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Anaerobic Digestion of Aqueous Pyrolysis Condensate

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Abstract

Lignocellulosic material can be subjected to pyrolysis to yield pyrolysis liquid, biochar, and gas. The pyrolysis liquid resulting from the condensation of vapours can be separated into a rich organic condensate and an acidic “aqueous pyrolysis condensate” (APC) which may be considered a waste. The target of this research is subjecting APC to anaerobic digestion to produce biogas that can be energy purposed, and to reduce the organic load of APC to acceptable levels for possible disposal. From this study, it was found that without any treatment, the inoculum requires 50 to 60 days of adaptation period to reduce the amount of inhibitors in APC and produce methane, along with 30 % of COD reduction. The addition of nutrient and biochar resulted in higher methane production from APC. This research showed the feasibility of anaerobically digesting APC to produce methane and reduce COD.

Keywords

Anaerobic Digestion, Pyrolysis, Lignocellulosic Biomass, Waste-to-Resource, Biomethane.

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List of Abbreviations

AD: Anaerobic Digestion

APC: Aqueous Pyrolysis Condensate

CFU: Colony Forming Units

COD: Chemical Oxygen Demand

DAD: Diode Array Detector

GC: Gas Chromatography

HPLC: High Performance Liquid Chromatography

ICP-OES: Inductively Coupled Plasma Optical Emission Spectrometry

RID: Refractive Index Detector

sCOD: soluble COD

VFA's: Volatile Fatty Acids

VS: Volatile Solids

VSS: Volatile Suspended Solids

TS: Total Solids

TSS: Total Suspended Solids

Chapter 1

1. Introduction

1.1 Introduction and Motivation

Biomass is an abundant resource on earth that ranges from agriculture and forestry to microbial systems (Himmel, 2008). Through biorefinery processes, biomass is converted and processed to yield renewable fuels and chemicals (Brown, 2007). Biomass conversion methods can be classified under two categories: thermochemical and biochemical processes. Thermochemical processes utilize heat to convert biomass to intermediates that can be processed to become useful products. Biochemical processes involve the use of microorganisms or enzymes (Himmel, 2008). Importantly, as in any other engineering process, biomass conversion into renewable fuels and chemicals generates waste.

Pyrolysis is one of the methods for fuel and chemicals production in thermochemical conversion technologies (Himmel, 2008). In pyrolysis, organic matter is thermochemically decomposed in an environment lacking oxygen (Monlau et al., 2016). This process yields three products: non-condensable gas, biochar, and pyrolysis liquid (Bridgwater, 2012). The non-condensable gases (i.e. carbon monoxide, methane, ethane and hydrogen) can generate heat and electricity (Li et al., 2013). Biochar can be useful in environmental remediation and pollutant biodegradation (Oliveira et al., 2017). In addition, extensive research is generating new biochar applications (Nanda et al., 2016). The pyrolysis liquid, which contains a high proportion of water, will phase

separate to give rise to an organic phase and an aqueous phase (Oasmaa & Kuoppala, 2008). The organic phase can be valuable because it is suitable for being used as fuel and for the production of a wide variety of chemicals after further processing (Li et al., 2013). On the other hand, the aqueous phase contains mostly water and water-soluble substances that are low in calorific value (Oasmaa & Kuoppala, 2008), including large quantities of acetic acid. The aqueous phase of pyrolysis liquid (APL), considered the waste stream of the process due to its high acidity and water content, may represent an environmental burden.

Because of the characteristics of the APL (high water and acid content), it requires additional processing for either utilization or disposal. However, this waste stream in particular, shows suitability for biochemical processes, i.e. anaerobic digestion (AD), which seems to have the potential not only as a method to handle APL, but also as a way to create value from it. AD, through a series of biological degradation processes carried out by a consortium of microorganisms, can degrade the organic load of APL meanwhile producing biogas, mostly composed of methane and carbon dioxide (Torri & Fabbri, 2014; Hubner & Mumme, 2015).

By coupling anaerobic digestion and pyrolysis, the waste stream from pyrolysis (APL) can be anaerobically digested to create biogas that serves energy purposes, while the digestate from AD, if any, can be pyrolyzed to create valuable chemicals and fuel, and the effluent may be returned to the fields for nutrient cycling. The integration of thermochemical and biochemical conversion methods creates an environment with minimal waste, and this is known as the “Circular Economy” (see **Figure 1.1**).

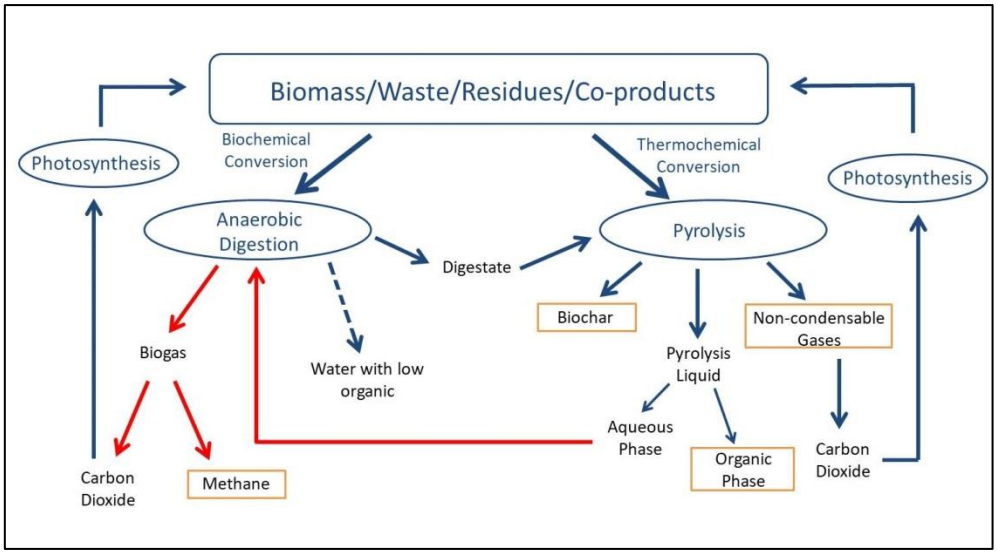

Figure 1.1 Circular Economy from integration of biochemical conversion and thermochemical conversion.

In 2014, Torri & Fabbri evaluated the anaerobic digestion of aqueous pyrolysis liquid and studied the addition of biochar as a solution for increasing the performance of the system. The research in this thesis differs from the work performed by Torri & Fabbri in 2014 mainly in the following. First, the aqueous pyrolysis liquid for this research was obtained from fractional condensation instead of phase separation as in Torri & Fabbri's study, hence the name aqueous pyrolysis condensate (APC). Second, the biomass pyrolyzed in this research was Birchwood rather than Corn Stalk. Different biomass might result in differences in the characteristics and composition of the aqueous pyrolysis liquid. Third, the anaerobic culture used for anaerobic digestion in Torri & Fabbri's study was from various sources including a corn silage digestion plant, distillery residues digestion plant and MSW (Municipal Solid Waste) digestion plant; however, the anaerobic culture used in this study is from a single source—a food waste digestion plant. Therefore, this study compares the performance of APC

anaerobic digestion, i.e. biogas production, against the results reported by Torri & Fabbri in 2014. Also, this thesis studied approaches to increase the biogas production relative to previous work.

Chapter 2

2. Background

This chapter aims to provide background information relevant to this research. The first three sections give background information on the three main concepts: lignocellulosic biomass, pyrolysis, and anaerobic digestion. Section 2.4 discusses what has already been done regarding the anaerobic digestion of aqueous pyrolysis liquid.

