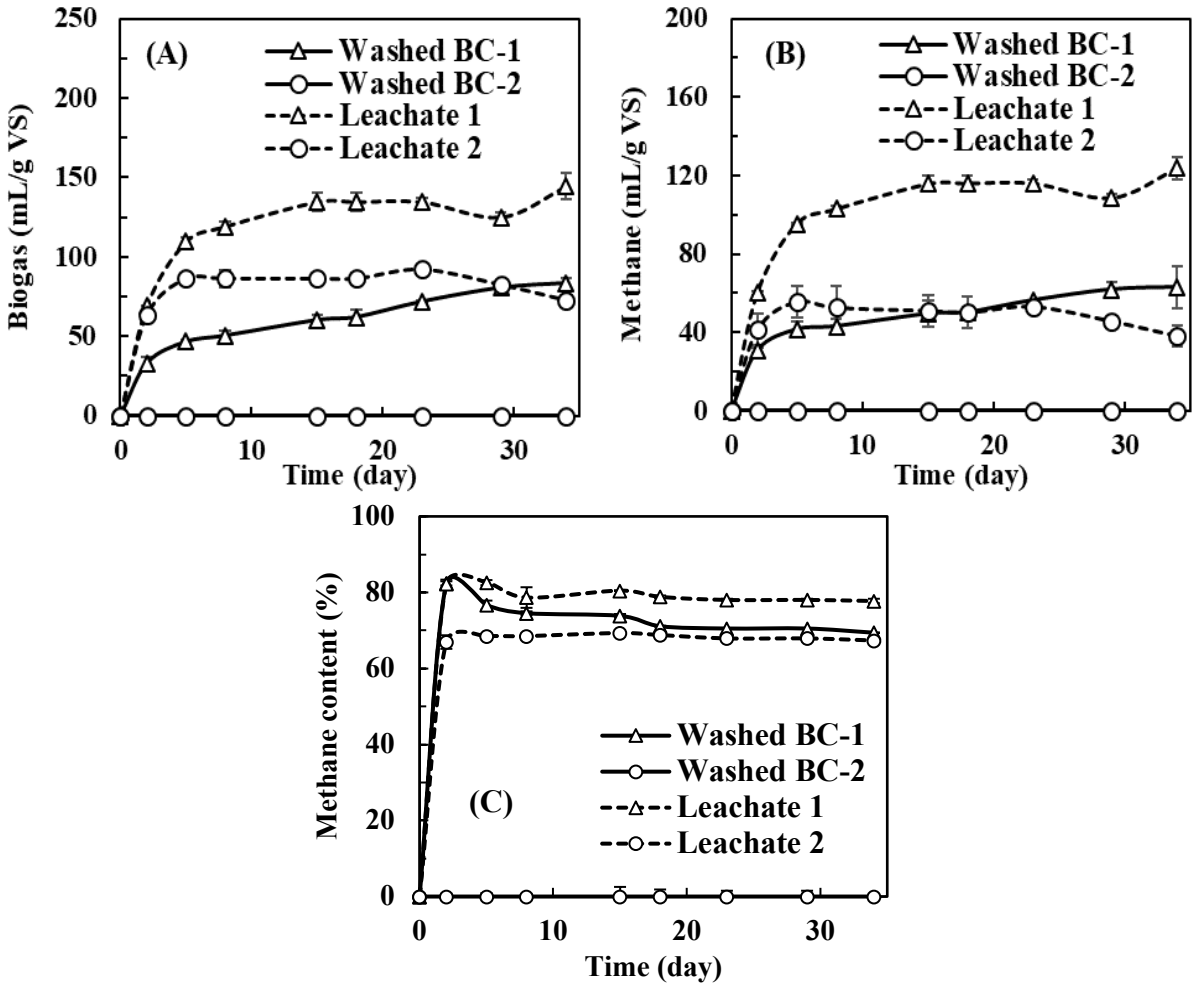

Figure 3.7. Cumulative biogas production (**A**); methane production (**B**); and methane content (**C**) of washed biochar and leachate during BMP tests. Washed biochar and leachate were mixed with the anaerobic digestion seed (sludge-free) and incubated at 37°C in the BMP test. The biogas and methane data were presented by subtracting the baseline date from the seed culture results.

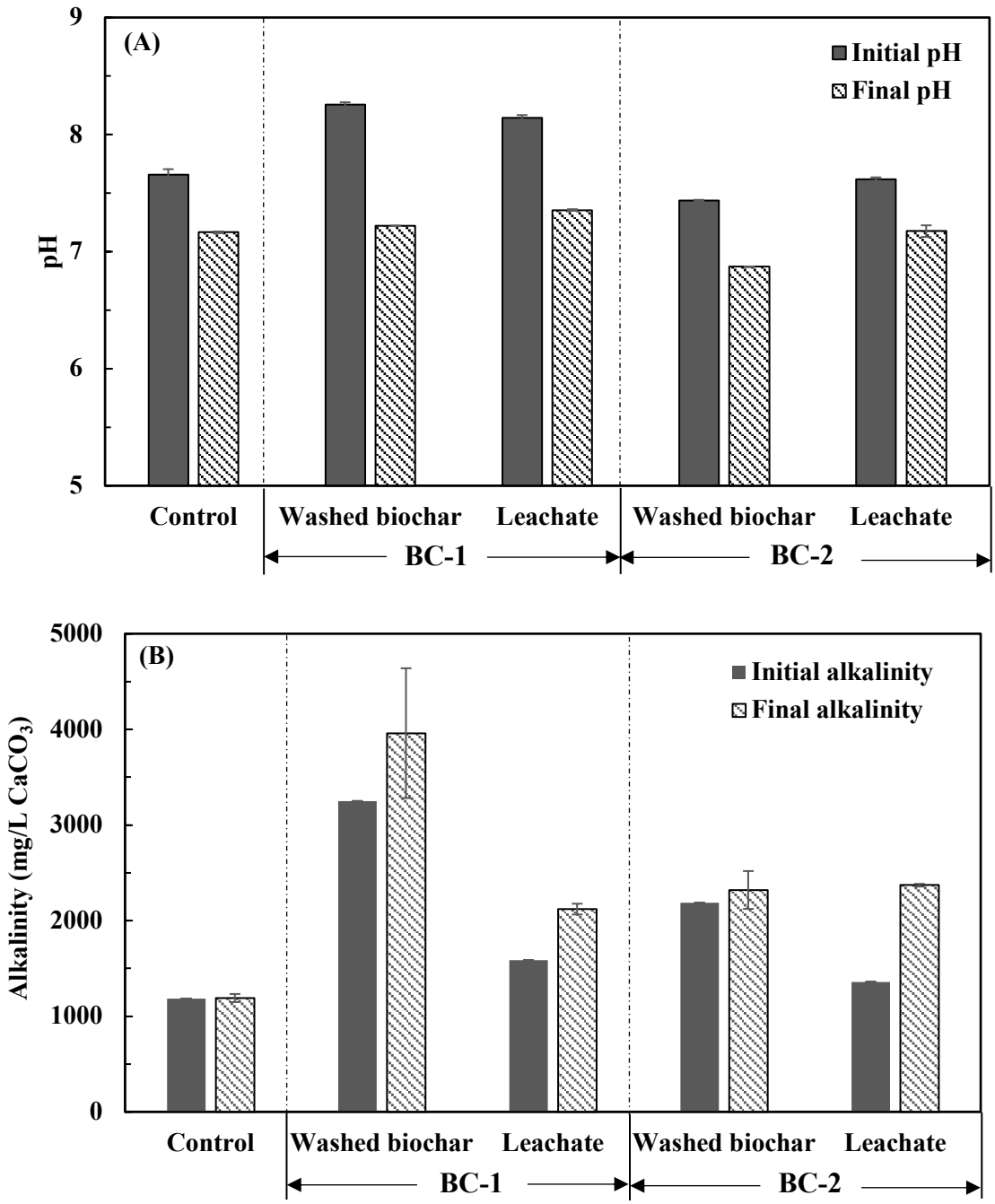

Figure 3.8. Characteristics of sludge before and after AD amended with washed biochar and leachate derived from two types of biochar used in this work. (**A**) Sludge pH; (**B**) sludge alkalinity.

The pH and alkalinity of the washed biochar and leachate are characterized in Figure 3.8A. Compared to the control, Washed BC-1 and Leachate-1 increased the initial pH and maintained a similar pH at the end of AD. For Washed BC-2 and Leachate-2, the initial pH did not change, while the final pH reduced particularly for the Washed BC-2. Figure 3.8B shows that Washed BC-1 increased the initial alkalinity and maintained this high level of alkalinity during AD, which could be the major reason for this material to enhance biogas and methane production (Figure 3.6A & B). The alkaline pH of biochar may be resulted from the high content of alkali and alkaline earth metals (K, Ca, and Mg), which convert CO_2 to bicarbonate and/or carbonate and thus provide high alkalinity. This would prevent the pH from dropping as organic acids were produced in the AD process. Moreover, maintaining CO_2 in the liquid phase facilitates methane formation through CO_2 reduction by hydrogenotrophic methanogens (Baek et al., 2018). In this reaction, DIET between syntrophic microorganisms plays a crucial role, indicating biochar probably promotes DIET to improve methane production.

3.4 Conclusion

This study revealed that mineral acid pretreatment of biomass to enhance pyrolytic sugar production had negative impact on biochar relative to its performance as an additive in AD. The properties of biochar produced from the acid-treated biomass were significantly different from the biochar produced from the raw biomass, and the properties difference resulted in a significantly different effects on biogas/methane production when the biochar materials were added to the AD system. Biochar made from raw corn stover enhanced methane production by up to 26.2% at 66.6 g/L loading, mainly attributing to the high buffering capacity and organic compounds (especially VFAs and sugars) contained in this type of biochar. Sulfuric acid

pretreatment of corn stover resulted in a biochar with high sulfur content with low alkalinity capacity, which caused inhibitory and low buffering capacity to the AD system. To overcome this negative effect, use of phosphoric acid (no sulfur) for biomass pretreatment; and addition of extra alkali to the AD along with biochar from pretreated biomass may be attempted in the future work. In addition, a microbial analysis is needed to better understand mechanisms of different biochar affects AD performance and microbial structure.

3.5 References

APHA;AWWA;WPCF, 2005. Standard methods for the examination of water and waste- water. American Public Health Association (APHA): Washington, DC, USA.

Arif, S., Liaquat, R., Adil, M., 2018. Applications of materials as additives in anaerobic digestion technology. Renew. Sustain. Energy Rev. 97, 354–366. https://doi.org/10.1016/j.rser.2018.08.039

Baek, G., Kim, Jaai, Kim, Jinsu, Lee, C., 2018. Role and potential of direct interspecies electron transfer in anaerobic digestion. Energies 11. https://doi.org/10.3390/en11010107

Chantarasukon, C., Tukkeeree, S., Rohrer, J., 2008. Determination of organic acids in wastewater using ion-exclusion chromatography and on-line carbonate removal (Application Note 291).

Cerato, A.B., Lutenegger, A.J., 2002. Determination of surface area of fine-grained soils by the ethylene glycol monoethyl ether (EGME) method. Geotech. Test. J. 25, 315–321. https://doi.org/10.1520/GTJ11087J

Cheah, S., Malone, S.C., Feik, C.J., 2014. Speciation of sulfur in biochar produced from pyrolysis and gasification of oak and corn stover. Environ. Sci. Technol. 48, 8474–8480. https://doi.org/10.1021/es500073r

Chen, S., Zhang, J., Wang, X., 2015. Effects of alkalinity sources on the stability of anaerobic digestion from food waste. Waste Manag. Res. 33, 1033–1040. https://doi.org/10.1177/0734242X15602965

Crombie, K., Mašek, O., Sohi, S.P., Brownsort, P., Cross, A., 2013. The effect of pyrolysis conditions on biochar stability as determined by three methods. GCB Bioenergy 5, 122–131. https://doi.org/10.1111/gcbb.12030

Dalluge, D.L., Daugaard, T., Johnston, P., Kuzhiyil, N., Wright, M.M., Brown, R.C., 2014. Continuous production of sugars from pyrolysis of acid-infused lignocellulosic biomass. Green Chem. 16, 4144–4155. https://doi.org/10.1039/c4gc00602j

EPA., U.S., 1994. Method 200.7:Determination of Metals and Trace Elements in Water and Wastes By Inductively Coupled Plasma-Atomic Emission Spectrometry. Revision 4.4, Cincinnati, OH. https://doi.org/10.1016/b978-0-8155-1398-8.50010-0

Fagbohungbe, M.O., Herbert, B.M.J., Hurst, L., Li, H., Usmani, S.Q., Semple, K.T., 2016. Impact of biochar on the anaerobic digestion of citrus peel waste. Bioresour. Technol. 216, 142–149. https://doi.org/10.1016/j.biortech.2016.04.106

Ghidotti, M., Fabbri, D., Hornung, A., 2017. Profiles of Volatile Organic Compounds in Biochar: Insights into Process Conditions and Quality Assessment. ACS Sustain. Chem. Eng. 5, 510–517. https://doi.org/10.1021/acssuschemeng.6b01869

Khanal, S.K., Huang, J.-C., 2006. Online Oxygen Control for Sulfide Oxidation in Anaerobic Treatment of High-Sulfate Wastewater. Water Environ. Res. 78, 397–408. https://doi.org/10.2175/106143006x98804

Kookana, R.S., Sarmah, A.K., Van Zwieten, L., Krull, E., Singh, B., 2011. Biochar application to soil. agronomic and environmental benefits and unintended consequences, 1st ed, Advances in Agronomy. Elsevier Inc. https://doi.org/10.1016/B978-0-12-385538-1.00003-2

Laird, D.A., Brown, R.C., Amonette, J.E., Lehmann, J., 2009. Review of the pyrolysis platform for coproducing bio-oil and biochar. Biofuels, Bioprod. Biorefining 6, 547–562. https://doi.org/10.1002/bbb

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. Bioresour. Technol. 249, 1009–1016. https://doi.org/10.1016/j.biortech.2017.11.002

Li, S., Harris, S., Anandhi, A., Chen, G., 2019. Predicting biochar properties and functions based on feedstock and pyrolysis temperature: A review and data syntheses. J. Clean. Prod. 215, 890–902. https://doi.org/10.1016/j.jclepro.2019.01.106

Li, W., Dang, Q., Smith, R., Brown, R.C., Wright, M.M., 2017. Techno-economic analysis of the stabilization of bio-oil fractions for insertion into petroleum refineries. ACS Sustain. Chem. Eng. 5, 1528–1537. https://doi.org/10.1021/acssuschemeng.6b02222

Li, Y., Chen, Y., Wu, J., 2019. Enhancement of methane production in anaerobic digestion process: A review. Appl. Energy 240, 120–137. https://doi.org/10.1016/j.apenergy.2019.01.243

Li, Y., Zhang, R., He, Y., Liu, X., Chen, C., Liu, G., 2014. Thermophilic solid-state anaerobic digestion of alkaline-pretreated corn stover. Energy and Fuels 28, 3759–3765. https://doi.org/10.1021/ef5005495

Madden, P., Al-Raei, A.M., Enright, A.M., Chinalia, F.A., de Beer, D., O'Flaherty, V., Collins, G., 2014. Effect of sulfate on low-temperature anaerobic digestion. Front. Microbiol. 5, 1–15. https://doi.org/10.3389/fmicb.2014.00376

Martín-González, L., Font, X., Vicent, T., 2013. Alkalinity ratios to identify process imbalances in anaerobic digesters treating source-sorted organic fraction of municipal wastes. Biochem. Eng. J. 76, 1–5. https://doi.org/10.1016/j.bej.2013.03.016

Mohan, D., Pittman, C.U., Steele, P.H., 2006. Pyrolysis of wood/biomass for bio-oil: A critical review. Energy and Fuels 20, 848–889. https://doi.org/10.1021/ef0502397

Mumme, J., Srocke, F., Heeg, K., Werner, M., 2014. Use of biochars in anaerobic digestion. Bioresour. Technol. 164, 189–197. https://doi.org/10.1016/j.biortech.2014.05.008

Mun, M., Cho, H., 2013. Mineral carbonation for carbon sequestration with industrial waste. Energy Procedia 37, 6999–7005. https://doi.org/10.1016/j.egypro.2013.06.633

Oudenhoven, S.R.G., Lievens, C., Westerhof, R.J.M., Kersten, S.R.A., 2015. Effect of temperature on the fast pyrolysis of organic-acid leached pinewood; the potential of low temperature pyrolysis. Biomass and Bioenergy 89, 78–90. https://doi.org/10.1016/j.biombioe.2015.12.019

Pan, J., Ma, J., Liu, X., Zhai, L., Ouyang, X., Liu, H., 2019. Effects of different types of biochar on the anaerobic digestion of chicken manure. Bioresour. Technol. 275, 258–265. https://doi.org/10.1016/j.biortech.2018.12.068