2.1 Lignocellulosic Biomass

Biomass, generally speaking, refers to biological organisms as well as any organic matter derived from them (Wang & Luo, 2017). Lignocellulosic biomass refers to the non-edible part of the plant biomass due to its non-starch and fibrous nature. It has received lots of attention due to its potential as raw material for the production of chemicals and fuels (Cherubini, 2010). As a renewable resource, lignocellulosic biomass can be classified as either agricultural resource or forest resource (Wang & Luo, 2017). Agricultural resources include crop residues, animal manures and food processing residues; while forest resources include logging residues and mill processing residues (Yang, 2007). Examples of biomass resources are shown in **Table 2.1**.

Table 2.1 Examples of Biomass Resources (Adapted from Wang & Keshwani, 2010)

Edible Biomass	Lignocellulosic Biomass (Agricultural)	Lignocellulosic Biomass (Forest)
Sugar Crops (sugarcane, sugar beet, sweet sorghum) Starch Crops (corn, wheat, potato, sweet potato)	Corn stover Wheat straw Rice straw	Softwood (pine, spruce) Hardwood (poplar, willow, oak)

The main components of the cell wall of lignocellulosic biomass are cellulose, hemicellulose and lignin (Wang & Luo, 2017). The distribution of these three components is illustrated in **Figure 2.1**. As seen in the figure, cellulose and hemicellulose are covered by lignin.

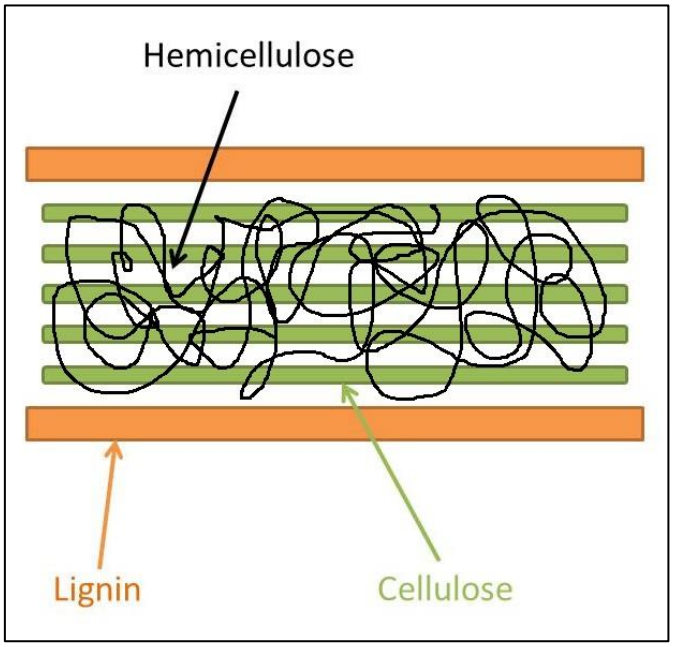

Figure 2.1 Distribution of cellulose, hemicellulose and lignin in the cell wall.

2.1.1 Cellulose and Hemicellulose

Cellulose forms the basic structure of plant cell walls. Cellulose is a linear homopolysaccharide composed of anhydroglucose units with a chemical formula of $(C_6H_{10}O_5)_n$ (Jonsson & Martin, 2015; Keshwani, 2010). In cellulose, the glucose units are linked by β-1,4-glycosidic bonds. The crystallinity of cellulose comes from the intramolecular and intermolecular hydrogen bonding of the OH groups in glucose. Its linear and crystalline structure makes cellulose difficult to hydrolyze by enzymes and break down into glucose monomers (Keshwani, 2010).

Hemicellulose is a branched heteropolysaccharide (Jonsson & Martin, 2015). The basic units of the heteropolysaccharide (see **Figure 2.2**) in hemicellulose include five-carbon sugars such as arabinose and xylose, six-carbon sugars like galactose, mannose, etc., and uronic acids. Hemicellulose can hold cellulose and lignin together by forming hydrogen bonds with cellulose and covalent bonds with lignin (Keshwani, 2010). In comparison to cellulose, hemicellulose is easier to break down due to a low degree of polymerization (Cherubini, 2010).

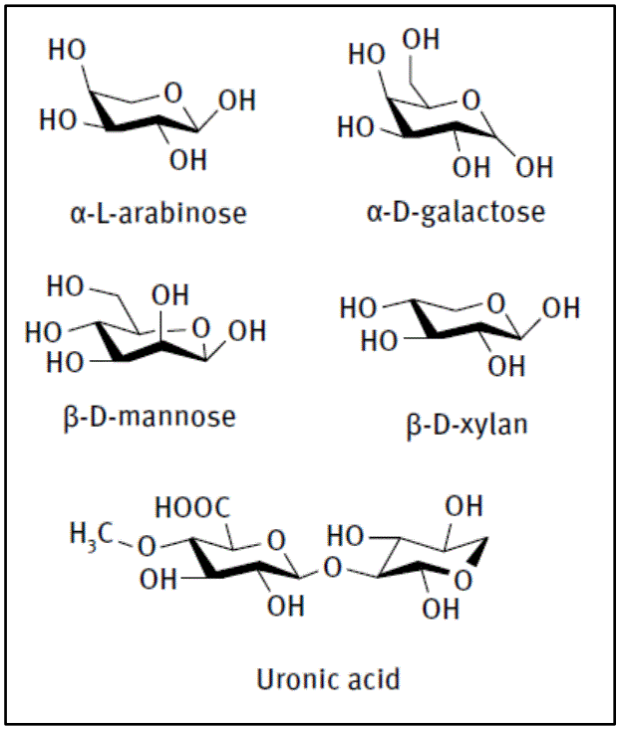

Figure 2.2 Basic units of hemicellulose (Adapted from Wang & Luo, 2017).

There are many applications for cellulose such as in pulp and paper, in the manufacturing process of insulation material and nitrocellulose (Keshwani, 2010). Cellulose and hemicellulose are commonly used in biochemical conversion processes, after pretreatment of the feedstock to free its sugars monomers, to produce valuable products like ethanol, xylitol, lactic acid, etc. (Dietrich et al., 2017).

The percentage of lignin, cellulose and hemicellulose present in lignocellulosic biomass varies from one type of biological specie to another (see **Table 2.2** for the percentage of cellulose and hemicellulose). This variation will contribute to the differences in the yield of chemical products from pyrolysis of various lignocellulosic biomasses.

Table 2.2 Percentage of cellulose and hemicellulose in different biomass (Adapted from Keshwani, 2010)

	Wood	Agricultural	Grass
Cellulose (% dry wt.)	40 ~ 50 %	30 ~ 45 %	25 ~ 50 %
Hemicellulose (% dry wt.)	25 ~ 30 %	20 ~ 25 %	15 ~ 30 %

2.1.2 Lignin

Lignin is a complex aromatic polymer composed of phenylpropanoid units (Jonsson & Martin, 2015). There are three basic structural units in lignin (**Figure 2.3**) and the functional groups that are present in the basic units vary from alcoholic hydroxyl and phenolic hydroxyl, to carbonyl and methoxyl, etc. (Wang & Luo, 2017). The presence of carbon-carbon and ether linkages in lignin creates a highly polymerized and complex structure, which makes it difficult to degrade. The amount of lignin in grasses and hardwoods are around 15 to 25 % dry wt. and 25 to 35 % dry wt. for softwoods (Keshwani, 2010; Wang & Luo, 2017).