Polin, J.P., Peterson, C.A., Whitmer, L.E., Smith, R.G., Brown, R.C., 2019. Process intensification of biomass fast pyrolysis through autothermal operation. Appl. Energy 249, 276–285. https://doi.org/10.1016/j.apenergy.2019.04.154

Qin, Y., Wang, H., Li, X., Cheng, J.J., Wu, W., 2017. Improving methane yield from organic fraction of municipal solid waste (OFMSW) with magnetic rice-straw biochar. Bioresour. Technol. 245, 1058–1066. https://doi.org/10.1016/j.biortech.2017.09.047

Raheem, A., Sikarwar, V.S., He, J., Dastyar, W., Dionysiou, D.D., Wang, W., Zhao, M., 2018. Opportunities and challenges in sustainable treatment and resource reuse of sewage sludge: A review. Chem. Eng. J. 337, 616–641. https://doi.org/10.1016/j.cej.2017.12.149

Romero-Güiza, M.S., Wahid, R., Hernández, V., Møller, H., Fernández, B., 2017. Improvement of wheat straw anaerobic digestion through alkali pre-treatment: Carbohydrates bioavailability evaluation and economic feasibility. Sci. Total Environ. 595, 651–659. https://doi.org/10.1016/j.scitotenv.2017.04.006

Sanchez-Monedero, M.A., Cayuela, M.L., Roig, A., Jindo, K., Mondini, C., Bolan, N., 2018. Role of biochar as an additive in organic waste composting. Bioresour. Technol. 247, 1155–1164. https://doi.org/10.1016/j.biortech.2017.09.193

Shen, Y., Forrester, S., Koval, J., Urgun-Demirtas, M., 2018. Yearlong semi-continuous operation of thermophilic two-stage anaerobic digesters amended with biochar for enhanced biomethane production. J. Clean. Prod. 167, 863–874. https://doi.org/10.1016/j.jclepro.2017.05.135

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. J. Clean. Prod. 135, 1054–1064. https://doi.org/10.1016/j.jclepro.2016.06.144

Shen, Y., Linville, J.L., Urgun-Demirtas, M., Schoene, R.P., Snyder, S.W., 2015. Producing pipeline-quality biomethane via anaerobic digestion of sludge amended with corn stover biochar with in-situ CO2 removal. Appl. Energy 158, 300–309. https://doi.org/10.1016/j.apenergy.2015.08.016

Smith, C.R., Hatcher, P.G., Kumar, S., Lee, J.W., 2016. Investigation into the Sources of Biochar Water-Soluble Organic Compounds and Their Potential Toxicity on Aquatic Microorganisms. ACS Sustain. Chem. Eng. 4, 2550–2558. https://doi.org/10.1021/acssuschemeng.5b01687

Sunyoto, N.M.S., Zhu, M., Zhang, Z., Zhang, D., 2016. Effect of biochar addition on hydrogen and methane production in two-phase anaerobic digestion of aqueous carbohydrates food waste. Bioresour. Technol. 219, 29–36. https://doi.org/10.1016/j.biortech.2016.07.089

Takashima, M., Nakakihara, E., Ikemoto, R., 2018. Effects of Thermal Pretreatment and Trace Metals on High- rate Thermophilic Anaerobic Digestion of Sewage Sludge. Iwa 144, 3–6. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001340.

USEPA, 2007. Method 3051A. Microwave assisted acid digestion of sediments, sludges, soils, and oils.

Wang, C., Liu, Y., Gao, X., Chen, H., Xu, X., Zhu, L., 2018. Role of biochar in the granulation of anaerobic sludge and improvement of electron transfer characteristics. Bioresour. Technol. 268, 28–35. https://doi.org/10.1016/j.biortech.2018.07.116

Wang, M., Zheng, Q., Shen, Q., Guo, S., 2013. The critical role of potassium in plant stress response. Int. J. Mol. Sci. 14, 7370–90. https://doi.org/10.3390/ijms14047370

Xie, S., Wickham, R., Nghiem, L.D., 2017. Synergistic effect from anaerobic co-digestion of sewage sludge and organic wastes. Int. Biodeterior. Biodegrad. 116, 191–197. https://doi.org/10.1016/j.ibiod.2016.10.037

Yang, S.L., Tang, Y.Q., Gou, M., Jiang, X., 2015. Effect of sulfate addition on methane production and sulfate reduction in a mesophilic acetate-fed anaerobic reactor. Appl. Microbiol. Biotechnol. 99, 3269–3277. https://doi.org/10.1007/s00253-014-6235-0

Yuan, H.Y., Ding, L.J., Zama, E.F., Liu, P.P., Hozzein, W.N., Zhu, Y.G., 2018. Biochar Modulates Methanogenesis through Electron Syntrophy of Microorganisms with Ethanol as

a Substrate. Environ. Sci. Technol. 52, 12198–12207. https://doi.org/10.1021/acs.est.8b04121

Zhou, H., Wen, Z., 2019. Solid-State Anaerobic Digestion for Waste Management and Biogas Production. Adv Biochem Eng Biotechnol. https://doi.org/10.1007/10

CHAPTER 4. BIOCHAR ALLEVIATED ACIDIFICATION IN ANAEROBIC CO-DIGESTION OF MUNICIPAL SLUDGE AND FOOD WASTE

Abstract

Food waste is an easily digestible feedstock in anaerobic digestion, which can cause acidification and inhibit the biogas production. Co-digestion of food waste with municipal sludge did not show any alleviation in the acidification due to insufficient buffering capacity. Biochar is a promising additive in alleviating the acidification in AD process mainly due to the high alkalinity. In this work, acid-buffering capacity of biochar was evaluated using 4 typical volatile fatty acids (VFAs), which proved the strong buffering capacity. When biochar was amended in the digesters, the beneficial effects were significant. At 2% and 4% biochar dose, the methane yield was increased by 619.1% and 772.5%, respectively. The increased methane yield was mainly due to strong pH buffering capacity and promoted VFAs consumption by biochar addition. Biochar addition at different doses also had distinct effects on the microbial structure. At 2% biochar dose, the archaeal community was predominated by hydrogenotrophic methanogens while 4% biochar doses facilitated the growth of aceticlastic methanogens.

4.1 Introduction

With the global economic development and population growth, food waste through the food supply chain is generated at an increasing rate. Annually, one third (about 1.3 billion tons) of food production is wasted in the world and the food waste in US is about 38 million tons (Li et al., 2017). Traditional treatments of food waste (landfilling, incineration, and composting) have negative environment impacts, such as uncontrolled greenhouse gas (GHG) emissions, contamination of water supplies through leaching of inorganic matter and low energy recovery (Lin et al., 2013).

Anaerobic digestion (AD) has been proposed to be an effective technology for renewable energy production and waste manage, which converts the biodegradable biomass into a renewable clean energy-biogas (mainly CH_4 and CO_2) in the absence of oxygen. The heat value of a good quality raw biogas containing up to 75% of methane ranges from 20,100–28,900 kcal/Nm^3 (Harasimowicz et al., 2007). Besides the clean energy, AD also produces a fertilizer-quality digestate for land application. Food waste is a promising substrate for AD, due to its high energy content, large quantity, and higher accessibility worldwide (Paritosh et al., 2017). However, high biodegradability of food waste usually leads to the fast accumulation of volatile fatty acids (VFAs) especially at high organic loading rate (OLR), resulting in low pH value and consequently inhibited methane production (Giwa et al., 2019a). Another associated problem is the low quality of the digestate produced if AD performance is inhibited (Fagbohungbe et al., 2017).

To improve the AD performance of easily acidified food waste, co-digestion of food waste with other substrates is thought to be an effective method. Co-digestion of food waste with other substrates such as waste activated sludge (Li et al., 2018) and animal manure (Wang et al.,

2017) can increase the buffering capacity, adjust C/N ratio, reduce the inhibition from excessive VFAs and increase methane yield subsequently. However, it is very hard to employ different substrates in the same spatial and temporal scale considering the economic and energy inputs from transportation and processing. For instance, food waste is abundant in urban areas while animal manure is available in rural areas. Besides, improving digester designs and operating strategies is another way to improve the methane yield in AD of food waste, in which two-stage digesters have been proposed. In two-stage digesters, the acidogenesis and methanogenesis are separated and independently controlled in two reactors to prevent the pH inhibition issues of one-stage systems (Li et al., 2017). However, Voelklein et al. (2016) found that the solubilization of the substrate in the first stage of a two-stage system was significantly affected by the OLR, which was inferior at high OLR (Shen et al., 2013). Meanwhile, the higher capital and operating cost and the extra complexity of the two-stage system would not be offset by the improved process efficiency. Besides, addition of trace elements to digesters have been reported to reduce VFA accumulation and stabilize digesters due to their function in key enzymes (Banks et al., 2012) (FitzGerald et al., 2019). One issue with the trace elements is that overdosing of them would cause precipitation and clogging or even inhibition on AD of food waste (Romero-Güiza et al., 2016).

Recently, biochar, a carbon rich material produced from pyrolysis of biomass, has been paid more attention in AD of food waste as it can enhance the methane production and stabilize the process. Due to redox-active property, biochar can facilitate direct interspecies electron transfer (DIET) thus promoting the syntrophic degradation of VFAs in co-digestion of activated sludge and food waste (Wang et al., 2018). Biochar was also reported to alleviate the inhibitory effect of high ammonia nitrogen concentration and increase the chemical oxygen demand (COD)

removal efficiency (Su et al., 2019). Besides, the porous structure of biochar provides a habitation to immobilize microorganisms and promote biofilm growth (Cai et al., 2016). Moreover, the alkaline nature of biochar was believed to increase the buffer capacity and resulted in increased methane production rate in co-digestion of food waste and waste activated sludge (Li et al., 2018). Shen et al. (2015) also proved that the high alkalinity of biochar can increase the methane content in-situ by reacting with CO_2 and H_2S. However, to the best of the authors' knowledge, the effects of biochar addition on high organic loading of carbohydrate-only food waste have not been reported. Meanwhile, few studies have been conducted so far on the long-term dynamic relationships between biochar and microbial populations in AD of food waste.

In order to better understand the role of biochar especially the buffering capacity in AD of food waste, easily acidified substrate corn starch was selected as substrate to co-digest with municipal sludge. In this research, high organic loading was intentionally employed to worsen the AD performance and achieve acidification. Different doses of biochar were added to the digesters to evaluate the effects on biogas production, VFA changes, pH, COD and total alkalinity. Variation of microbial community structure was also evaluated using a high-throughput sequencing technology to understand the mechanism of stable and efficient methane production at high OLR.

4.2 Materials and methods

4.2.1 Experimental materials: Anaerobic inoculum, sludge, corn starch and biochar

Anaerobic inoculum, sludge and corn starch were obtained from the anaerobic digesters at Grain Processing Corporation (Muscatine, IA). Inoculum and sludge were stored in air-tight bottles at 4 °C before use. Thereafter, total solids (TS) and volatile solids (VS) of the inoculum

and sludge were determined according to the standard methods (APHA et al., 2005). The TS of inoculum and sludge were 50.38 and 64.32 g/L, respectively; the VS of inoculum and sludge were 27.73 and 43.88 g/L, respectively. Biochar was made from corn stover, which was dried and reduced in particle size using a hammer mill with a 1/8-in. screen. The pyrolysis was conducted at 500 °C for about 10s in a continuous pyrolysis process development unit described in a previous study (Polin et al., 2019). The basic characteristics of biochar and starch were listed in Table 4.1.

Table 4.1. Characteristics of corn stover biochar and corn starch.

Parameters	Biochar	Starch
Moisture	6.10	7.81
Volatile matter	29.27	81.48
Fixed carbon	44.21	92.19
Ash	20.42	0
C	59.45	42.86
H	2.74	5.86
N	1.27	0.32
S	0.07	0.05
O	16.05	50.92

4.2.2 Determining the buffering capacity of biochar to VFAs

In order to check the buffering capacity of biochar to VFAs, biochar was mixed with deionized water at the proportion of 2% and 4% (w/w). Four typical short chain VFAs, including acetic acid, propionic acid, isobutyric acid and butyric acid, were choosen to titrate the biochar mixture. The VFAs solutions were prepared at 0.2 mol/L with titration of 200 μL each time. The pH value of the mixture was recorded immediately after each titration until pH was lower than 5.0. The consumed acids for decreasing the pH from initial pH to 5.5 were defined as the buffering capacity of biochar to the corresponding VFAs (Wang et al., 2017).

4.2.3 Anaerobic digestion experimentation

The first AD experiment was conducted to observe the effects of substrate loadings on biogas production. The AD experiment was conducted in 150mL serum bottles with 100mL working volume at mesophilic temperature (37 °C). In each condition, the bottle contained 20mL inoculum, 40mL sludge and 40mL water. The control group contained no starch. The bottles contained 0.1g, 0.2g, 0.4g and 0.6g starch were named 1g/L, 2g/L, 4g/L and 6g/L, respectively. Each bottle was purged with nitrogen for 3mins before AD experiments began. All the experiments were operated at 100 rpm agitation and incubated for 148d.

The second AD experiment was conducted to observe the effects of biochar on biogas production at high substrate loading (10g/L). The AD experiment was conducted in 150mL serum bottles with 100mL working volume at mesophilic temperature (37 °C). The experiment was in batch mode at four conditions as summarized in Table 4.2. Control group (GA) contained inoculum and sludge with water making up to 100mL. GB group was co-digestion of sludge and starch at 1% w/v. GC and GD groups were amended with two dosages of corn stover biochar at 2% w/v and 4% w/v. Each experimental condition was tested in four replicates, with two for biogas analysis and other two for liquid sampling during different time intervals. Each bottle was purged with nitrogen for 3 mins before AD experiments began. All the experiments were operated at 100 rpm agitation.

Table 4.2. Batch AD experimental conditions.

Sample	Inoculum (mL)	Sludge (mL)	Starch (g)	Biochar (g)	Water (mL)
GA	20	40	0	0	40
GB	20	40	1	0	40
GC	20	40	1	2	40
GD	20	40	1	4	40

4.2.4 Analytical methods

The element contents were analyzed using TruSpec Micro CHNS analyzer (LECO Corporation, MI, USA). Moisture, ash content, volatile matter and fixed carbon were analyzed using ASTM D3172-89. Volatile fatty acids (VFA) concentrations were determined in the soluble fraction, by ion chromatography (IC) (Thermo Fisher Scientific, MA, USA) according to the standard method (Chantarasukon et al., 2008). Chemical oxygen demand (COD) was determined using TNTplus Vial Test Kit with DRB200 Digital Reactor Block (Hach, CO, USA). Total alkalinity was measured by Hach test kit (Method 8203) (CO, USA).

Biogas production was measured in a water replacement setting. Biogas composition was analyzed by a gas chromatography equipped with a thermal conductivity detector (GC-TCD). The HP-PLOT/Q column is 30 m in length, 0.320 mm inner diameter with a 0.02 mm film thickness. Helium (99.999% purity) was used as the carrier gas with a constant flow rate of 2 mL/min. The temperature of the detector and column was 100 °C and 30 °C, respectively.

2.5 DNA extraction, PCR, and high-throughput sequencing

High-throughput sequencing technology has been applied to fully explore the microbial community in AD of corn starch. Anaerobic sludge samples at different conditions were collected and freeze-dried for sequencing. Total genomic DNA was extracted using the CTAB/SDS method (Sambrook and Russel, 2001). DNA concentration and purity was monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1ng/μL using sterile water. The V4-5 region of the bacterial 16S rRNA was amplified by PCR using the primers 515F (5'-GTGCC AGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'); and the V8 region of the archaeal 16S rRNA was amplified using the primers 1106F (5'-TTWAGTA GGCAACGAGC-3') and 1378R (5'-

TGTGCAAGGAGCAGGGAC-3'). The PCR reactions were conducted with 15 μL Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM each of the primers, 10 μL template DNA (1 ng/ μL). The amplification program was as follows: initial activation at 98 °C for 1 min followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and extension at 72 °C for 30 s. The PCR extension ended with a final extension at 72 °C for 5 min (Zhou et al., 2019). The PCR products were mixed with 1× loading buffer and detected on 2% agarose gel electrophoresis. Then, the PCR products were purified with Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) and asseseed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina NovaSeq platform and 250 bp paired-end reads were generated. The raw sequencing data was processed and analyzed following the previous report (Zhou et al., 2018).