Lignin compounds are useful as adhesive agents and raw materials for other chemical synthesis (Keshwani, 2010). In the pulp and paper industry, lignin can be combusted to provide energy. However, when it comes to biochemical conversion processes, lignin is not a suitable feedstock because its monomers cannot be fermented or digested. Importantly, the thermochemical degradation of lignin results in phenolic compounds

which have been reported in previous studies to be inhibitory for many microorganisms (Wang et al., 1991).

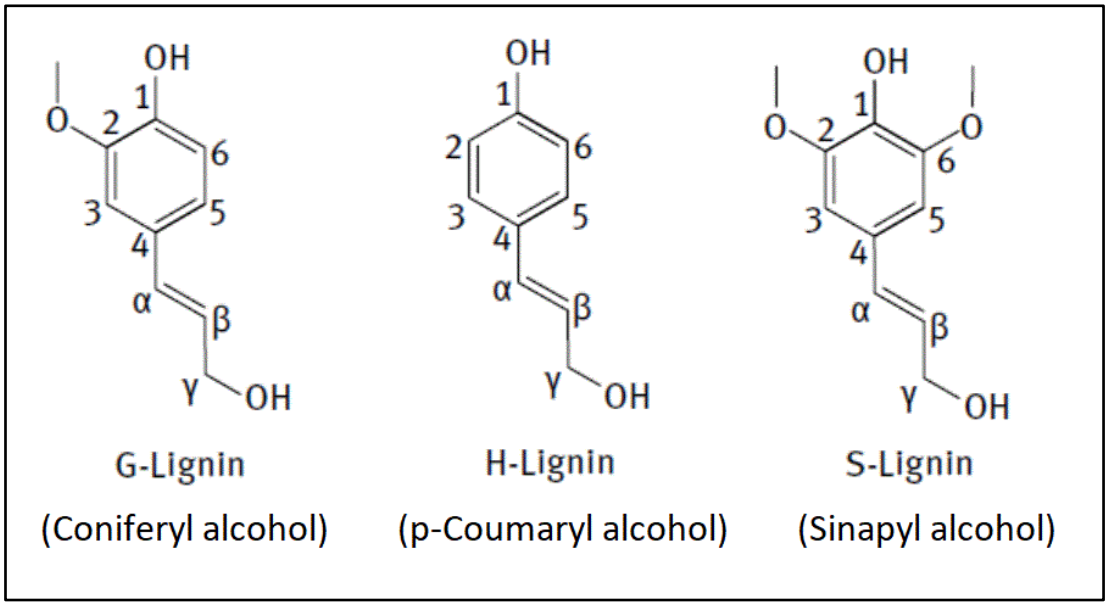

Figure 2.3 Three basic structural units in lignin (Adapted from Wang & Luo, 2017).

2.2 Thermochemical Conversion - Pyrolysis

As mentioned in the Introduction, pyrolysis is a thermochemical decomposition process in which the organics, mainly biomass, are thermally cracked in the absence of oxygen or in the presence of quantities of oxygen well below the stoichiometric level required for combustion. The temperatures for pyrolysis usually range from 400 ℃ to 600 ℃ (Basu, 2010). In general, biomass is fed into a pyrolysis reactor (typically a fluidized bed reactor) that contains hot solids as the heat source and mixing medium for thermal cracking (Basu, 2010). As a result of biomass decomposition, char,

condensable and non-condensable gases are formed. The condensable gases can be condensed into bio-oil while the non-condensable gases remain as gas which can be burned to supply heat to the pyrolysis reactor (Basu, 2010).

Pyrolysis of biomass undergoes primary decomposition reactions and gas-phase secondary tar-cracking reactions. Primary decomposition yields the volatiles and the char and the secondary reaction gives rise to three products: liquid, char and gas (Basu, 2010). Pyrolysis can be classified into either fast or slow pyrolysis. The heating rate of fast pyrolysis is high while the heating rate of slow pyrolysis is low. Fast pyrolysis is the method commonly used for bio-oil production because it generates higher yields of bio-oil (about 60 to 75 %) due to short vapour residence time which limits secondary decomposition reactions into gas. Slow pyrolysis involves longer vapour residence times and yields char and gases as the main products (Basu, 2010, Jameel et al., 2010).

The product of pyrolysis is dependent on many different parameters such as the biomass chemical and physical properties, and the process conditions at which the pyrolysis is operated (heating rate, pyrolysis temperature, and the residence time). The chemical composition affects the yield of each product; for instance, pyrolyzing biomass that contains more cellulose will yield more condensable vapours whereas the pyrolysis of biomass containing more hemicellulose will have a greater yield of non-condensable gas. Pyrolysis temperature is also an important parameter as it can affect the relative amounts of char, gas and bio-oil yielded. Under higher pyrolysis temperature, less char and more gas will be produced. The effects of heating rate and residence time on product yield have been discussed in the previous paragraph.

2.2.1 Pyrolysis of Cellulose

In thermal cracking, dehydration, condensation and depolymerisation occur simultaneously to break down larger molecular structures into smaller ones. The major chemical products of cellulose pyrolysis include levoglucosan, hydroxyacetaldehyde, hydroxyacetone, 5-HMF, furfural, etc. (Diebold & Bridgwater, 1997). Other chemical products from the pyrolysis of cellulose are acids, like acetic and propionic acid. Under typical pyrolysis conditions, the following condensable volatile compounds from pyrolysis of cellulose can be detected: acids, aldehydes, ketones, esters, ethers and phenols (Wang & Luo, 2017).

Given that other parameters are kept constant, at lower temperature, the pyrolysis of cellulose generates biochar as the main product. As pyrolysis temperature increases, the production of condensable and non-condensable gases also increases. The production of condensable gases increases with temperature until it reaches a maximum temperature (around 600 ℃), and then starts to decrease due to the progressive additional fragmentation of the molecules into permanent gases. The yield of levoglucosan decreases with increasing pyrolysis temperature whereas the yields of hydroxyacetaldehyde and hydroxyacetone increase with temperature (Wang & Luo, 2017).

The gas phase residence time is controlled by the inert carrier gas flow rate. By increasing the carrier gas flow rate, the residence time is decreased. One can increase the residence time to increase the yield of hydroxyacetaldehyde and hydroxyacetone due to the additional decomposition of levoglucosan. The yield of 5-HMF and other furans will decrease when the residence time is increased (Wang & Luo, 2017).

2.2.2 Pyrolysis of Hemicellulose

As mentioned previously, hemicellulose is composed of different monosaccharides: xylose, arabinose, mannose, and galactose, etc. Pyrolysis of those monosaccharides gives acids (i.e. formic, acetic, propionic), furans (i.e. furfural, 5-HMF), ketones, alcohols, and anhydro-sugars. The product of pyrolysis of hemicellulose generates high concentrations of furfural and 5-HMF due to the thermal cracking of 5-carbon and 6-carbon sugars (Wang & Luo, 2017).

Hemicellulose can decompose rapidly under lower temperature compared to cellulose and lignin. The liquid yield from pyrolysis of hemicellulose increases with increasing pyrolysis temperature until it reaches a maximum yield. The composition analysis of liquids shows that it includes acids, aldehydes, ketones, furans, etc. At pyrolysis temperatures lower than 300 ℃, the major products are furfural and saccharides; however, at temperatures higher than 300 ℃, the major products are ketones and aldehydes (Liu et al., 2010). Longer residence times will favour the formation of acids and decrease furan yield.

2.2.3 Pyrolysis of Lignin

Lignin decomposes more slowly in comparison to cellulose and hemicellulose. The pyrolysis oil contains mostly phenolic compounds, following by aldehydes and acids. Examples of phenolic compounds from pyrolysis of lignin are guaiacols and syringols. Pyrolysis of lignin produces more chars compared to pyrolysis of cellulose and hemicellulose. **Figure 2.4** shows some typical phenolic compounds produced from pyrolysis of lignin.

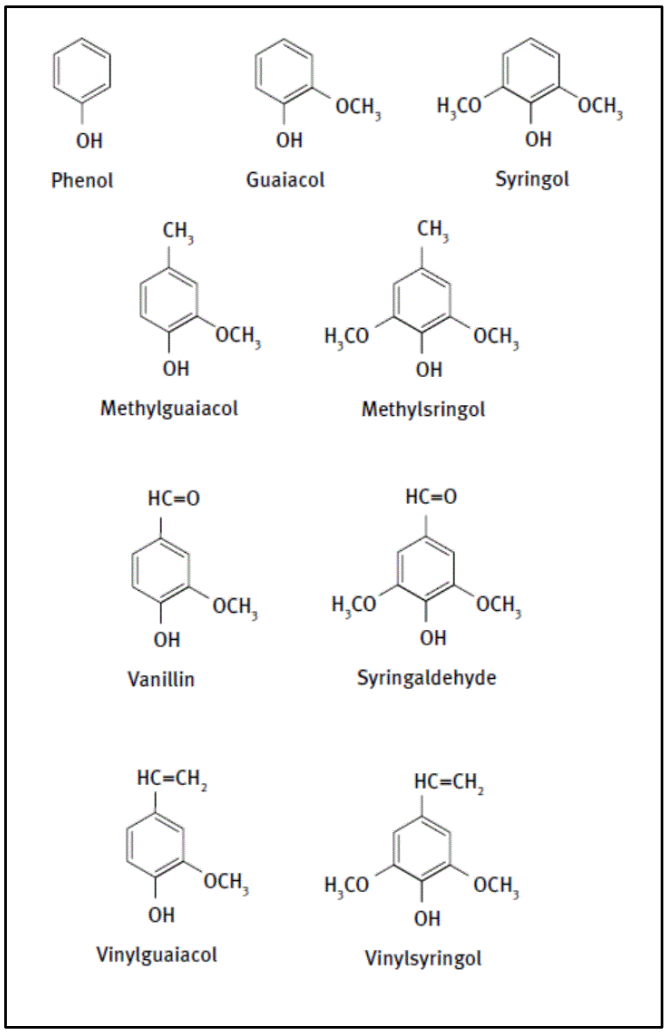

Figure 2.4 Typical phenolic compounds produced from pyrolysis of lignin (Adapted from Wang & Luo, 2017).

The yield of guaiacols and syringols in pyrolysis liquid increases at first with temperature and then decreases; the yield of phenols and catechols continue to increase with temperature, resulting from the decomposition of guaiacols and syringols (Wang & Luo, 2017). The yield of phenolics without methoxyl group was found to increase with residence time but the yield of guaiacols decreases with residence time after reaching their maximum yield (Jegers et al., 1985).

2.3 Biochemical Conversion - Anaerobic Digestion (AD)

2.3.1 Reaction Pathways

According to McCarty (1964), there are two stages in anaerobic treatment: (a) a waste conversion stage in which complex organics are converted to organic acids by acid forming bacteria, and (b) a waste stabilization stage carried out by methane forming bacteria to produce methane and carbon dioxide from organic acids. To be more specific, there are four biochemical reactions throughout the entire anaerobic digestion: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

In hydrolysis, the complex polymers, such as carbohydrates, proteins and fats are broken down by microorganisms into their corresponding monomers (monosaccharides, amino acids and fatty acids). The microbes in acidogenesis would use the products from the hydrolysis reaction to produce short-chain organic acids (or called volatile fatty acids, i.e. formic acid and acetic acid) and alcohols as the major products. The microbes performing acetogenesis in turn use the alcohol and volatile fatty acids (VFA's) as their substrate to form acetates and hydrogen. Lastly, the methanogens use the products from previous reactions to produce biogas which is mostly composed of methane and carbon dioxide (Zieminski & Frac, 2012). There are two major mechanisms for methane production: acetic acid cleavage (Reaction 1) which accounts for two-thirds of total methane production, and carbon dioxide reduction (Reaction 2) which accounts for one-third of methane production. The methane in biogas can be used for power generation.

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad [1]$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad [2]$$

2.3.2 Influential Factors (Temperature and pH)

There are multiple parameters that can affect anaerobic digestion: temperature, pH, presence of inhibitors, amounts of substrate and nutrients available for the digestion (McCarty, 1964). Any disturbance in the above parameters would cause an imbalance in the process. In anaerobic digestion, the product from one step becomes the substrate of the following step (microbial syntrophy). If an imbalance occurs in the process, which often causes the accumulation of products from one step, the whole anaerobic digestion will be inhibited. Therefore, it is very important to carefully monitor and control the parameters.

Temperature and pH are important parameters because they can affect the growth of microbes and the enzymatic activity for each step of the anaerobic digestion; hence the anaerobic digestion should be carried out at optimal temperature and pH. The optimum temperature for mesophilic anaerobes is in the range of 29 ℃ to 37 ℃, while the optimum temperature for thermophilic anaerobes is between 48 ℃ and 57 ℃ (McCarty, 1964). At higher temperatures, the metabolic rate of the microbes is higher but this might also increase the amount of intermediate products such as VFA's, which increases the risk of inhibition (Chen et al, 2008).

Methanogens are very sensitive to pH. The optimum pH for anaerobic digestion is 6.6 to 7.6 (McCarty, 1964). When pH falls below 6.2, the acidic environment is toxic to the methanogens (McCarty, 1964). The pH is important not only as a controlling

parameter, also as an indication of inhibition in the anaerobic digestion. Previous literatures have suggested that pH and volatile fatty acid concentrations are stress indicators (Ahring et al., 1995). A decrease in pH is an indication of VFA accumulation.

Nitrogen and phosphorus are two of the most important macronutrients required by biological processes; they are available in the form of NH_4^+-N (ammonical-nitrogen) and HPO_4^--P (orthophosphate-phosphorus). Micronutrients such as cobalt, iron, and nickel are also required by the methane-forming bacteria to produce methane from acetate (Gerardi, 2003). Although these macro and micronutrients are essential to microorganisms, if they are present at an amount higher than the threshold levels, they could cause inhibition in AD.

2.3.3 Inhibitors

The process of anaerobic digestion is also affected by the presence of inhibitors. McCarty (1964) discussed common inhibitors (alkali and alkaline-earth salt, ammonia, sulphides, heavy metals, etc.) in anaerobic digestion and how to control toxic materials. To control the toxicity of the anaerobic digestion system, one can dilute the medium until the toxicity is below the threshold level, or add materials that can either precipitate out the toxic compounds (complex formation) or antagonize the toxicity.