4.3 Results and discussion

4.3.1 Buffering capacity of biochar to VFAs

Four typical types of VFAs in the digestate, including acetic acid, propionic acid, isobutyric acid and butyric acid, were employed to titrate the biochar for measuring the buffering capacities to these VFAs. According to the dissociation constant of acetic acid, propionic acid, isobutyric acid and butyric acid, the calculated pH of these acids at 0.02mol/L was 3.23, 3.29, 3.28, and 3.27, respectively. As shown in Fig.4.1, the initial pH of 2% and 4% biochar was quite high (above 9.5) due to the alkaline nature of the biochar. With the titration of 2% biochar, the pH gradually decreased. At 0.02mol/L concentration of four VFAs, the pH values of 2% biochar were all higher than 5, indicating buffering capacities of biochar to these VFAs. When the buffering capacity of 4% biochar was measured, the decrease of pH with four VFAs titration was

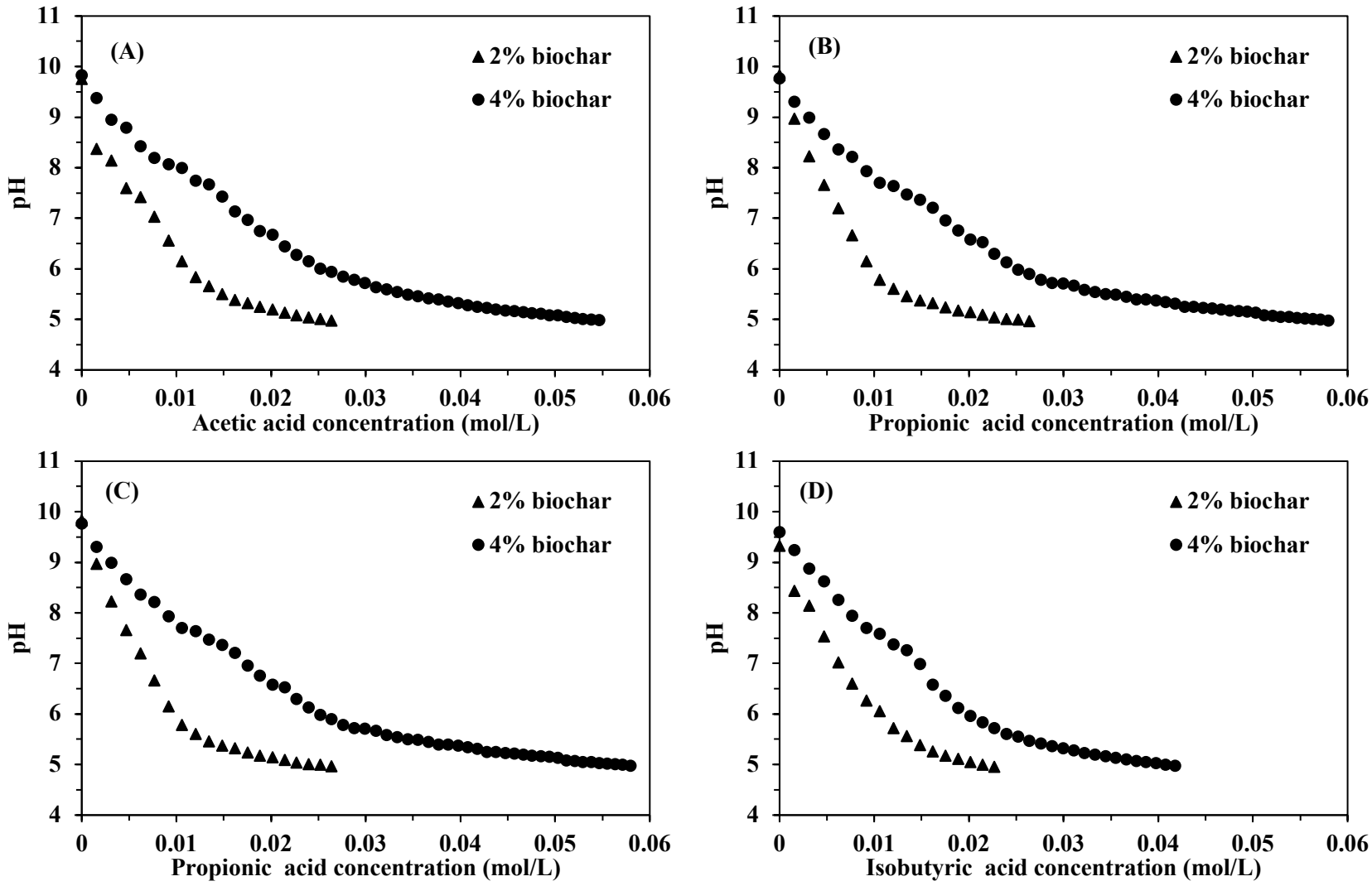

Figure 4.1. Buffering curves of acetic acid, propionic acid, isobutyric acid, and butyric acid for biochar.

much slower than that of 2% biochar. Apparently, the higher biochar loading resulted in stronger buffering capacity. It was thought the alkali and alkaline-earth metals (including Na, K, Ca, Mg) were the main elements attributed to the alkalinity of biochar. As reported by Wang et al. (Wang et al., 2017), the buffering capacity of biochar was source from the following reaction:

$$Ca(Mg)CO_3 + C_xH_yCOOH \rightleftarrows [C_xH_yCOO]_2Ca(Mg) + H_2O + CO_2 \quad (1)$$

The buffering capacities of biochar to four VFAs were calculated and shown in Table 4.3. To decrease the pH from initial to 5.5 by 2% biochar, the consumed concentrations of acetic acid, propionic acid, isobutyric acid and butyric acid were 888.9 mg/L, 994.0 mg/L, 1240.8 mg/L and 1240.8 mg/L, respectively. The buffering capacity of 4% biochar to four VFAs can be improved to 2066.2 mg/L, 2548.3 mg/L, 2270.4 mg/L and 2270.4 mg/L, respectively.

Table 4.3. Calculated buffering capacities of biochar to VFAs.

Sample	Acetic acid mg/L	Propionic acid mg/L	Isobutyric acid mg/L	Butyric acid mg/L
2% biochar	888.9	994.0	1240.8	1240.8
4% biochar	2066.2	2548.3	2270.4	2270.4

Overall, these results proved that biochar had high buffering capacity to four VFAs, providing possibility to buffer the VFAs produced in AD of easily acidified feedstock and stable the process.

4.3.2 Biochar addition in anaerobic digestion

4.3.2.1 Biogas and methane production

The actual effects of biochar on AD of starch was further investigated by adding to the digesters. In order to intentionally acidify the AD system, a high organic loading of 10g/L starch and a low proportion of biochar (2% and 4%) were employed for digestion. The daily and cumulative biogas and methane yields were shown in Fig. 4.2.

As indicated in Fig. 4.2A, sludge only digester had steady biogas production in the first 23 days with the maximum daily biogas appeared at the 8th day. Other conditions with starch had the maximum daily biogas at the first day, which was 34.7, 38.0 and 35.3 mL/g TS for starch+sludge, 2% biochar, and 4% biochar, respectively. However, the majority of the biogas was CO_2. For the starch+sludge, the biogas production was nearly 0 after the first 5 days. With addition of biochar, the biogas production lasted for approximately 80 days, indicating adding biochar can enhance the AD performance. After the first 3 days of high biogas yield, digesters with 2% biochar and 4% biochar both had a low daily biogas yield period. The digester with 4% biochar resumed the biogas production from 23rd day, while 2% biochar restarted until 40th day. These results suggested the differences between buffering capacity of 2% and 4% biochar. The cumulative biogas yield was recorded (Fig. 4.2B), which showed the differences between four conditions. Adding starch into the digesters (starch+sludge group) reduced the biogas yield by 53.0% compared to the sludge-only group, indicating the inhibition of organic fraction conversion caused by the acidification. However, adding biochar greatly increased the biogas yield, which was 223.3mL/g TS for 2% biochar and 276.6 mL/g TS for 4% biochar. Compared to the starch+sludge group, 2% biochar and 4% biochar increased the biogas yield by 234.4% and 314.1%, respectively. The result partially implied that better AD performance was closely related to stronger buffering capacity.

As shown in Fig. 4.2C, the daily methane peak appeared at the first day for groups with starch while it appeared at the 8th day for sludge-only group. Although the daily biogas yield was extremely high at the first day (Fig. 4.2A), CO_2 was the major composition in the biogas since starch was easily digestible that the acidogenesis and acetogenesis steps were too fast, which resulted in a relatively low daily methane yield (Fig. 4.2C). Similar to the biogas

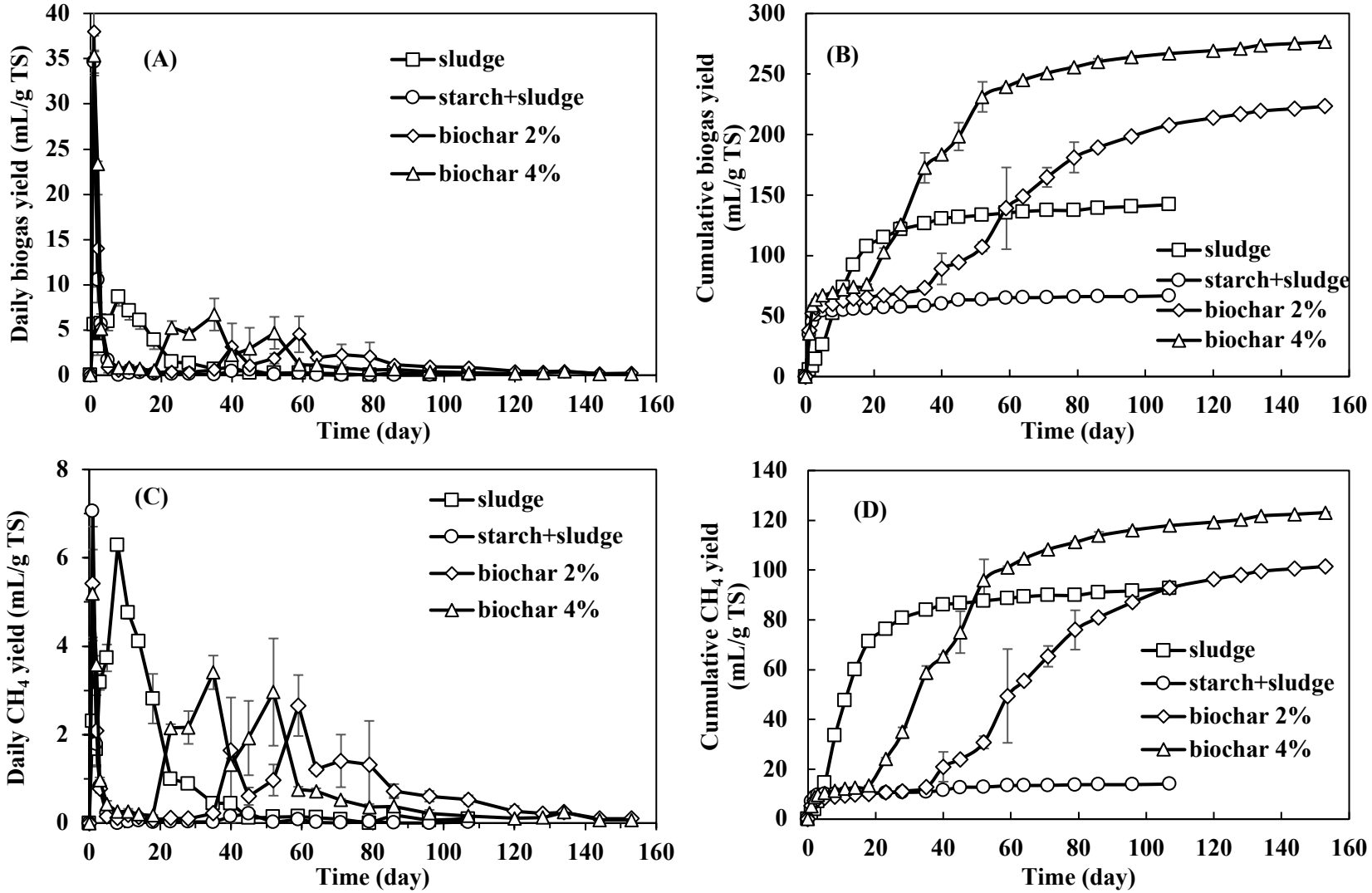

Figure 4.2. Time-course profiles of batch AD experiments with biochar addition: (**A**) Daily biogas yield; (**B**) cumulative biogas yield; (**C**) daily CH_4 yield; (**D**) cumulative CH_4 yield.

production, the methane yield of starch+sludge group gradually reduced and even stopped after the 5th day, which resulted in a quite low methane yield of 14.1mL/g TS (Fig. 4.2D). The sludge-only group steadily produced methane for 28 days, with a high methane content (average 60.7%) and a cumulative methane yield of 92.7mL/g TS. The group of 2% biochar has a cumulative methane yield of 101.5 mL/g TS, which is 9.5% higher than sludge-only group and 619.1% higher than starch+sludge group. The improvement of methane yield by 4% biochar group was more obvious, which is 32.8% higher than sludge-only group and 772.5% higher than starch+sludge group.

4.3.2.2 Sludge characteristics

The pH of the digesters greatly affects the stability of the AD process and subsequently the biogas yield, which is strongly dependent on the buffering capacity. In order to better understand the effects of buffering capacity of biochar on AD of starch, the evolution of pH and VFAs along with other sludge characteristics was investigated (Figs. 4.3 & 4.4).

As shown in Fig. 4.3A, addition of biochar increased the initial pH to 7.05 and 7.50 for 2% and 4% biochar loadings, which was higher than that of sludge-only (pH 6.79) and starch+sludge (pH 6.60). Since starch is easily digestible, it was no surprising to see the pH in the groups with starch dropped very quickly in the first 2 days. The pH values of starch+sludge, 2% biochar and 4% biochar were 4.56, 4.93 and 5.29, respectively; while the pH of sludge only slightly increased to 7.07 and stayed above 7 for the whole process. Then the pH of the starch+sludge group kept in this low level and never recovered for the whole digestion process.

With addition of biochar, pH began to change after a period of time. The 4% biochar group began to recover the pH after 23 days, which was also the same time to recover biogas yield (Fig. 4.2). After 45 days, the pH value reached to 7.18 and stayed above that until the end

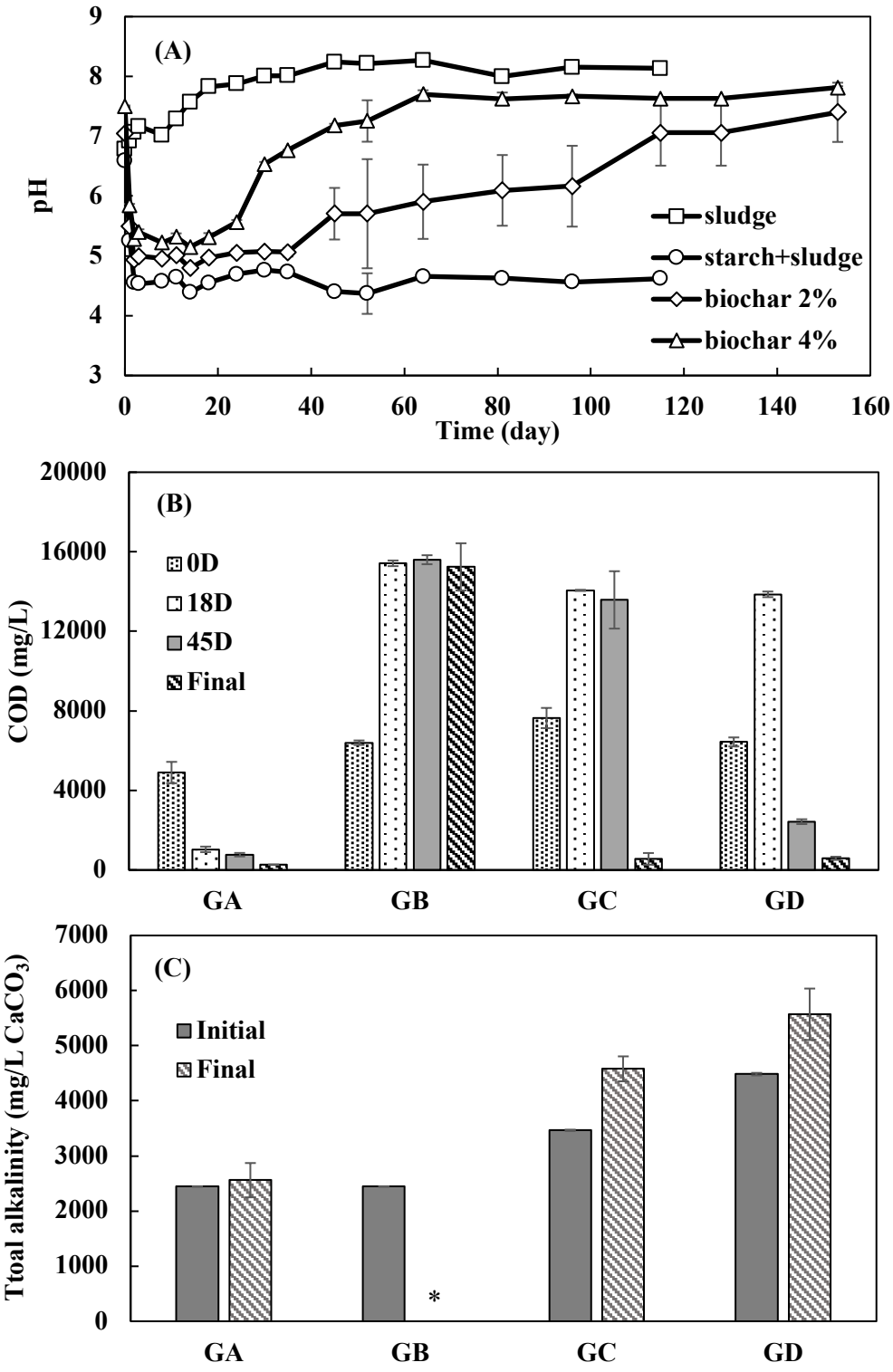

Figure 4.3. Sludge characteristics during batch AD: (**A**) pH; (**B**) COD; (**C**) total alkalinity.
* Final pH <4.5 resulted in undetectable total alkalinity.GA: sludge only; GB: starch + sludge; GC: 2% biochar; GD: 4% biochar.