Ammonia toxicity is a common cause of inhibition in anaerobic treatments that has protein- or urea-rich feed. There are two forms of ammonia (ammonium ion NH_4^+ or ammonia gas NH_3) and the dominant form is dependent on the pH of the environment. The dissociation constant of ammonia is 9.25 (Haynes, 2014). When the pH is higher

than 9.25, there the formation of NH_3 is favoured which causes NH_3 to be the dominant form; when the pH is lower than 9.25 where there is more H^+, NH_4^+ becomes the dominant form. Free ammonia is very toxic to methanogens whereas ammonium is not as toxic. The anaerobic treatment is inhibited when the ammonia nitrogen concentration (which includes the concentration of both NH_4^+ and NH_3) is above 1,500 to 3,000 mg/L at pH higher than 7.6, and becomes toxic when the concentration is above 3,000 mg/L (McCarty, 1964). Ammonia can be removed by either stripping or precipitation (Kabdasli et al., 2000).

Hydrogen sulfide (H_2S), resulting from the reduction of sulfate and degradation of amino acids that contain sulfur, is very toxic to methanogens because it can inhibit their metabolic activity. Like ammonia, hydrogen sulfide toxicity is also dependent on pH. Decrease in pH will favour the formation of hydrogen sulfide. It was reported that 200 mg/L of hydrogen sulphide is enough to cause toxicity. When sulfide gets precipitate out by the addition of metals (such as iron or lead), it will not be able to diffuse into the cell and cause inhibition (Gerardi, 2003).

Small amounts of alkali and alkaline-earth salts can stimulate the growth and activities of the microbes; however, when the amount is above a certain limit, it becomes toxic and inhibitory. High levels of salts will create osmotic pressure and cause dehydration of bacterial cells (Yerkes et al., 1997). It seems that the salt toxicity is associated with cations and not anions and those cations come from the addition of chemicals for pH adjustment. The inhibitory level of cations in alkali and alkaline earth salts varies from literature to literature. **Table 2.3** lists the inhibitory level of the cations. It is proposed that the presence of an antagonist ion would reduce the

inhibition. For example, K^+, Mg^{2+} and Ca^{2+} are the antagonist ions for Na^+ and their presence in the medium has been shown to increase the tolerance of anaerobes to Na^+ (Soto et al., 1993).

Table 2.3 Inhibitory Level of Cations

Metal Ions	Stimulatory Level (McCarty, 1964)	Inhibitory Level (References)
Ca^{2+}	100 ~ 200 mg/L	Moderately inhibitory at 2,500 ~ 4,000 mg/L Strongly inhibitory at 8,000 mg/L (Kugelman & McCarty, 1964)
K^+	200 ~ 400 mg/L	Strongly inhibitory at 12,000 mg/L (McCarty, 1964)
Mg^{2+}	75 ~ 150 mg/L	Growth cease at 400 mg/L (Schmidt & Ahring, 1993)
Na^+	100 ~ 200 mg/L	Moderately inhibitory at 3,500 ~ 5,500 mg/L Strongly inhibitory at 8,000 mg/L (McCarty, 1964)

Salts containing heavy metals such as copper, zinc and nickel often cause anaerobic digestion to fail. Heavy metals inhibit microbial cells through the inactivation of

enzymes. As mentioned when discussing sulfide toxicity, heavy metals can combine with sulfide to form an insoluble salt which will not cause toxicity (Gerardi, 2003; Chen et al., 2008).

In addition to the above inhibitors, there are many other compounds that are inhibitory to anaerobic digestion: benzene ring compounds (Yang & Speece, 1986; Bhattacharya et al., 1996) and phenolic compounds (Sierra-Alvarez & Lettinga, 1991; Fang et al., 1995; Fang & Chan, 1997; Shin & Kwon, 1998), aldehydes (Gonzales-Gil et al., 2002), ketones (Playne & Smith, 1983), etc. The organic compounds that are most commonly reported to be inhibitory are the aromatic compounds (Wang et al., 2009).

The inhibitors that are likely to be found in aqueous pyrolysis liquid are the phenolics, aldehydes, ketones, furans, etc. resulting from the pyrolysis of cellulose, hemicellulose and lignin. Previous research study has found that threshold level of phenol to biogranules, which are cell aggregates, ranges from 850 to 1,700 mg/L (Fang & Chan, 1997). In 1983, Fedorak & Hrudey found that inhibition of methane by cresols occurs when the concentration of cresols is more than 1,000 mg/L. A phenol concentration of 1000 mg/L will decrease the gas production to 44 %; 3000 mg/L to 11 % and 10,000 mg/L to 4 %. Phenolic compounds are toxic towards microbial cells through the disruption of membranes (Mills et al., 2009). Aldehydes such as formaldehyde exhibit toxicity through denaturation of polynucleotides (Haselkorn & Doty, 1961). Furfural and HMF have cytotoxic effects towards bacteria and yeast by creating mutations in the DNA (Hadi et al., 1989). Research involving the study of inhibitors has showed that the inhibition of many of these compounds can be overcome by acclimatization.

2.4 AD of Aqueous Pyrolysis Liquid

Previous analysis showed APL contains water, acids and other substances like phenols and furans, etc. (Oasmaa & Kuoppala, 2008). The acids can be used by the microorganisms in the acetogenesis step of AD to produce acetate and hydrogen. However, substances such as phenols are known to be inhibitory to microorganisms (Fang & Chan, 1997). According to Wastewater Systems Effluent Regulations under the *Fisheries Act*, the level of carbonaceous biochemical oxygen demand (cBOD) in the wastewater stream permitted for direct disposal via the system's final discharge point is no more than 25 mg/L. The European Union effluent regulations (1991) stated that the regulated COD value is 125 mg/L and the percent of COD reduction required is 75 %. This means that the COD content in APL has to be reduced to less than 125 mg/L before direct disposal.

There are a few research studies investigating the feasibility of aqueous pyrolysis liquid AD and ways to increase its efficiency. As mentioned before, Torri & Fabbri (2014) found that the addition of biochar along with acclimatization of the microbial consortia makes the anaerobic digestion of aqueous pyrolysis liquid feasible. In their experiment, the inoculum (anaerobic microbial culture with other substances) is first adapted by adding 15 g of aqueous pyrolysis liquid (APL) from the pyrolysis of corn stalk pellets at 400 ℃, at the beginning of adaptation, and 22 g at every 60 days for a period of 200 days. In the adaptation experiment, Torri & Fabbri found that APL had significantly inhibited the biogas production and it took more than 20 days for

methane production to start increasing. The second addition of APL took the system 50 days for methane production to start. They also did a micro-batch test using the adapted inoculum comparing the biogas production from APL-only, APL with nutrient, and APL with biochar. From the micro-batch test, they concluded that the addition of nutrient did not have a significant improvement on biogas production, but the addition of biochar did.

In 2015, Hubner and Mumme subjected the aqueous pyrolysis liquor (from pyrolysis of digestates) that were produced at different temperatures (330 ℃, 430 ℃, and 530 ℃) to anaerobic digestion using an un-adapted inoculum. They found that the liquor from pyrolysis at 330 ℃ had greater methane yield than the pyrolysis liquor produced at 430 ℃ and the pyrolysis liquor from pyrolysis at 530 ℃ had the lowest methane yield. In their experiment, they found that furfural and 5-HMF were completely removed after anaerobic digestion, and up to 63 % of COD was removed, which showed the feasibility of using anaerobic digestion for degradation of organics present in the investigated aqueous pyrolysis liquor.

2.5 Research Objectives

Lignocellulosic biomass, such as forestry and agricultural residues, can be depolymerized via pyrolysis to yield syngas, bio-oil and biochar. The aqueous pyrolysis condensate (APC) contains acids and other organic compounds that are environmentally unfriendly and costly for disposal. AD may offer a suitable method for treatment of APC; however, the organics present (phenolics, ketones, aldehydes,

etc.) in the APC are inhibitors of anaerobic microorganisms; therefore, adaptation of microbial system or toxic mitigation techniques would be required to perform a successful AD of APC.