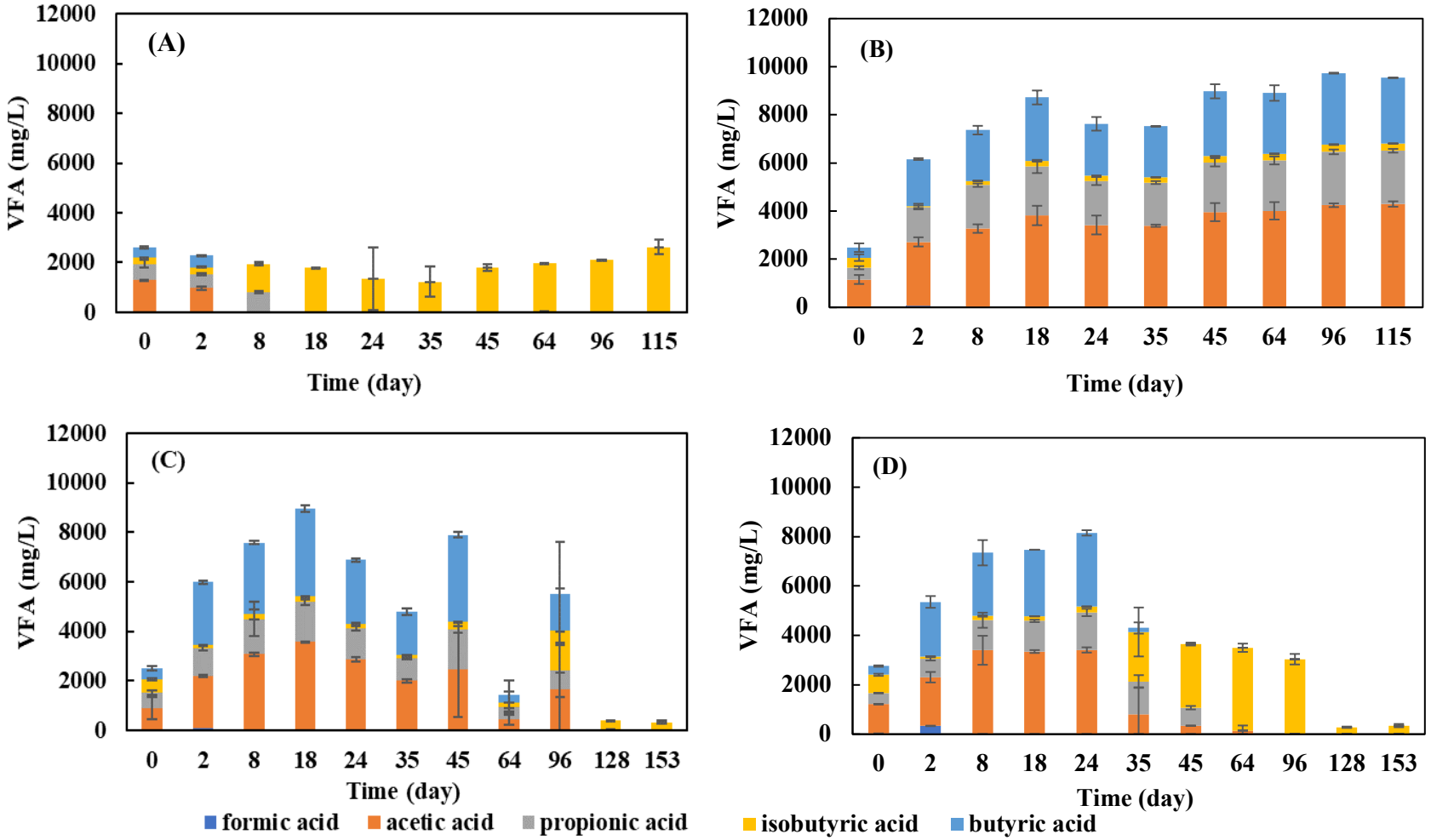

Figure 4.4. Volatile fatty acids (VFA) profiles during batch AD: (**A**) sludge; (**B**) starch + sludge; (**C**) starch + sludge + 2% biochar; (**D**) starch + sludge.

of the digestion. However, for 2% biochar group, it took longer to recover the pH. From 45th day, the pH began to increase and reached 7.06 until 115 days. The results showed that biochar can relieve the acidification of starch by recovering the low pH due to buffering capacity. In an AD system, COD typically reflects the organic matters present in the sludge and the efficiency of AD can be evaluated using COD reduction (Meegoda et al., 2018). In the sludge-only group, the low final COD represented the sludge contained mainly biodegradable fraction (Fig. 4.3B). With addition of starch, COD was greatly increased and reached to 15420, 14060 and 13860 mg/L for starch+sludge, 2% biochar and 4% biochar on 18th day. COD of starch+sludge group stayed high after 18 days, showing the strong inhibition to the anaerobic bacteria resulted in the cease of the organic decomposition, which was also proved by the low biogas yield (Fig. 4.2). Addition of biochar facilitated the COD reduction that both 2% and 4% loading resulted in a very low final COD, although 4% biochar consumed COD faster that COD was almost depleted at 45th day while that of 2% biochar group was still very high.

Alkalinity is the direct indicator of buffering capacity of the AD systems, where higher alkalinity values indicate a greater capacity for resisting pH changes (Zhou and Wen, 2019). As shown in Fig. 4.3C, biochar addition increased the initial alkalinity of the digesters due to the strong buffering capacity of the biochar. After digestion, starch+sludge group had an undetectable alkalinity due to the pH dropped below 4.5. Both 2% and 4% biochar groups resulted in a slightly increased alkalinity, demonstrating sufficient buffering capacity to the system. The increased final alkalinity would be attributed to the release of alkali and alkaline earth metals from biochar and formation of ammonia during AD, consuming CO_2 to form HCO_3^- /CO_3^{2-} (Shen et al., 2016).

VFAs are important intermediates in the AD process, while accumulation of VFAs beyond a threshold concentration may significantly lower the pH and consequently inhibit the methanogensis for efficient methane production (Wang et al., 2019). For the sludge-only group, the VFAs concentration stayed low during the whole process, in which the majority of the final type was isobutyric acid. The VFAs concentration was sharply increased with starch addition on the 2nd day (Figs. 4.4 B, C & D), which was corresponding to the sudden decrease of pH (Fig. 4.3A). The VFAs in starch+sludge group slightly increased with the decomposing of starch to produce VFAs, among which the most abundant type was acetic acid followed by butyric acid. The average concentration of VFAs in this group was 8284.2 mg/L from day 2 to day 115, which was higher than the reported threshold level of 6000 mg/L (Y. Li et al., 2016). The high VFAs concentration well responded to the low pH and high COD (Fig. 4.3). Biochar facilitate the consumption of VFAs at both loadings. For 2% biochar group, the first decrease of VFAs appeared on 35th day, corresponding to the recovering of biogas production (Fig. 4.2) and pH (Fig. 4.3A). There was a fluctuation of VFAs concentration between day 64 and day 96. Then after 96 days, the VFAs was almost depleted and the biogas production was also slowed down (Fig. 4.2). The 4% biochar group had more stable performance that the VFAs concentration gradually decreased since 35th day and almost depleted at the end of the digestion.

Collectively, the results showed that high loading of starch in the digesters caused acidification and inhibited the biogas yield, while addition of biochar greatly increased the buffering capacity and biogas and methane yield, meanwhile facilitating COD reduction and VFAs consumption.

4.3.3 Effect of biochar on microbial community structures

4.3.3.1 Diversity and richness of bacterial and archaeal communities

To reveal the microbial community structure in AD of starch, digestion sludge samples from different conditions retrieved at 0, 18, 45 and 96 days were used for DNA extraction and amplification and subsequent sequencing of a region of bacterial and archaeal 16S rRNA. After removing the low-quality sequences and chimeras, the clean reads for bacteria and archaea ranged between 60,923–81,090 and 80,636–97,759, respectively (data not shown). All of the sequences were aligned and clustered to calculate operational taxonomic units (OTUs) using 97% sequence identity as a cutoff. The good coverage of all the samples was above 99.4% (Table 4.4), indicating a high reliability of the data. The alpha diversity of the samples was represented as Shannon index, Simpson index, Chao1, and ACE estimation (Table 4.4).

The bacterial OTUs of sludge-0D sample was 1028, indicating the complexity of feed inoculum and sludge that might be attributed to the wide spectrum of substances of the original wastewater sludge. The OTUs numbers of sludge-45D and 4% biochar-96D were reduced compared to sludge-0D, which meant that some of the bacterial species faded away when the AD process was at late stage that no more biogas was produced. The OTUs numbers of other samples were all higher than the initial sludge-0D, showing a highly diversified bacterial community. The Shannon index of 2% biochar-18D and 4% biochar-18D was the highest, possibly due to biochar addition facilitated more varieties of bacteria at the early AD process stage. Compared to that of the bacterial community, the OTUs of archaeal community were much lower, probably because only limited methanogenic groups account for a small proportion of the microflora in anaerobic digesters (Liu et al., 2016). While the bacteria OTUs number of 4% biochar-96D sample decreased, the archaea OUTs number of this sample was the only

Table 4.4. Richness and diversity indexes of bacterial and archaeal community.

Sample	Bacteria						Archaea					
	Coverage[a]	OTUs	Shannon	Simpson	chao1	ACE	Coverage	OTUs	Shannon	Simpson	chao1	ACE
Sludge 0D	99.7%	1028	6.31	0.95	1053	1094	100%	111	3.68	0.87	119	117
Sludge 45D	99.6%	897	6.29	0.97	995	1039	100%	89	3.36	0.83	92	94
Starch 18D	99.5%	1248	6.21	0.95	1351	1417	100%	90	3.50	0.82	95	93
2% biochar 18D	99.4%	1271	6.75	0.97	1426	1446	100%	93	3.65	0.84	95	97
4% biochar 18D	99.5%	1191	6.63	0.96	1302	1327	100%	94	3.70	0.85	95	96
2% biochar 96D	99.5%	1066	5.80	0.92	1197	1210	100%	87	3.94	0.90	88	88
4% biochar 96D	99.7%	836	6.24	0.96	895	906	100%	112	2.56	0.62	117	122

increased one compared to sludge-0D. For the 2% biochar samples, the Shannon index increased from 3.65 to 3.94 for 18D and 96D samples, respectively, which indicated a more diverse archaea community. However, the Shannon index of 4% biochar samples decreased from 3.70 to 2.56 for 18D and 96D samples although the OTUs slightly increased. Overall, results in Table4.4 suggested that the biochar addition and loading, and the digestion time substantially influenced the diversity of microbial communities.

4.3.3.2 Bacterial community

Details regarding the bacterial phylum composition of each sample are shown in Fig. 4.5. More than 10 bacterial phyla were detected and only those with relevant abundance more than 1% were presented. The most abundant phyla were Bacteroidetes (38.6%) and Proteobacteria (22.2%) in the initial sample sludge-0D. Bacteroidetes are mainly involved in the hydrolysis of complex macromolecular organic matter, such as degradation of carbohydrates into monosaccharides, lipids into lower fatty acids, and alcohols and proteins into amino acids and some organic acids (Zhao et al., 2017). Proteobacteria are involved in the degradation of organic matters and was important consumer of glucose and various VFAs (Liu et al., 2016), playing a crucial role in key steps – hydrolysis and acetogenesis. With the culture progression, the abundance of both phyla was greatly decreased in sludge-45D sample, possibly due to lack of substrates to facilitate the growth of these phyla. Instead, the abundance of Firmicutes and Chloroflexi were increase, from 8.8% to 25.0% and from 9.6% to 26.8%, respectively. However, the bacterial community structures in all the digesters with starch were very different from the sludge-only digester. For both starch-18D and 2% biochar-18D samples, the predominant phylum was Firmicutes, which is widely reported gram-positive bacteria in anaerobic digesters (Zhou et al., 2019). Firmicutes can hydrolysis complex macromolecules

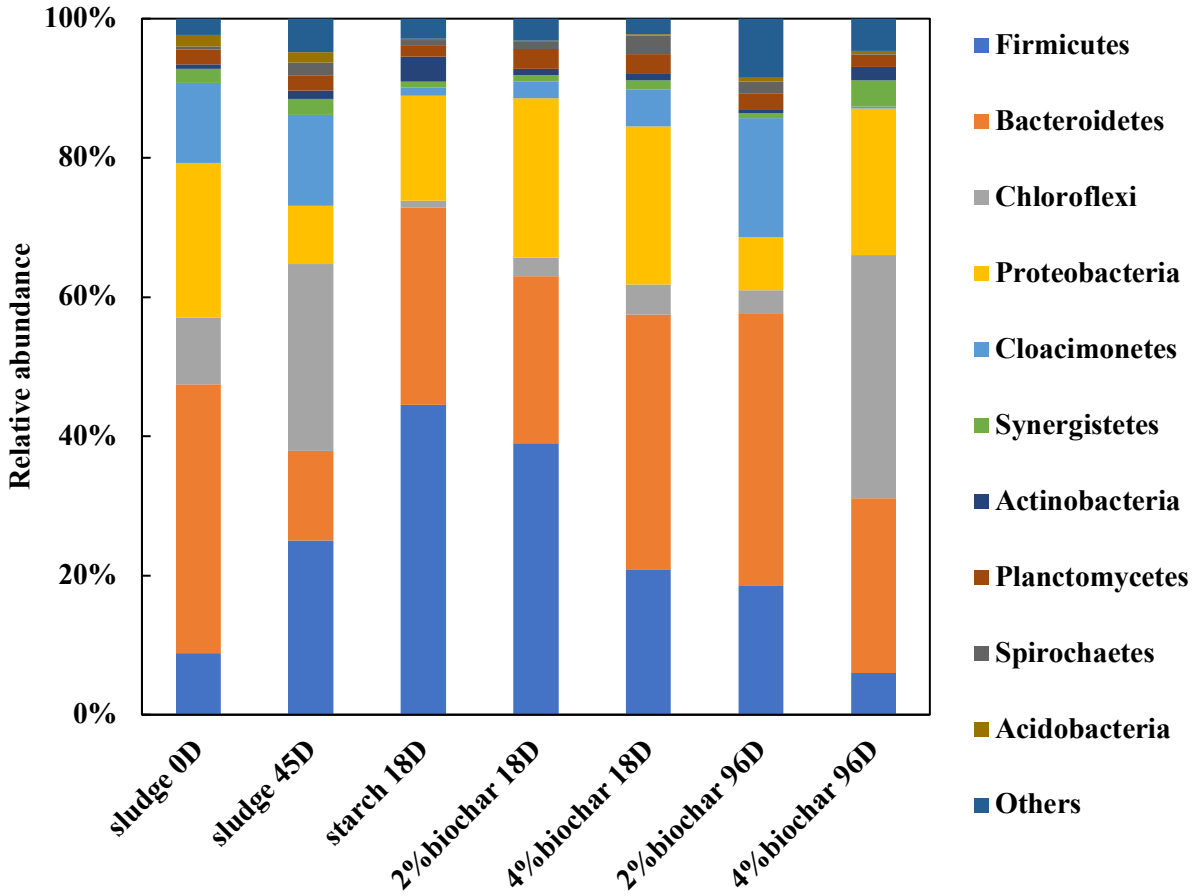

Figure 4.5. Characteristics of bacterial community at phylum level (relative abundance higher than 1% was presented).

(proteins, cellulose, hemicellulose, fats) by secreting a variety of proteases, cellulases, lipases and other extracellular enzymes (Zhao et al., 2017). The high abundance of Firmicutes was closely related to its role in degrading cellulose, consuming VFAs or adapting to low pH environment (Li et al., 2016). At the late digestion stage (96D), the abundance of Firmicutes in both 2% biochar and 4% biochar groups were decreased due to the depleting of the starch. Both at 18D and 96D, there were obvious differences between bacterial community of 2% biochar and 4% biochar samples. For example, the predominant phylum of 4% biochar sample was Bacteroidetes at 18D while Firmicutes in 2% biochar sample. Then at 96D, Bacteroidetes increased and became the most abundant phylum in 2% biochar sample while Chloroflexi predominated in 4% biochar sample. Chloroflexi has been reported as a glucose utilizer (Liu et al., 2016) and is also capable of H_2-oxidizing homoacetogenesis (Nobu et al., 2015). This phenomenon indicated different loadings of biochar had various effects on the bacterial community structures.

4.3.3.3 Archaeal community

Although archaea were less abundant than bacteria, they were playing a critical role in AD. Methanogens consumed the intermediated metabolized by bacteria and converted VFAs to produce CH_4 and CO_2. Details regarding the genus distribution of the major archaea in each sample are shown in Fig. 4.6. The dominant genus were Methanosaeta in the sludge-only samples at both 0D and 45D, the abundance of which were 45.6% and 41.4%, respectively. Methanosaeta is an aceticlastic methanogen, consuming electrons derived from the oxidation of propionate or butyrate to acetate (Wang and Li, 2016). The second abundant genus was Methanosphaerula, a hydrogenotrophic methanogen using H_2/CO_2 to produce CH_4. Thus,

acetotrophic methanogens were predominant in the sludge-only digesters, as acetotrophic methanogens were over 4-fold more abundant than those of hydrogenotrophic methanogens.

Addition of starch in the digesters greatly changed the archaeal community structures. In the starch-18D sample, the abundance of Methanosaeta (16.5%) was reduced compared to the sludge-only samples while Methanosphaerula (17.8%) became the dominant genera. Besides Methanosphaerula, both Methanobrevibacter (4.0%) and Methanobacterium (2.8%) are hydrogenotrophic methanogens, which surpassed aceticlastic methanogens to become the predominant methanogens in the starch-18D sample. Similarly, for both 2% and 4% biochar samples at 18D, the abundance of aceticlastic Methanosaeta was reduced compared to sludge-only samples, while the abundance of hydrogenotrophic methanogens (including Methanobrevibacter, Methanosphaerula and Methanobacterium) gradually increased. After a long-run, samples at 96D showed large differences. In 2% biochar-96D sample, hydrogenotrophic Methanobrevibacter (26.9%) was the most abundant, followed by Methanosaeta. Methanobrevibacter has been reported that they can adapt to high VFA concentrations (Bayrakdar et al., 2017), which corresponding to the relatively high VFAs in the digester shown in Fig. 4.4C. It was a common genus found in AD of fruit and vegetable (Bouallagui et al., 2004), restaurant food waste (Giwa et al., 2019b) and industrial food waste (Ike et al., 2010). However, in 4% biochar-96D sample, Methanosaeta was dominating the archaeal community with the abundance of 76.8%. Thus, higher biochar loading favored the growth of aceticlastic methanogens.

4.4 Conclusion

Corn stover derived biochar in this study showed excellent efficiency in maintaining a long-term anaerobic-codigestion process at high OLR of starch (10g/L) and enhanced methane yield significantly. Moreover, biochar addition alleviated the acidification caused by accumulation of VFAs by recovering pH to a normal range and promoted the consumption of VFAs. Higher biochar loading showed superior beneficial effects in AD due to increased buffering capacity. Biochar addition also changed the microbial community structures in the digesters. The archaeal community was predominated by hydrogenotrophic methanogens at 2% biochar addition while 4% biochar addition facilitated the growth of aceticlastic methanogens.

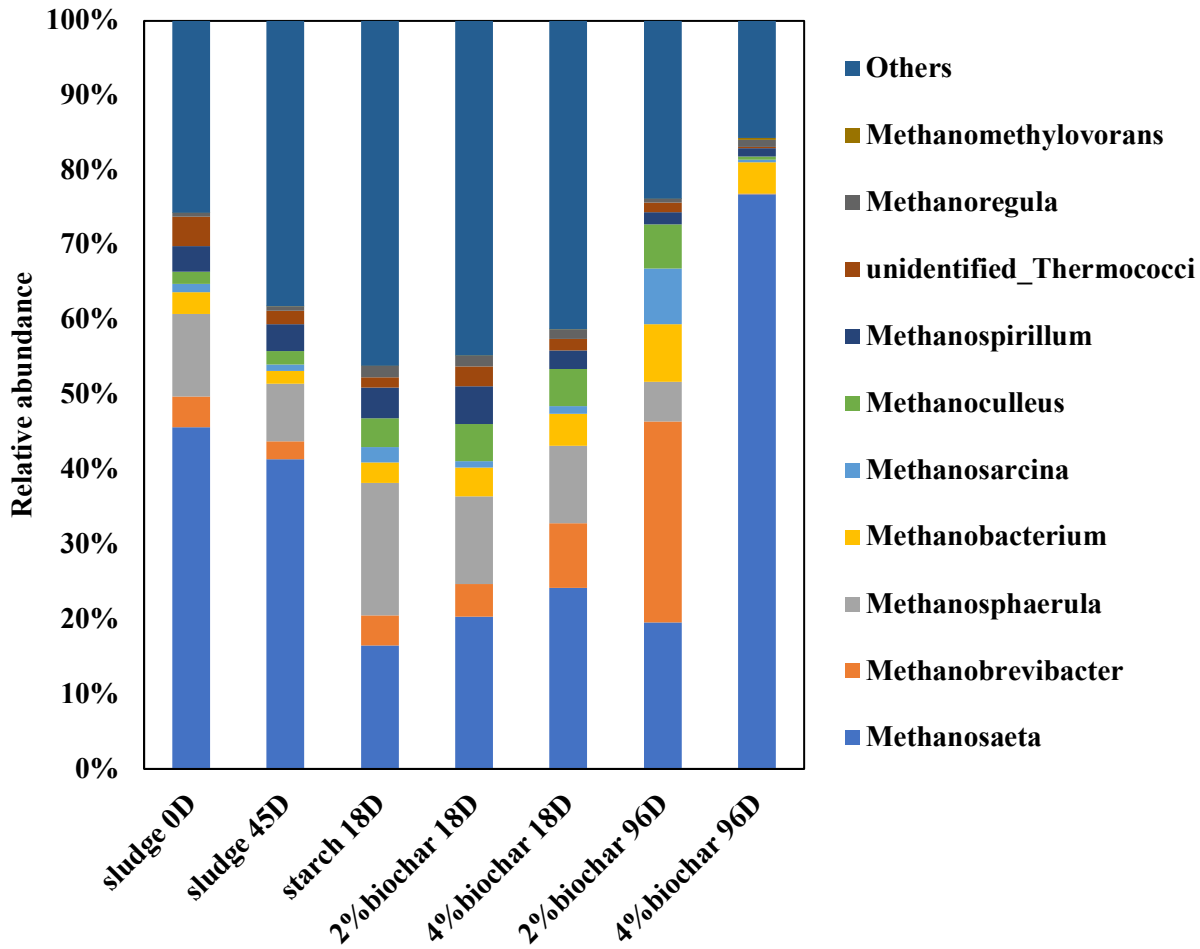

Figure 4.6. Characteristics of archaeal community at genera level (Only top 10 genera were presented).

4.5 References

APHA;AWWA;WPCF, 2005. Standard methods for the examination of water and waste- water. American Public Health Association (APHA): Washington, DC, USA.

Banks, C.J., Zhang, Y., Jiang, Y., Heaven, S., 2012. Trace element requirements for stable food waste digestion at elevated ammonia concentrations. Bioresour. Technol. 104, 127–135. https://doi.org/10.1016/j.biortech.2011.10.068

Bayrakdar, A., Sürmeli, R.Ö., Çalli, B., 2017. Dry anaerobic digestion of chicken manure coupled with membrane separation of ammonia. Bioresour. Technol. 244, 816–823. https://doi.org/10.1016/j.biortech.2017.08.047

Bouallagui, H., Torrijos, M., Godon, J.J., Moletta, R., Ben Cheikh, R., Touhami, Y., Delgenes, J.P., Hamdi, M., 2004. Microbial monitoring by molecular tools of a two-phase anaerobic bioreactor treating fruit and vegetable wastes. Biotechnol. Lett. 26, 857–862. https://doi.org/10.1023/B:BILE.0000025892.19733.18

Chantarasukon, C., Tukkeeree, S., Rohrer, J., 2008. Determination of organic acids in wastewater using ion-exclusion chromatography and on-line carbonate removal (Application Note 291).

Cai, J., He, P., Wang, Y., Shao, L., Lü, F., 2016. Effects and optimization of the use of biochar in anaerobic digestion of food wastes. Waste Manag. Res. 34, 409–416. https://doi.org/10.1177/0734242X16634196

Fagbohungbe, M.O., Herbert, B.M.J., Hurst, L., Ibeto, C.N., Li, H., Usmani, S.Q., Semple, K.T., 2017. The challenges of anaerobic digestion and the role of biochar in optimizing anaerobic digestion. Waste Manag. 61, 236–249. https://doi.org/10.1016/j.wasman.2016.11.028

FitzGerald, J.A., Wall, D.M., Jackson, S.A., Murphy, J.D., Dobson, A.D.W., 2019. Trace element supplementation is associated with increases in fermenting bacteria in biogas mono-digestion of grass silage. Renew. Energy 138, 980–986. https://doi.org/10.1016/j.renene.2019.02.051

Giwa, A.S., Heng, X., Chang, F., Wu, J., Li, Y., Ali, N., Ding, S., Wang, K., 2019a. Effect of biochar on reactor performance and methane generation during the anaerobic digestion of food waste treatment at long-run operations. J. Environ. Chem. Eng. 7, 103067. https://doi.org/10.1016/j.jece.2019.103067

Giwa, A.S., Xu, H., Chang, F., Wu, J., Li, Y., Ali, N., Ding, S., Wang, K., 2019b. Effect of biochar on reactor performance and methane generation during the anaerobic digestion of food waste treatment at long-run operations. J. Environ. Chem. Eng. 7, 103067. https://doi.org/10.1016/j.jece.2019.103067

Harasimowicz, M., Orluk, P., Zakrzewska-Trznadel, G., Chmielewski, A.G., 2007. Application of polyimide membranes for biogas purification and enrichment. J. Hazard. Mater. https://doi.org/10.1016/j.jhazmat.2007.01.098

Ike, M., Inoue, D., Miyano, T., Liu, T.T., Sei, K., Soda, S., Kadoshin, S., 2010. Microbial population dynamics during startup of a full-scale anaerobic digester treating industrial food waste in Kyoto eco-energy project. Bioresour. Technol. 101, 3952–3957. https://doi.org/10.1016/j.biortech.2010.01.028

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. Bioresour. Technol. 249, 1009–1016. https://doi.org/10.1016/j.biortech.2017.11.002

Li, Y., Shi, J., Nelson, M.C., Chen, P.H., Graf, J., Li, Y., Yu, Z., 2016. Impact of different ratios of feedstock to liquid anaerobic digestion effluent on the performance and microbiome of solid-state anaerobic digesters digesting corn stover. Bioresour. Technol. 200, 744–752. https://doi.org/10.1016/j.biortech.2015.10.078

Li, Y., Li, Yu, Zhang, D., Li, G., Lu, J., Li, S., 2016. Solid state anaerobic co-digestion of tomato residues with dairy manure and corn stover for biogas production. Bioresour. Technol. 217, 50–55. https://doi.org/10.1016/j.biortech.2016.01.111

Li, Y., Xu, F., Yang, L., Ge, X., Li, Yangyang, 2017. Anaerobic digestion of food waste – Challenges and opportunities. Bioresour. Technol. 247, 1047–1058. https://doi.org/10.1016/j.biortech.2017.09.020

Lin, C.S.K., Pfaltzgraff, L.A., Herrero-Davila, L., Mubofu, E.B., Abderrahim, S., Clark, J.H., Koutinas, A.A., Kopsahelis, N., Stamatelatou, K., Dickson, F., Thankappan, S., Mohamed, Z., Brocklesby, R., Luque, R., 2013. Food waste as a valuable resource for the production of chemicals, materials and fuels. Current situation and global perspective. Energy Environ. Sci. 6, 426–464. https://doi.org/10.1039/c2ee23440h

Liu, C., Li, H., Zhang, Y., Si, D., Chen, Q., 2016. Evolution of microbial community along with increasing solid concentration during high-solids anaerobic digestion of sewage sludge. Bioresour. Technol. 216, 87–94. https://doi.org/10.1016/j.biortech.2016.05.048

Meegoda, J.N., Li, B., Patel, K., Wang, L.B., 2018. A review of the processes, parameters, and optimization of anaerobic digestion. Int. J. Environ. Res. Public Health 15. https://doi.org/10.3390/ijerph15102224

Nobu, M.K., Narihiro, T., Rinke, C., Kamagata, Y., Tringe, S.G., Woyke, T., Liu, W.T., 2015. Microbial dark matter ecogenomics reveals complex synergistic networks in a methanogenic bioreactor. ISME J. 9, 1710–1722. https://doi.org/10.1038/ismej.2014.256

Paritosh, K., Kushwaha, S.K., Yadav, M., Pareek, N., Chawade, A., Vivekanand, V., 2017. Food Waste to Energy: An Overview of Sustainable Approaches for Food Waste Management and Nutrient Recycling. Biomed Res. Int. 2017. https://doi.org/10.1155/2017/2370927

Polin, J.P., Peterson, C.A., Whitmer, L.E., Smith, R.G., Brown, R.C., 2019. Process intensification of biomass fast pyrolysis through autothermal operation. Appl. Energy 249, 276–285. https://doi.org/10.1016/j.apenergy.2019.04.154

Romero-Güiza, M.S., Vila, J., Mata-Alvarez, J., Chimenos, J.M., Astals, S., 2016. The role of additives on anaerobic digestion: A review. Renew. Sustain. Energy Rev. 58, 1486–1499. https://doi.org/10.1016/j.rser.2015.12.094

Sambrook, J., Russel, D.W., 2001. Molecular cloning: A Laboratory manual. Q. Rev. Biol. 76(3), 348–349.

Shen, F., Yuan, H., Pang, Y., Chen, S., Zhu, B., Zou, D., Liu, Y., Ma, J., Yu, L., Li, X., 2013. Performances of anaerobic co-digestion of fruit & vegetable waste (FVW) and food waste (FW): Single-phase vs. two-phase. Bioresour. Technol. 144, 80–85. https://doi.org/10.1016/j.biortech.2013.06.099

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. J. Clean. Prod. 135, 1054–1064. https://doi.org/10.1016/j.jclepro.2016.06.144

Shen, Y., Linville, J.L., Urgun-Demirtas, M., Schoene, R.P., Snyder, S.W., 2015. Producing pipeline-quality biomethane via anaerobic digestion of sludge amended with corn stover biochar with in-situ CO2 removal. Appl. Energy 158, 300–309. https://doi.org/10.1016/j.apenergy.2015.08.016

Su, C., Zhao, L., Liao, L., Qin, J., Lu, Y., Deng, Q., Chen, M., Huang, Z., 2019. Application of biochar in a CIC reactor to relieve ammonia nitrogen stress and promote microbial community during food waste treatment. J. Clean. Prod. 209, 353–362. https://doi.org/10.1016/j.jclepro.2018.10.269

Voelklein, M.A., Jacob, A., O' Shea, R., Murphy, J.D., 2016. Assessment of increasing loading rate on two-stage digestion of food waste. Bioresour. Technol. 202, 172–180. https://doi.org/10.1016/j.biortech.2015.12.001

Wang, D., Ai, J., Shen, F., Yang, G., Zhang, Y., Deng, S., Zhang, J., Zeng, Y., Song, C., 2017. Improving anaerobic digestion of easy-acidification substrates by promoting buffering capacity using biochar derived from vermicompost. Bioresour. Technol. 227, 286–296. https://doi.org/10.1016/j.biortech.2016.12.060

Wang, G., Li, Q., Gao, X., Wang, X.C., 2019. Sawdust-Derived Biochar Much Mitigates VFAs Accumulation and Improves Microbial Activities to Enhance Methane Production in Thermophilic Anaerobic Digestion. ACS Sustain. Chem. Eng. 7, 2141–2150. https://doi.org/10.1021/acssuschemeng.8b04789

Wang, G., Li, Q., Gao, X., Wang, X.C., 2018. Synergetic promotion of syntrophic methane production from anaerobic digestion of complex organic wastes by biochar: Performance and associated mechanisms. Bioresour. Technol. 250, 812–820. https://doi.org/10.1016/j.biortech.2017.12.004

Wang, J., Li, Y., 2016. Synergistic pretreatment of waste activated sludge using CaO2 in combination with microwave irradiation to enhance methane production during anaerobic digestion. Appl. Energy 183, 1123–1132. https://doi.org/10.1016/j.apenergy.2016.09.042

Zhao, X., Liu, Jinhuan, Liu, Jingjing, Yang, F., Zhu, W., Yuan, X., Hu, Y., Cui, Z., Wang, X., 2017. Effect of ensiling and silage additives on biogas production and microbial community dynamics during anaerobic digestion of switchgrass. Bioresour. Technol. 241, 349–359. https://doi.org/10.1016/j.biortech.2017.03.183

Zhou, H., Brown, R.C., Wen, Z., 2019. Anaerobic digestion of aqueous phase from pyrolysis of biomass: Reducing toxicity and improving microbial tolerance. Bioresour. Technol. 292, 121976. https://doi.org/10.1016/j.biortech.2019.121976

Zhou, H., Sheng, Y., Zhao, X., Gross, M., Wen, Z., 2018. Treatment of acidic sulfate-containing wastewater using revolving algae biofilm reactors: Sulfur removal performance and microbial community characterization. Bioresour. Technol. 264, 24–34. https://doi.org/10.1016/j.biortech.2018.05.051

Zhou, H., Wen, Z., 2019. Solid-State Anaerobic Digestion for Waste Management and Biogas Production. Adv Biochem Eng Biotechnol. https://doi.org/10.1007/10

CHAPTER 5. HYDROXYCINNAMIC ACIDS EXTRACTION AS AN EEFECTIVE PRETREATMENT IN ENHANCING ANAEROBIC DIGESTION OF CORN STOVER

Abstract

Corn stover is an abundant lignocellulosic biomass for energy production in the United States. Due to the recalcitrant structure of lignocellulose, the effective conversion of cellulose and hemicellulose was hindered by the presence of lignin. In order to remove the lignin and improve the economy of the anaerobic digestion (AD) process of corn stover, a novel pretreatment was investigated in this study. The high-value hydroxycinnamic acids (HCAs) in the lignin was extracted through a mild alkaline pretreatment, leaving the residual biomass more digestible for microorganisms in AD. It was concluded that 33.5 wt.% of HCAs can be extracted from corn stover on a lignin basis and approximately 6.0 wt.% on a biomass basis. This simple extraction, employing a mild alkaline solution of water, ethanol, and sodium hydroxide, also results in significant delignification of the biomass, dramatically improving its digestibility. In the batch AD test, it was found that corn stover after HCA extraction can increase the biogas and methane yield by 31.7% and 46.2% compared to the raw corn stover, respectively. Meanwhile, different organic loading rate (OLR) had a great impact on the methane yield in which reducing the OLR from 40 g/L to 12.5 g/L can even increase the biogas and methane yield by 56.0% and 89.9%, respectively. Overall, HCAs extraction can be an effective pretreatment to increase the biomass hydrolysis rate, reduce the lag phase and improve the methane production rate and yield. This combined process provides a multifaceted approach to improve economics of bioenergy from anaerobic digestion and a pathway for producing renewable chemicals from agricultural biomass waste.