The main objective of this research was to confirm whether AD can indeed process aqueous pyrolysis condensate (APC) to reduce its organic content and make it suitable for disposal while producing a value added product, namely biogas for energy generation and to better understand the mechanisms involved in such process; moreover, the secondary objective included the study of the adaptation of anaerobic microbial consortia to the presence of toxic substances from APC.

Chapter 3

3. Materials and Methods

This chapter begins with the description of the sources of APC and of the AD inoculum used in this study, and then discusses the methodologies used to perform analyses and experiments.

3.1 APC and AD Inoculum

3.1.1 APC

The APC used in this research (see **Figure 3.1**) was provided by the Institute for Chemical and Fuels from Alternative Resources (ICFAR) resulting from the pyrolysis of Birch bark sawdust. The process used was an autothermal fast pyrolysis in which carefully controlled amounts of oxygen were introduced in the reactor in order to generate sufficient thermal energy by oxidation to self-sustain the pyrolysis process. The ground and dry biomass was fed to the pyrolysis reactor (bubbling fluidized bed reactor) using N_2 as pulse gas and continuous carrier gas. The biomass was thermally cracked at 500 ℃ inside the pyrolysis reactor which contained hot fluidized sand as the inert bed material for an efficient mixing and heat transfer. The vapour was condensed into three different condensers maintained at different temperatures. The first two condensers were part of the condensation unit for separating the organic phase (bio-oil) and were maintained at temperatures above 120 °C to prevent the

condensation of water. The APC was collected in the last condenser that was placed in an ice bath (Li et al., 2014).

Figure 3.1 Aqueous Pyrolysis Condensate (APC) from pyrolysis of Birch bark sawdust at 500 °C.

Although the complete composition analysis was not performed on APC in this study, previous studies suggested the aqueous phase of pyrolysis liquid contains volatile fatty acids, hydroxyacetaldehyde, small oxygenated compounds, hydroxyacids, anhydrosugars, phenols, furans and some nitrogen compounds (Torri & Fabbri, 2014; Hubner & Mumme, 2015).

3.1.2 AD Inoculum

The AD inoculum (anaerobic microbial culture with other substances) used throughout this research study was provided by Storm Fisher, London, Ontario, Canada, and came from single stage reactors digesting the Organic Fraction of Municipal Solid Waste (OFMSW). The operating conditions of the such reactors are as follows: temperature regime is mesophilic with an average of 38 °C, average pH of

7.8, residence time of 56 days, COD of 30.0 to 50.0 g/L, 22.9 g/L of TS and 15.7 g/L of VS. The AD inoculum was stored at 4 ℃ after collection from Storm Fisher.

3.2 Analysis of APC and AD Inoculum

3.2.1 COD, TS, VS, TSS, VSS Analyses

AD is the degradation of organic matter which results in biogas generation; therefore, the content of organic matter in the feedstock may affect it. In order to know how much of total organics and inorganics are present in the APC and in the inoculum, Total Solid (TS) – organic and inorganic matter, Volatile Solid (VS) – organic matter, Total Suspended Solid (TSS) and Volatile Suspended Solid (VSS) analyses were performed. Holliger et al. (2016) suggested COD is used as an indicative value to estimate total gas production; thus, analysis on total and soluble Chemical Oxygen Demand (COD) were also conducted.

COD (Adapted from *Standard Methods for Examination of Water and Wastewater*, APHA, 1999)

The sample was homogenized using a blender, then 2.0 mL of sample was transferred to the digestion vial (COD Digestion Vial High Range Plus, Hach, USA); the vial was well mixed and incubated for 2 hours at 150 ℃. After incubation, the vial was let to cool at room temperature, the organic matter formed complexes resulted in change of color. The change in color was related to COD content which was read straight from a

colorimeter previously calibrated. To measure the soluble COD (sCOD) of APC, a filtration step is required prior to transferring the content into the digestion vial.

TS, VS, TSS and VSS (Adapted from the *Standard Methods for Examination of Water and Wastewater*, APHA, 1999)

Total Solids (TS)

The evaporating dish was heated at 105 ℃ in oven for 1 hour and the weight was measured after cooling down to room temperature inside a desiccator. A measured volume of sample was transferred to the pre-weighed evaporating dish and placed in oven at 105 ℃ for at least 1 hour for water evaporation, then the samples were cooled down to room temperature inside of a desiccator. The heating and cooling procedure was repeated until the measured weight was constant. The final weight was recorded, and TS can be calculated using Equation 1.

$$TS\ in\ mg/L = \frac{(W_2 - W_1)\ in\ mg}{sample\ volume\ in\ mL} \times 1000 \qquad [1]$$

Where: W_1 = weight of dish; W_2 = weight of dried residue + dish.

Volatile Solids (VS)

The evaporating dish was ignited at 550 ℃ in a muffle oven for 1 hour and the weight was measured after cooling down to room temperature inside a desiccator. The residue from TS analysis was ignited at 550 ℃ for 15 to 20 minutes, and cooled down to room temperature inside a desiccator. The procedure of igniting and cooling was

repeated until the weight measured became constant. The final weight recorded could then be used to calculate VS using Equation 2.

$$VS\ in\ mg/L = \frac{(W_2 - W_3)\ in\ mg}{sample\ volume\ in\ mL} \times 1000 \qquad [2]$$

Where: W_2 = weight of residue + dish before ignition; W_3 = weight of residue + dish after ignition.

Total Suspended Solids (TSS)

The glass fibre filter used for filtration was washed with distilled water and de-watered by the application of vacuum before being placed on an evaporating dish. The evaporating dish, along with the filter, was heated at 105 ℃ in oven for 1 hour and the weight was measured after cooling down to room temperature inside a desiccator. A measured volume of sample was transferred to the pre-weighed evaporating dish and placed in an oven at 105 ℃ for at least 1 hour for water evaporation, then the samples were cooled down to room temperature inside of a desiccator. The heating and cooling procedure was repeated until the measured weight is constant. The final weight was recorded, and TSS can be calculated using Equation 3.

$$TSS\ in\ mg/L = \frac{(W_5 - W_4)\ in\ mg}{sample\ volume\ in\ mL} \times 1000 \qquad [3]$$

Where: W_4 = weight of dish + filter; W_5 = weight of dried residue + dish + filter.

Volatile Suspended Solids (VSS)

The glass fibre filter was prepared as stated in TSS analysis. The evaporating dish, along with the filter, was ignited at 550 ℃ in a muffle oven for 1 hour and the weight

was measured after cooling down to room temperature inside a desiccator. The residue from TSS analysis was ignited at 550 ℃ for 15 to 20 minutes, and cooled down to room temperature inside a desiccator. The procedure of igniting and cooling was repeated until the weight measured becomes constant. The final weight recorded can be used to calculate VSS using Equation 4.

$$VSS\ in\ mg/L = \frac{(W_5 - W_6)\ in\ mg}{sample\ volume\ in\ mL} \times 1000 \qquad [4]$$

Where: W_5 = weight of residue + dish + filter before ignition; W_6 = weight of residue + dish + filter after ignition.