5.1 Introduction

Bioenergy production from renewable biomass is becoming crucial to address the growing demand for energy and reducing greenhouse gas (GHG) emissions, owing to the unavoidable depletion of fossil fuel and the environmental consequences of global warming (Bolado-Rodríguez et al., 2016). Corn stover (CS) is one of the most abundant lignocellulosic biomass in the United States. According to the US *Billion-Ton Update* report (United States Department of Energy, 2011), about 120 million tons of dry corn stover is projected to be available for bio-based products in year 2022 in the United States when the farm gate price for corn stover is US $60 per dry short ton ($66 per dry Mg). Generally, 3 types of energy can be produced from lignocellulosic biomass through biochemical or thermochemical processing: liquid fuels such as bioethanol or biobutanol, gaseous fuels such as biogas, and electricity by combustion (Menon and Rao, 2012).

Biogas composing of mainly methane and carbon dioxide, can be produced through anaerobic digestion (AD), one of the most efficient technologies to convert biomass with high energy recovery and environmental benefits (Bolado-Rodríguez et al., 2016). Biogas is a clean and renewable form of energy, which has the advantages of easy implementation and being feasible for small-scale farms. Corn stover can be used as a sustainable substrate for AD. However, the complex structure of cellulose, hemicellulose and lignin made CS very recalcitrant for degradation. Lignin is poorly biodegradable in anaerobic reactors, and also limits the microorganisms to access to the fermentable sugars, thus reducing the methane yield (Liu et al., 2018). In order to enhance the accessibility of lignocellulosic compounds in CS, pretreatment prior to AD is essential to improve biodegradability and biogas production of CS.

Pretreatment is not only costly in its own right but also has a pervasive impact on the cost of all other processing operations, including preceding pretreatment, the handling of the liquid stream generated and the solids from pretreatment, waste management, and potential co-products production (Menon and Rao, 2012). To successfully implement the biogas production process, the first impediment to be solved is the efficient removal of lignin in CS through a cost-effective pretreatment process. One intriguing possibility is to recover high value hydroxycinnamic acids (HCAs) from lignin while removing lignin from CS. HCAs can be used in production of food flavoring additives, sunscreens, cosmetics, acrylic acids, and styrene (Johnston, 2017), with the value of $500-600/kg. With corn stover containing up to 6% HCAs, a ton of corn stover could yield up to $36,000 in HCAs.

Extraction of HCAs from corn stover lignin as pretreatment in AD of CS poses many challenges. Lignin is a complex polymer synthesized mainly from three hydroxycinnamyl alcohols differing in their degree of methoxylation: p-coumaryl, coniferyl, and sinapyl alcohols (Rencoret et al., 2011). Breaking it down into recoverable monomers is difficult. A wide range of lignin depolymerization approaches have been investigated including enzymatic, catalytic, and thermal, but different degrees of aromatic ring saturation can cause issues upgrading to fuels and chemicals (Beckham et al., 2016). Furthermore, the highly reactive fragments of lignin depolymerization condense to form new, refractory carbon-carbon bonds that both reduce monomer yields and make more difficult further upgrading of fractionated lignin (Renders et al., 2017). Thus, lignin-first concept was emerged with the goal of first recovering essentially unmodified lignin from biomass rather than accepting it as a highly modified polymeric co-product of cellulosic sugar production (Renders et al., 2017).

Alkaline pretreatment (NaOH) has been reported to be effective in increasing degree of conversion of glucan to glucose before subsequent biological processing (Murciano Martínez et al., 2016). This pretreatment not only partially delignifies the biomass but also allows for the extraction of high value HCA by breaking down HCAs cross-linkers in the cell wall and dissolving HCAs in an alcohol and water solution. However, the effect of HCAs extraction as pretreatment in AD of corn stover is rarely reported. The goal of this research is utilizing a mild alkaline pretreatment method to directly extract high value HCAs, namely coumaric and ferulic acids, from corn stover. Concurrently, the HCA extracted corn stover will be evaluated as feedstock in AD. Fig. 5.1 schematic illustrates the method for recovery of high value materials upstream of corn stover and subsequent AD process for biofuel production.

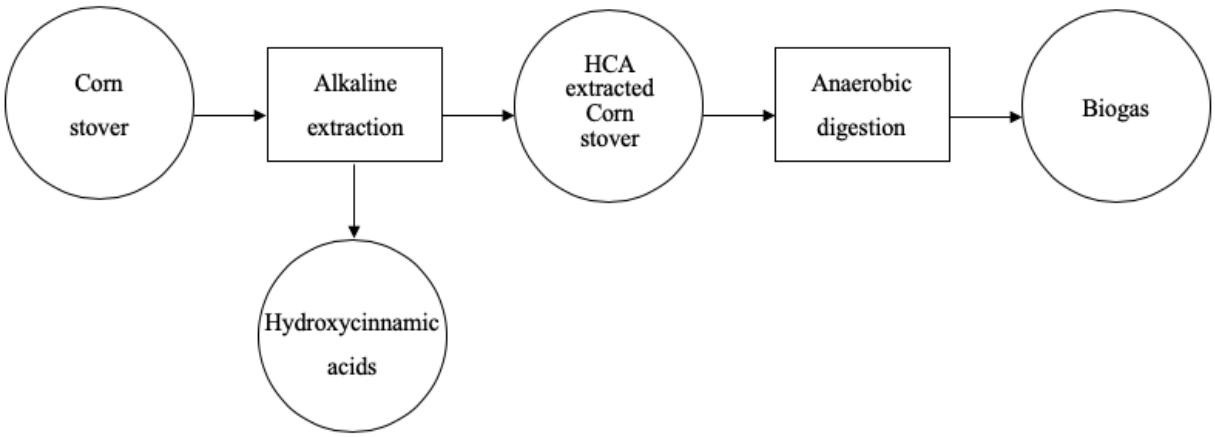

Figure 5.1. Schematic of the alkaline extraction and anaerobic digestion process.

5.2 Materials and methods

5.2.1 Corn Stover Samples

The corn stover used in this study was from the Biocentury Research Farm (BCRF) harvested in Ames, IA. The corn stover used in the extraction process was milled to roughly 1/16” -1/8” particle size. The biomass was dried to less than 10% moisture.

5.2.2 Hydroxycinnamic Acid (HCA) Extraction

Removing the HCAs coumaric and ferulic acids directly from corn stover was accomplished by using a sodium hydroxide solution and refluxing for approximately two hours. The solution used was 62.5% ethanol and 37.5% 18.2 Ω deionized water with sodium hydroxide (Buranov and Mazza, 2009; Hertog et al., 1992; Johnston, 2017; Sun et al., 2002). The procedure used for the 6L batches is as follows – 190 grams of corn stover, 4800mL of the ethanol and water solution, 1200mL of 4N sodium hydroxide. After the solution was refluxed for two hours, the solution was decanted and filtered with a 0.45µm glass microfiber filter. The quantitative concentration of HCAs extracted was determined by a high-performance liquid chromatograph (HPLC) and a diode array detector (DAD) set at 263nm. The HCA corn stover was subsequently washed with deionized water and dried for further analysis. The washing step is critical for removing the elemental sodium from the sodium hydroxide treated corn stover. After the washing step the biomass was tested on the ICP to determine the sodium concentration. The sodium concentration should be similar to the levels found in typical corn stover. This process details an upstream process for removing the high value chemicals. The HCA extraction was tested in triplicate and injected in triplicate on the HPLC. The nature of the error bars is from a 95% confidence interval.

Besides the typical HCA extracted corn stover, two other types of corn stover were also tested in the following anaerobic digestion. The washing step was eliminated so that the ash was maintained to provide alkalinity to the AD system. The first type (unwashed HCA CS-1) was pretreated with NaOH, water and ethanol, while the second type (unwashed HCA CS-2) was pretreated with NaOH and water.

5.2.3 Anaerobic digestion setup

Anaerobic bacterial inoculum was obtained from an anaerobic digester at Water Pollution Control Facility at Muscatine, IA. The materials were stored in air-tight bottles at 4°C prior to use. Total solids (TS) and volatile solids (VS) of the inoculum were 27.3 g/L and 15.0g/L, respectively. The pH of the inoculum was 6.84. AD experiments were performed through a biomethane potential (BMP) test in 125 mL serum bottles with 80 mL working volume. The bottles were incubated in a shaker at 37°C with 100 rpm. Each condition was conducted in duplicate.

The first BMP test was to evaluate the effect of HCA extraction of corn stover on AD performance. Each serum bottle contained 60mL inoculum and 20mL DI water. The treatment without corn stover addition was tested as control. Raw corn stover was added at loading of 3.2g/bottle (40 g/L). HCA extracted corn stover (HCA CS) was respectively added to each bottle at loading of 3.2g/bottle and 2.6g/bottle (32.5 g/L), which were based on the same biomass amount and biodegradable content (organic matters except lignin and ash), respectively.

The second BMP test was to evaluate the washing step on AD of HCA corn stover. Two types of unwashed HCA extracted corn stover were tested in this batch. The first type (unwashed HCA CS-1) was pretreated with NaOH, water and ethanol; while the second type (unwashed HCA CS-2) was pretreated with NaOH and water. Each serum bottle contained 60mL inoculum and 20mL DI water. The treatment without corn stover addition was tested as control. Unwashed HCA CS-1 was added at loading of 3.2 g/bottle (40 g/L) and 1 g/bottle (12.5 g/L), respectively. Unwashed HCA CS-2 was added at loading of 3.2 g/bottle.

5.2.4 Analysis

Coumaric and ferulic acid standards were purchased from Sigma Aldrich (St. Louis, MO, USA) and had purities of ⩾99.0%. The methanol used was HPLC grade and sub-micron filtered from Fisher Scientific with an assay of ⩾99.9%. The deionized water was 18.2 MΩ from a Thermo Scientific/Barnstead Micro Pure UV (Waltham, MA). A complete lignocellulosic characterization and summary was performed by Celignis Limited (limerick, Ireland) on both the untreated corn stover and the HCA extracted corn stover. The test methods used for the lignocellulosic summary were comparable to the National Renewable Energy Laboratory (NREL) TP-510-42618 "Determination of Structural Carbohydrates and Lignin in Biomass". The concentration of glucan was determined by Celignis Biomass Analysis Laboratory (University of Limerick, Ireland) using method based off of NREL Laboratory Analytical Procedure (LAP) TP-510-42618 protocol. Corn stover from the same super sack was extracted into two different batches. These two batches HCA extracted corn stover was tested for glucan concentrations. The glucan concentration on an ash free basis was multiplied by 1.11 to determine the glucose concentration (Ghosh and Brown, 2018).

Total solids (TS) and volatile solids (VS) were measured based on the standard methods (APHA et al., 2005). Total biogas was measured in a water replacement setting. Biogas composition was analyzed by a gas chromatography (GC) equipped with a thermal conductivity detector. The HP-PLOT/Q column has 30 m long, 0.320 mm inner diameter with a 0.02 mm film thickness. Helium (99.999% purity) was used as the carrier gas with a constant flow rate of 2 mL/min. The temperature of the detector and column was 100 °C and 30 °C, respectively.

5.2.5 Kinetic simulation

The first-order model (shown in Eq. (1)) (Li et al., 2013) or Cone model (shown in Eq. (2)) (Zhen et al., 2016) can be used to calculate the hydrolysis rate of organic matter and the cumulative methane yield.

$$P = P_{\max}[1-\exp(-kt)] \quad (1)$$

$$P = \frac{P_{\max}}{1+(kt)^{-n}} \quad (2)$$

Besides the hydrolysis rate constant and the accumulative methane yield, the duration of the lag-phase is also a crucial indicator reflecting the efficiency of anaerobic digestion, which can be estimated with the modified Gompertz model (Li et al., 2014):

$$P = P_{\max} \times \exp\left\{-\exp\left[\frac{R_{\max} \times e}{P_{\max}}(\lambda - t)+1\right]\right\} \quad (3)$$

where P is the cumulative methane production (mL/g VS), $P_{\max}$ is the maximum methane potential (mL/g VS), k (day^{-1}) stands for the first-order rate constant, and t (day) refers to the digestion time, n is the shape factor, R_{max} is the maximum methane production rate (mL/gVS d^{-1}), e is Euler's Number (2.718), λ is the lag phase time (day). Parameters in all models were regressed from experimental data using non-linear regression algorithm in MATLAB R2019a software.

5.3 Results and discussion

5.3.1 HCA Extraction

Fig. 5.2 displays the composition of the untreated and treated (HCA extracted) corn stover. The results show a decrease in lignin content, where Klason lignin reduced from 14.97% to 4.32% and acid soluble lignin from 1.86 % to 0.82 % after the extraction. The total glucan increased from 34.41 wt.% to 63.61 wt.% which would be expected from the alkaline extraction

method. Meanwhile the ash content decreased from 10.93% to 6.74% due to the washing step in the extraction process. The process is opening up the lignin structure causing partial delignification with removal of HCAs which sequentially provides accessibility to the carbohydrate. The HCAs are the cross-linkers between the lignin and carbohydrate and the glucan in corn stover becomes more accessible to microbial conversion.

Fig. 5.3 displays the HCA concentration on both a corn stover and lignin wt. % basis. The yields of coumaric and ferulic acids were 24% and 9.5% on a lignin basis, respectively. The calculations were based on a lignin concentration of 16.8 wt.% (including Klason lignin and acid soluble lignin). Overall, this alkaline extraction method can delignifiy the biomass with a mild alkaline treatment and extract coumaric and ferulic acids from the lignin leaving the biomass intact for further biological conversion processes.

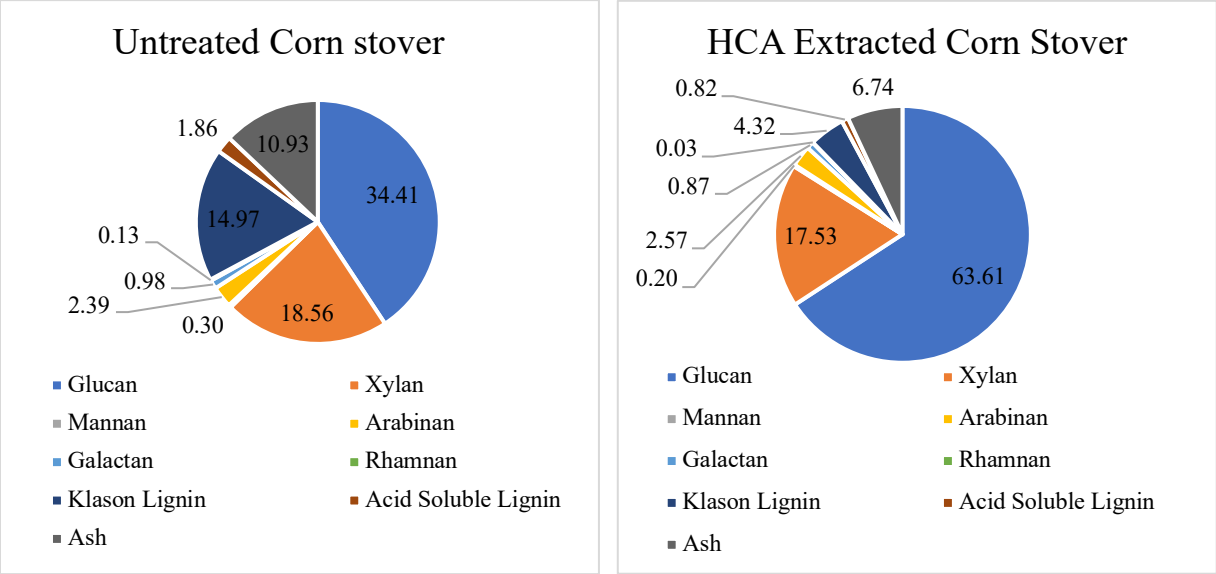

Figure 5.2. Composition of untreated corn stover and HCA extracted corn stover (wt.%).