3.2.2 pH and Elemental Analyses

Another parameter that can affect the biogas yield of a given feedstock is its chemical composition. It was reported in the literature that a feedstock carbon to nitrogen ratio (C:N) of 25:1 is the best for biogas generation and the recommended range for AD is 20:1 to 35:1 (Gerardi, 2003; Kwietniewska & Tys, 2014). Elemental analysis was performed to get the ratio of C, H, N, S and O in APC.

Elemental

The elemental (C, H, and N) analysis was done using an Elemental Analyzer (Thermo Scientific FlashEA 1112 HT).

pH

The pH of APC was measured using a pH meter (VWR Symphony SB70P). During the digestion process, the pH of the AD inoculum was checked using pH test strips (Micro Essential Lab Hydrion™ Micro Dispenser).

3.2.3 Glucose and VFA Quantification Using HPLC

Before AD, it is important to know how much substrate and AD intermediates are present in APC. Glucose is a common product from the hydrolysis of complex organic matter; therefore, it was used as a positive control and its concentration was monitored in this study. A decrease in glucose concentration during AD is desired because it shows that acidogenesis is not inhibited since the conversion of organic monomers, such as glucose, to volatile fatty acids (VFA's) is occurring. As mentioned earlier, VFA's are intermediate products of AD and their accumulation is an indicator of AD inhibition.

Quantification of glucose and VFA's in APC was performed using HPLC (Agilent 1260 LC) as described in Luque et al., 2016. The column used was a Hi-Plex H column kept at 60 ℃. The mobile phase used was 5.0 mM H_2SO_4 with a flow rate of 0.7 mL/min for 80 minutes; the compounds were detected using RID (Refractive Index Detector) with detector temperature around 50 ℃.

3.2.4 Common AD Inhibitors Analyses

Common inhibitors of anaerobic digestion reported in the literatures are ammonia, sulphides, light metal ions, heavy metal ions, etc. (Chen et al, 2008). Analyses on some

of those compounds were performed before carrying out AD. In addition, phenolic compounds are known to be inhibitory to microbial activity and there were studies that investigate the toxicity of phenolic compounds on anaerobes (Wang et al., 1991; Fang & Chan, 1997). Their aromatic structures give rise to the chromophoric property which can be determined by HPLC.

Ammonium Content

The ammonium concentration was measured by a colorimetric method using test kit (Ammonium Test, MColortest^{TM}, EMD Millipore Corp., Billerica, MA, USA) as described by Lee et al., 2018.

Metal Content

ICP-OES (Vista-Pro CCD Simultaneous, Varian) was used to analyze following metals: Al, Ca, Cd, Cr, Cu, Fe, K, Mg, Na, Ni, Pb, and Zn. The method used for digestion of metal was described by Hseu, 2004.

Sulphide Content

The amount of sulphide was estimated from the ICP-OES analysis of sulfur.

Phenolics Content

Total phenolic was quantified colorimetrically using the Folin Ciocalteau method as described by Rover & Brown, 2013.

Inhibitors with Chromophoric Properties

Many compounds (aromatics, aldehydes, etc.) that were reported in the literatures as inhibitors of AD can be detected by UV due to their chromophoric properties; for instance, compounds with nitro group can be detected at 271 nm, aldehyde groups at 290 nm, and esters at 205 nm (Pavia et al., 2009).

The amount of inhibitors with chromophoric properties was estimated using HPLC under the same operation condition as described by Luque et al., 2016. The chromatogram of the sample in the wavelength range of 200 nm to 340 nm was recorded by DAD. The peaks of the chromatogram are a relative measure of the amount of chromophoric compounds. The total area under the peak was obtained by a numerical integration over time and wavelength, and the value obtained was normalized with the concentration of acetic acid in the sample (Equation 5).

$$\frac{IV}{AA} = \int_{t=0min}^{t=80min} \int_{\lambda=200nm}^{\lambda=340nm} S_{DAD} dt d\lambda / C_{AA} \qquad [5]$$

3.3 General Experimental Procedure

The inoculation procedures were adapted from Holliger et al., 2016. Before using the inoculum for experiments, the inoculum was kept at 37 ℃ in an incubator for a period of time which is known as “degassing period” to allow the microbes to degrade endogenous organics. The length of degassing period varied from one batch of inoculum to another depending on the amount of endogenous organics present. The degassed inoculum and substrate were poured into the reactor vessels, and flushed

with N_2 to assure an anaerobic environment. No neutralization was performed throughout the entire study as the alkalinity of inoculum was able to keep the pH of the mixture in a range of 6.5 to 8.5. The reactor vessels were serum bottles capped with rubber septa and sealed using aluminum caps.

3.3.1 Study the Effects of Different Dilutions of APC on Biogas Composition

In this experiment, inoculum was degassed for a month. At the time of inoculation, the inoculum was diluted by 5 times using distilled water to a final volume of 52.5 mL in each 126 mL-reactor vessel, with 7.5 mL of feedstock.

Feedstock Preparation

In the positive control, 7.5 mL of feedstock was made of 1.5 g/L of glucose solution. In the experimental conditions, APCs were diluted to final volume of 7.5 mL with different dilutions. The dilutions were reported as final concentration taking in consideration the added volume of both the inoculum and the feedstock. For example, 1:100 APC means that the APC was diluted 100 times in total of 60 mL of working volume. Another way that was used in this study to express the APC concentration was by expressing it in terms of acetic acid concentration (as shown in **Table 3.1**).

Table 3.1 Acetic Acid Concentration in Different Dilutions of APC

Dilution factor of APC	Acetic Acid Concentration (g/L)
1:100	1.0
1:200	0.5
1:400	0.2
1:800	0.1

3.3.2 Study the Adaptation of AD Inoculum to APC

Two experiments were performed as duplicate in this study. In the first experiment, the inoculum used was degassed for four months, then transferred to 250 mL-reactor vessels where 75 mL of original inoculum was diluted with a solution made of distilled water and three different feedstocks, to a final volume of 150 mL. The three different feedstocks used were: 1.0 g/L of glucose, 1.0 g/L of acetic acid, 1.0 g/L of acetic acid equivalent APC. Glucose was the suggested model substrate for determining the activity of acidogenic microbial population in the reactor vessels, whereas acetic acid is the feedstock chosen for determining the activity of acetoclastic microbial population (Angelidaki et al., 2009). In this way, the inoculum was tested in-vivo for acidogenesis and acetogenesis stages. On Day 27 following the inoculation, 1.0 g/L of glucose was added to the three reactors.

In the second experiment, the inoculum used was degassed for four months, then transferred to 250 mL-reactor vessels where 75 mL of original inoculum was diluted with a solution made of distilled water and four different feedstocks to a final volume of 150 mL. The four different feedstocks used were: 1.0 g/L of glucose, 1.0 g/L of

acetic acid, 1.0 g/L of acetic acid equivalent APC, and 0.5 g/L of acetic acid equivalent APC. However, there was no glucose addition on Day 27 after the inoculation. The extra feedstock (0.5 g/L of acetic acid equivalent APC) was to determine whether lower inhibitor concentration will shorten adaptation period or not.

3.3.3 Study the Effects of Addition of Biochar and Nutrient on Biogas Production

The inoculum was degassed for two months, and then 30 mL of degassed inoculum was added to reactor vessels, a blank (30 mL of inoculum) and a glucose control (0.3 g of glucose added to 30 mL of inoculum) were made. The experimental conditions used for the rest of the reactor vessels were: 1) APC ONLY— 1.3 g/L of acetic acid equivalent of APC added to the reactor vessel containing 30 mL of inoculum, 2) APC + Nutrients— 60 mg of ammonium chloride and 25 mg of potassium phosphate and 1.3 g/L of acetic acid equivalent of APC was added to the reactor vessel containing 30 mL of inoculum, 3) APC + Biochar— 1.5 g of biochar and 1.3 g/L of acetic acid equivalent of APC was added to the reactor vessel containing 30 mL of inoculum. All the 3 experiment were carried out in triplicates.