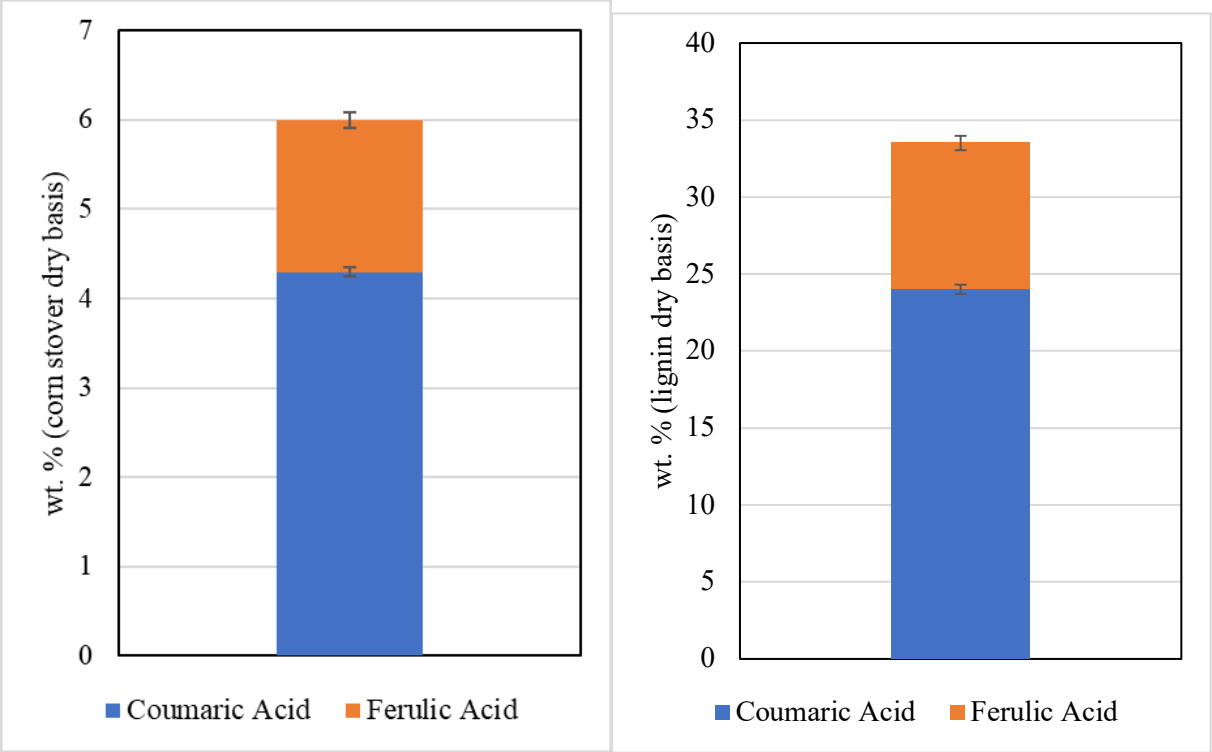

Figure 5.3. Hydroxycinnamic acid concentrations in corn stover.

Fig. 5.4 displays the complete set of ICP-OES results after the biomass samples were digested. The most abundant element was potassium in the raw corn stover since K-rich fertilizers are commonly used for corn cultivation (Wang et al., 2013). After the extraction process, the contents of calcium and manganese were increased while all other elements (including magnesium, phosphorus, sulfur and sodium) were reduced. The sodium was readily washed out after the extraction to the typical levels in corn stover biomass.

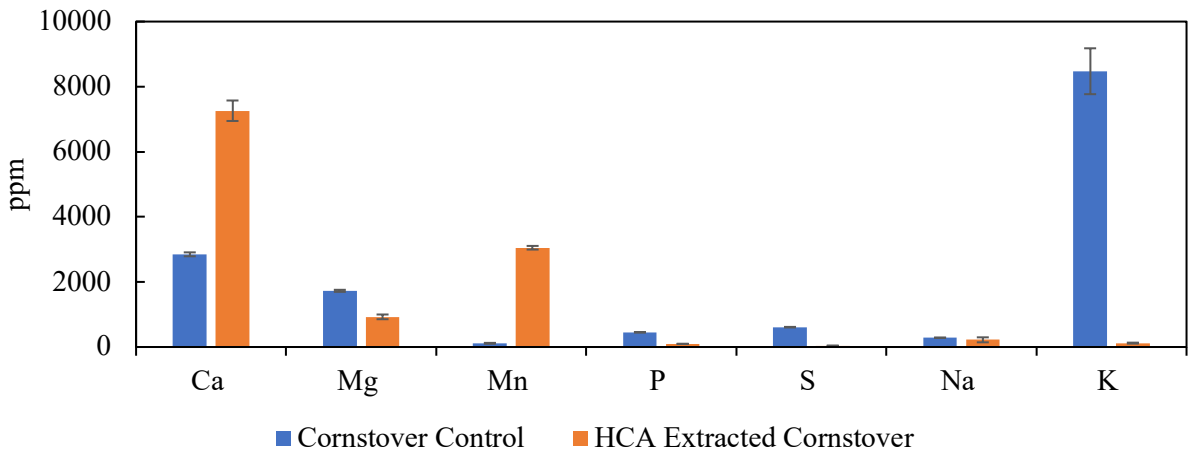

Figure 5.4. Displays the comparison of the ICP-OES analysis of the typical corn stover elemental and HCA extracted corn stover.

3.2 AD of HCA extracted corn stover

The effect of typical HCA extraction of corn stover was first studied in the batch AD test. Since the HCA extraction reduced the amount of both lignin and ash (Fig. 5.2) in corn stover, the biodegradable content in HCA CS (88.12%) was higher than that in raw CS (72.24%). As shown in Fig. 5.5A, cumulative biogas production of HCA CS at both loadings (40g/L and 32.5g/L) was higher than control and raw CS at the first 9 days, which indicated the deconstructed lignocellulosic structure was more accessible for the microorganisms. However, the biogas production rate of HCA CS was greatly slowed down afterward. The biogas production of raw CS was steady and surpassed all other three conditions after 9 days. At the end of the batch test, raw CS produced significantly higher biogas production than the control ($p<0.01$) while HCA CS at two loadings produced comparable amount of biogas to the control. Fig. 5.5B showed that the methane production of raw CS was also significantly improved, while HCA CS resulted in reduced methane production with extremely low methane content (25.04% ~ 45.71%). The main reason would be that HCA CS pretreated with alkaline made it easily digestable for microbes,

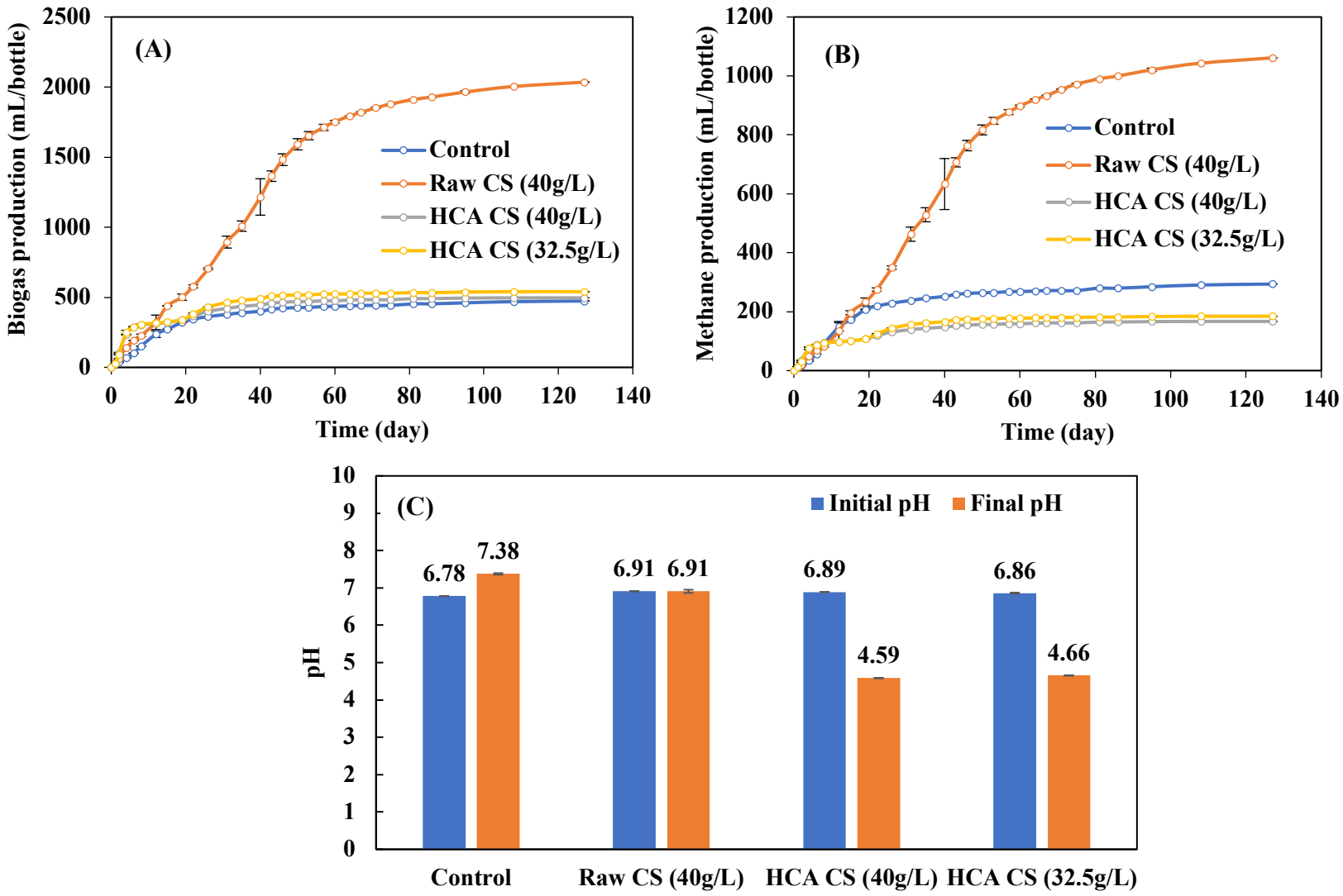

Figure 5.5. Profiles of the first batch of AD. **(A)** Cumulative biogas production; **(B)** cumulative methane production; **(C)** pH berfore and after AD. Raw CS: raw corn stover; HCA CS: HCA extracted corn stover.

which caused accumulation of volatile fatty acids (VFAs) and reduced pH. The imbalance between acetogenesis and methanogenesis resulted in the reduced biogas and methane production. The pH results in Fig. 5.5C clearly suggested that HCA CS resulted in very low final pH, proving that low pH was the main reason caused inhibition. The digesters had insufficient buffering capacity to maintain a stable pH.

To maintain the buffering capacity of the HCA extracted corn stover, the washing step was eliminated to get the unwashed HCA extracted corn stover. Based on the differences of the solvents used in the extraction step, two types of unwashed HCA CS were achieved. The first type (unwashed HCA CS-1) was pretreated with NaOH, water and ethanol, while the second type (unwashed HCA CS-2) was pretreated with NaOH and water. HCA was less soluble in water while more soluble in ethanol, thus the lignin content in unwashed HCA CS-1 was lower than that in unwashed HCA CS-2. As shown in Fig. 5.6A, no obvious inhibition was observed in the biogas production. Unwashed HCA CS-1 at two loadings both produced biogas faster than control or unwashed HCA CS-2. At lower loading of 1g unwashed HCA CS-1, it took around 30days to reach the plateau phase; at higher loading of 40g/L, it took longer (about 50 days) to reach the plateau phase. For unwashed HCA CS-2, there was almost no biogas production during the first 12 days. One possible reason would be that the high initial pH (9.81) caused the environmental stress to the microorganisms; another possible reason might be due to the exist of remaining lignin in the feedstock which made the structure of corn stover more recalcitrant for microbes' access. From 15th day, the biogas production began to gradually increase, and it took around 63days to reach the plateau phase. The trend of methane production (Fig. 5.6B) was quite similar to the biogas production. All three conditions resulted in significant higher methane production than control, indicating no inhibition was caused. As shown in Fig. 5.6C, unwashed

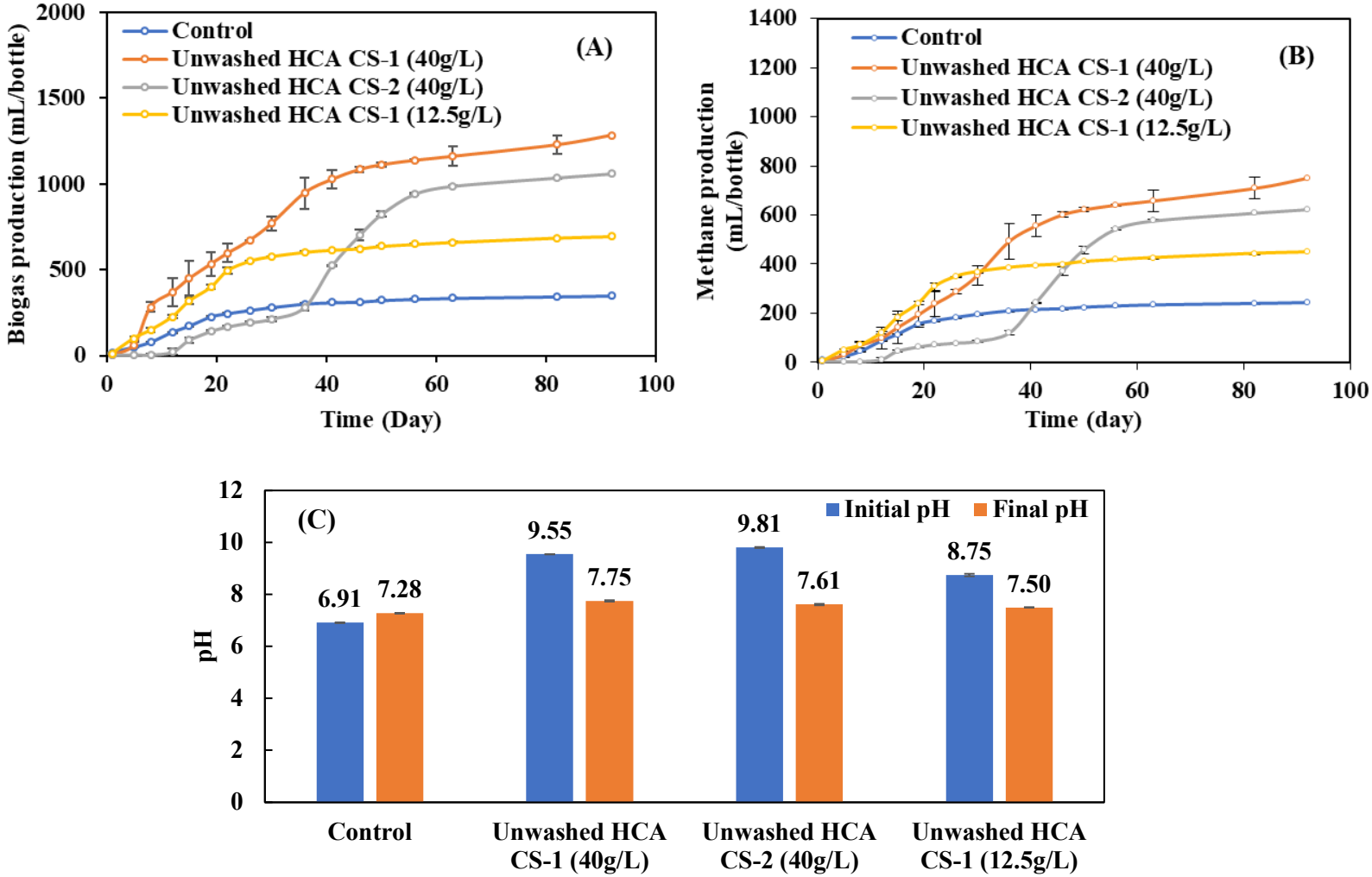

Figure 5.6. Profiles of the second batch of AD. **(A)** Cumulative biogas production; **(B)** cumulative methane production; **(C)** pH berfore and after AD. Unwashed HCA CS-1: unwashed HCA extracted corn stover pretreated with NaOH, water and ethanol; Unwashed HCA CS-2: unwashed HCA extracted corn stover pretreated with NaOH, and water.

corn stover resulted in very high initial pH in the digesters, especially by unwashed HCA CS-2. The final pH in all the digesters were above 7, which was favored by the anaerobic microbes.

Table 5.1. Volatile solid contents of different corn stover samples.

Sample	VS (wt%)
Raw corn stover	82.82
HCA CS	87.87
Unwashed HCA CS-1	39.09
Unwashed HCA CS-2	44.47

Due to different pretreatment, the VS of different corn stover samples varied greatly. As shown in Table 5.1, the VS of raw corn stover was 82.82%. The result was quite similar to the previous reported corn stover VS (82.98%) (Liu et al., 2018). HCA corn stover had higher VS content (87.87%) due to the removal of ash in the washing step. However, for both unwashed HCA corn stover samples, the VS content was much lower due to the exist of the excess salt formed by introducing NaOH in the pretreatment step. Unwashed HCA CS-1 was extracted by ethanol, which resulted in lower lignin content and subsequent lower VS than HCA CS-2. To normalize the biogas and methane yield from different corn stover samples, the yield was calculated based on the VS of corn stover (Table 5.2). Compared to the raw corn stover, HCA CS at both loadings (40g/L and 32.5g/L) significantly reduced the biogas and methane yield, corresponding to the inhibition shown in Figs. 5.5A&B. Unwashed HCA CS-1 increased both biogas and methane yield due to the removal of lignin. Interestingly, lower loading (12.5g/L) of feedstock had higher biogas and methane yield than that with higher loading (40g/L). Although unwashed HCA CS-2 showed increased production in Figs. 5.6A&B compared to raw corn stover, the yield was decreased based on the VS.

Table 5.2 Net biogas and methane yield based on volatile solids content.

Samples	Biogas		CH_4	
	Yield (mL/g VS)	Increase	Yield (mL/g VS)	Increase
Raw corn stover (40g/L)	567.5		277.7	
HCA CS (40g/L)	12.1	-97.9%	-42.1	-115.2%
HCA CS (32.5g/L)	33.4	-94.1%	-43.9	-115.8%
Unwashed HCA CS-1 (40g/L)	747.4	31.7%	406.0	46.2%
Unwashed HCA CS-1 (12.5g/L)	885.1	56.0%	527.3	89.9%
Unwashed HCA CS-2 (40g/L)	500.0	-11.9%	267.5	-3.7%

5.3.3 Estimation of model parameters by kinetic modeling

The hydrolysis rate, methane production rate, lag phase and maximum methane yield are important in evaluating the performance of AD (Li et al., 2013). In order to assess these kinetic parameters, first-order, Cone, and modified Gompertz model were used in this study. Although these classical models have been widely used to predict the methane yield in multiple lab-scale and plant-scale digesters (Zhen et al., 2016; Li et al., 2015; Xie et al., 2011), the suitability and precision of models largely depend on the experimental conditions, operating parameters, inoculum sources and feedstock types. Moreover, the reliability of different models might differ for the same experiment conditions.

As shown in Table 5.3, the estimated parameters of the studied models of digesters under different conditions vary differently. For the first-order model, the determination coefficient (R^2) ranged from 0.8257 to 0.9714, which was lower than that in Cone model and Modified Gompertz model. This indicated that the assumption of the first-order model might not suit well for all the experiment conditions in this work. Similar results were found by Li et al. (2014), where a low R^2 of 0.751 in the first-order model was obtained in the thermophilic AD of alkaline-pretreated corn stover. For the Cone model and Modified Gompertz model, the R^2 ranged from 0.9767 to 0.9948 and from 0.9055 to 0.9982, respectively, indicating that methane yield could well be

explained by these two models. On the basis of the results of the Cone model, HCA extraction could significantly increase *k* compared to raw corn stover (0.091 day^{-1} of HCA CS versus 0.031 day^{-1} of raw CS at OLR of 40 g/L), where a higher *k* value indicates higher hydrolysis rate. However, higher hydrolysis rate did not result in higher maximum methane potential (P_{max}), which was corresponding to the inhibition observed in Fig. 5.5B. The main reason would be that fast hydrolysis rate caused accumulation of VFAs formation and reduced pH in digester, which inhibited the methanogens (Zhen et al., 2015). For the unwashed HCA CS, only HCA CS-1 increased the hydrolysis rate. The only difference between unwashed HCA CS-1 and unwashed HCA CS-2 was the ethanol utilization in the extraction step, which reflected that the ethanol played an important role in extracting lignin and subsequently increasing the biodigestibility of the corn stover. Meanwhile, unwashed HCA CS-1 had the highest P_{max} at OLR of 12.5 g/L. For the Modified Gompertz model, the maximum methane production rate (R_{max}) of HCA CS was the lowest, since sever inhibition was observed in Fig. 5.5B. Unwashed HCA CS-1 at OLR of 12.5g/L had the highest R_{max}, which was 123.4% higher than that of raw CS. The highest lag phase time (λ) of 28 day was found in the unwashed HCA CS-2 group, probably due to the lowest hydrolysis rate (as shown in Cone model). For unwashed HCA CS-1, λ at both OLRs were lower than that raw CS, indicating faster startup. Overall, Cone model and Modified Gompertz model could describe the real process more accurately in this study, giving insight into important parameters in the AD process.

5.4 Conclusion

HCAs extraction through a mild alkaline method could be an effective pretreatment to enhance the AD efficiency of corn stover. The biogas and methane yields of HCA extracted corn

Table 5.3. Parameters of the three models under different conditions.

		Raw CS (40 g/L)	HCA CS (40 g/L)	HCA CS (32.5 g/L)	Unwashed HCA CS-1 (40 g/L)	Unwashed HCA CS-2 (40 g/L)	Unwashed HCA CS-1 (12.5 g/L)
First-order	P_{max} (mL/g VS)	319.8	42.6	56.2	334.6	240.4	364.5
	k (day^{-1})	0.023	0.085	0.068	0.027	0.020	0.041
	R^2	0.9714	0.9541	0.9659	0.9604	0.8257	0.9710
Cone	P_{max} (mL/g VS)	302.7	53.4	70.0	347.4	275.2	373.3
	k (day^{-1})	0.031	0.091	0.074	0.036	0.023	0.056
	n	2.621	0.802	0.868	2.604	5.812	1.898
	R^2	0.9948	0.9821	0.9767	0.9910	0.9892	0.9896
Modified Gompertz	P_{max} (mL/g VS)	300.5	41.6	55.2	344.7	276.1	331.9
	R_{max} (mL/g VS day^{-1})	5.95	2.41	2.35	8.04	9.01	13.29
	λ (day)	7.97	0.00	0.00	7.78	28.00	4.11
	R^2	0.9982	0.9055	0.9357	0.9960	0.9857	0.9942

stover were found to be 747.4 and 406.0 mL/g VS, respectively, which were 31.7% and 46.2%higher than the raw corn stover. Besides, HCA extracted corn stover had higher hydrolysis rate and shorter lag phase than the raw corn stover. Meanwhile, reducing the OLR can further increase the biogas and methane yield. Collectively, it was proved that HCA extraction could be a feasible pretreatment to enhance the bioenergy production from agricultural biomass waste, not only increasing the biogas yield, but also proving high value renewable chemicals.

5.5 References

APHA; AWWA; WPCF, 2005. Standard methods for the examination of water and waste- water. American Public Health Association (APHA): Washington, DC, USA.

Beckham, G.T., Johnson, C.W., Karp, E.M., Salvachúa, D., Vardon, D.R., 2016. Opportunities and challenges in biological lignin valorization. Curr. Opin. Biotechnol. 42, 40–53. https://doi.org/10.1016/j.copbio.2016.02.030

Bolado-Rodríguez, S., Toquero, C., Martín-Juárez, J., Travaini, R., García-Encina, P.A., 2016. Effect of thermal, acid, alkaline and alkaline-peroxide pretreatments on the biochemical methane potential and kinetics of the anaerobic digestion of wheat straw and sugarcane bagasse. Bioresour. Technol. 201, 182–190. https://doi.org/10.1016/j.biortech.2015.11.047

Buranov, A.U., Mazza, G., 2009. Extraction and purification of ferulic acid from flax shives, wheat and corn bran by alkaline hydrolysis and pressurised solvents. Food Chem. 115, 1542–1548. https://doi.org/10.1016/j.foodchem.2009.01.059

Ghosh, A., Brown, R.C., 2018. Production of solubilized carbohydrates from lignocellulosic biomass using solvent liquefaction. Chem. Enginneering Doctor of, 231. https://doi.org/10.31274/etd-180810-5987

Hertog, M.G.L., Hollman, P.C.H., Venema, D.P., 1992. Optimization of a Quantitative HPLC Determination of Potentially Anticarcinogenic Flavonoids in Vegetables and Fruits. J. Agric. Food Chem. 40, 1591–1598. https://doi.org/10.1021/jf00021a023

Johnston, P.A., 2017. Thermochemical methylation of lignin to produce high value aromatic compounds. Mech. Eng. Doctor of, 101.

Li, K., Liu, R., Sun, C., 2015. Comparison of anaerobic digestion characteristics and kinetics of four livestock manures with different substrate concentrations. Bioresour. Technol. 198, 133–140. https://doi.org/10.1016/j.biortech.2015.08.151

Li, Y., Feng, L., Zhang, R., He, Y., Liu, X., Xiao, X., Ma, X., Chen, C., Liu, G., 2013. Influence of inoculum source and pre-incubation on bio-methane potential of chicken manure and corn stover. Appl. Biochem. Biotechnol. 171, 117–127. https://doi.org/10.1007/s12010-013-0335-7

Li, Y., Zhang, R., He, Y., Liu, X., Chen, C., Liu, G., 2014. Thermophilic solid-state anaerobic digestion of alkaline-pretreated corn stover. Energy and Fuels 28, 3759–3765. https://doi.org/10.1021/ef5005495

Liu, C.M., Wachemo, A.C., Yuan, H.R., Zou, D.X., Liu, Y.P., Zhang, L., Pang, Y.Z., Li, X.J., 2018. Evaluation of methane yield using acidogenic effluent of NaOH pretreated corn stover in anaerobic digestion. Renew. Energy 116, 224–233. https://doi.org/10.1016/j.renene.2017.07.001

Menon, V., Rao, M., 2012. Trends in bioconversion of lignocellulose: Biofuels, platform chemicals & biorefinery concept. Prog. Energy Combust. Sci. 38, 522–550. https://doi.org/10.1016/j.pecs.2012.02.002

Murciano Martínez, P., Punt, A.M., Kabel, M.A., Gruppen, H., 2016. Deconstruction of lignin linked p-coumarates, ferulates and xylan by NaOH enhances the enzymatic conversion of glucan. Bioresour. Technol. 216, 44–51. https://doi.org/10.1016/j.biortech.2016.05.040

Rencoret, J., Gutiérrez, A., Nieto, L., Jiménez-Barbero, J., Faulds, C.B., Kim, H., Ralph, J., Martínez, Á.T., del Río, J.C., 2011. Lignin composition and structure in young versus adult eucalyptus globulus plants. Plant Physiol. 155, 667–682. https://doi.org/10.1104/pp.110.167254

Renders, T., Van Den Bosch, S., Koelewijn, S.F., Schutyser, W., Sels, B.F., 2017. Lignin-first biomass fractionation: The advent of active stabilisation strategies. Energy Environ. Sci. 10, 1551–1557. https://doi.org/10.1039/c7ee01298e

Sun, R.C., Sun, X.F., Wang, S.Q., Zhu, W., Wang, X.Y., 2002. Ester and ether linkages between hydroxycinnamic acids and lignins from wheat, rice, rye, and barley straws, maize stems, and fast-growing poplar wood. Ind. Crops Prod. 15, 179–188. https://doi.org/10.1016/S0926-6690(01)00112-1

United States Department of Energy, 2011. U.S. Billion-ton Update: Biomass Supply for a Bioenergy and Bioproducts Industry.

Wang, M., Zheng, Q., Shen, Q., Guo, S., 2013. The critical role of potassium in plant stress response. Int. J. Mol. Sci. 14, 7370–90. https://doi.org/10.3390/ijms14047370

Xie, S., Frost, J.P., Lawlor, P.G., Wu, G., Zhan, X., 2011. Effects of thermo-chemical pre-treatment of grass silage on methane production by anaerobic digestion. Bioresour. Technol. 102, 8748–8755. https://doi.org/10.1016/j.biortech.2011.07.078

Zhen, G., Lu, X., Kobayashi, T., Kumar, G., Xu, K., 2016. Anaerobic co-digestion on improving methane production from mixed microalgae (Scenedesmus sp., Chlorella sp.) and food

waste: Kinetic modeling and synergistic impact evaluation. Chem. Eng. J. 299, 332–341. https://doi.org/10.1016/j.cej.2016.04.118

Zhen, G., Lu, X., Kobayashi, T., Li, Y.Y., Xu, K., Zhao, Y., 2015. Mesophilic anaerobic co-digestion of waste activated sludge and Egeria densa: Performance assessment and kinetic analysis. Appl. Energy 148, 78–86. https://doi.org/10.1016/j.apenergy.2015.03.038

CHAPTER 6. GENERAL CONCLUSION

6.1 Conclusions

This work investigated various strategies to enhance anerobic digestion of different feedstocks, including traditional methods like pretreatment and co-digestion, as well as novel hybrid processing with thermochemical pathway. The topic was discussed from five chapters including one published paper, one submitted paper and two in preparation.

Chapter 1 provided the background and literature review of basic concepts of anaerobic digestion and thermochemical pathway-fast pyrolysis. It gave a comprehensive review about the main factors in anaerobic digestion, the process operations and enhancement methods. The combination of anaerobic digestion with fast pyrolysis provided possibility of a hybrid processing, which can be achieved in three ways: anaerobic digestion of fast pyrolysis main product, byproduct in fast pyrolysis-biochar as additive in anerobic digestion, and fast pyrolysis of digestate.

Chapter 2 explored the hybrid processing by using aqueous phase from fast pyrolysis of corn stover as substrates in anaerobic digestion. It suggested that the aqueous phase was rich in toxic compounds where pretreatment was essential. Overliming was the most promising pretreatment not only removed most of the inhibitors but also increased the pH of the aqueous phase. Moreover, directed evolution was proved to be effective in enhancing the toxicity tolerance of the microbial comsortium so that higher loading of aqueous phase can be digested in the process.

Chapter 3 compared two types of biochar made from fast pyrolysis of corn stover in different ways. The biochar made from pretreated biomass was different in element contents, ash content, pH, alkalinity and surface area comparing to the one made from raw biomass. When two

types of biochar were applied in anaerobic digestion, they had very distinct effects on the digester performance. It turned out that the main characteristics of biochar contributing to improved biomethane yield include high pH and alkalinity, high residual organic compounds. Other element such as sulfur introduced in the biomass pretreatment step was the main inhibitory to the reduced biomethane yield.

Chapter 4 expanded the application of proper biochar in alleviating the acidification of anaerobic co-digestion of food waste and municipal sludge. The high buffering capacity of the biochar to volatile fatty acids provided a promising solution to anerobic digestion process with easily digestible feedstock. It was proved that biochar addition can recover pH from acidic condition, promote consumption of volatile fatty acids, provide sufficient alkalinity. The microbial analysis suggested that biochar addition with various loadings had different effects in the facilitating specific species.

Chapter 5 proposed a novel hydroxycinnamic acids extraction method as pretreatment in anaerobic digestion of corn stover. Extraction of hydroxycinnamic acids not only recovered high value chemicals but also deconstructed the recalcitrant lignocellulosic structure of corn stover. The base used to extract hydroxycinnamic acids can also serve as additional alkalinity in the digester. The results were very promising with hydroxycinnamic acids yields up to 6% of the biomass and biomethane yield improved by 46.2% compared to raw corn stover.

6.2 Future work

The field of anaerobic digestion has been studied for decades. However, there are still challenges need to be solved. Future work for this dissertation can involve the following parts. First, developing novel bioreactor to improve the efficiency of anaerobic digestion process.

Exploring hybrid processing of anaerobic digestion with other pathways can broaden possibility of biofuel production from different sources. Secondly, in terms of scaling up of the process, we need a deeper study and analysis of potential challenges including feedstock sources, digester capacity, operational problems, and local policies. Finally, techno-economic and life cycle analysis of different scenarios serve an important role for scaling up the process. Methodologies and researches to convert lab-scale process to pilot-scale are essential for the development of economical biofuel technology.