3.4 Biogas Volume Measurement

The biogas produced was measured every two days for two weeks after inoculation, then, the measurement occurred once a week. The total volume of biogas produced was measured using a water displacement setup in which the water was acidified to pH less

than 4 to ensure no carbon dioxide was dissolved in water. The apparatus is shown in **Figure 3.2**. Biogas volume measurement is carried out at room temperature (22 ℃) and approximately 1 atm.

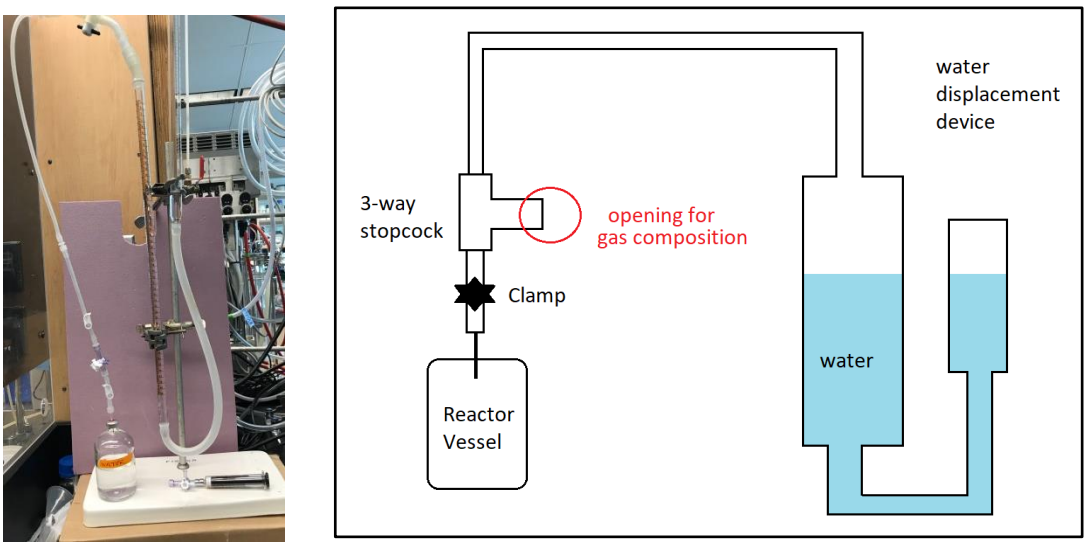

Figure 3.2 Water Displacement Device for biogas volume measurement. (Left: Actual Setup. Right: Schematic Diagram)

The apparatus possessed a three-way stopcock fixed to the needle and connected the water displacement device to the bottle (**Figure 3.3**) which gave the flexibility to measure gas volume, as well as sampling for biogas composition analysis. The gas for composition analysis was always drawn before measuring the volume of biogas produced. To take sample for biogas composition analysis, the reactor bottle was connected to the water displacement device as shown in **Figure 3.2**, the stopcock was turned until the "off" mark pointed towards the tubing connected to the burette, then the gas-tight syringe (with another three-way stopcock attached to the end of it) was connected to the horizontal opening (**Figure 3.4**), the clamp between the reactor vessel and the three-way stopcock was opened, draw a defined amount of gas (usually 5 to 10

mL), then the stopcock was turned until the “off” mark was pointing towards the gas-tight syringe (**Figure 3.5**). The stopcock on the gas-tight syringe was closed and the gas-tight syringe was disconnected from the water displacement device. Since the clamp located between the reactor and the stopcock was already opened, the rest of gases from the bottle would flow into the water displacement device to displace the water in the burette; the volume of gas drawn for composition analysis was added to the volume displaced in the device to obtain the total amount of biogas produced.

Figure 3.3 Connecting point of water displacement device for biogas volume measurement.

Figure 3.4 Stopcock "off" position when drawing gas sample for composition analysis.

Figure 3.5 Stopcock "off" position when finishes drawing gas sample for composition analysis.

3.5 Biogas Composition Analysis

The composition of the biogas produced was determined by drawing 5 to 10 mL of gas and injecting it into the GC (Agilent 7820A GC). The two major gases of biogas

(CH_4, and CO_2) were detected by the TCD (thermal conductivity detector). The front detector was operating at 300 ℃ and the back detector was operating at 250 ℃. The front inlet temperature was at 250 ℃. Helium gas was the carrier gas used. The oven temperature was at 180 ℃.

3.6 Community Level Physiological Profiling (CLPP)

The aim of using CLPP in this research was to show whether there was a difference in microbial composition of the inoculum based on their carbon source utilization pattern. CLPP is carried out in BIOLOG™ microplates, which are 96-well plates where each well of the plate contains a different carbon source as well as a redox dye. The redox dye results a change in colour that can be detected by spectrophotometry when the metabolic activity of microbial community is active. Based on the difference in carbon source utilization pattern before and after microbial adaptation to APC, CLPP can show that the microbial population composition has changed.

The BIOLOG™ plates used in this research were AN MicroPlates™. The carbon source in each well is as illustrated in **Figure 3.6a** and **Figure 3.6b**. The redox dye in AN MicroPlates™ is tetrazolium violet, which gets reduced to formazan as a result of NADH production in cell respiration. Previous research showed that formazan production does not occur until the cell density is between 10^5 and 10^8 cells/mL (Konopka et al., 1998; Garland et al., 2001). To determine the cell density of the inoculum, plating was used to do CFU (Colony Forming Units) count. The inoculum

was grown on an anaerobic agar plate adapted from the plate media used by Solera et al., 2011, for five to six days.

The inoculum was diluted to suitable cell density with N_2-flushed-distilled water; 100 µL of diluted cell suspension was transferred into each well of the plate. The plate was incubated at 37 ℃ and the absorbance reading was periodically taken using the microplate reader (Infinite M200 Pro, Tecan).

A1 Water	A2 N-Acetyl-D-Galactosamine	A3 N-Acetyl-D-Glucosamine	A4 N-Acetyl-β-D-Mannosamine	A5 Adonitol	A6 Amygdalin
B1 Dulcitol	B2 I-Erythritol	B3 D-Fructose	B4 L-Fucose	B5 D-Galactose	B6 D-Galacturonic Acid
C1 Glycerol	C2 D,L-α-Glycerol Phosphate	C3 m-Inositol	C4 α-D-Lactose	C5 Lactulose	C6 Maltose
D1 α-Methyl-D-Galactoside	D2 β-Methyl-D-Galactoside	D3 α-Methyl-D-Glucoside	D4 β-Methyl-D-Glucoside	D5 Palatinose	D6 D-Raffinose
E1 Turanose	E2 Acetic Acid	E3 Formic Acid	E4 Fumaric Acid	E5 Glyoxylic Acid	E6 α-Hydroxybutyric Acid
F1 D-Lactic Acid Methyl Ester	F2 D-Malic Acid	F3 L-Malic Acid	F4 Propionic Acid	F5 Pyruvic Acid	F6 Pyruvic Acid Methyl Ester
G1 Alaninamide	G2 L-Alanine	G3 L-Alanyl-L-Glutamine	G4 L-Alanyl-L-Histidine	G5 L-Alanyl-L-Threonine	G6 L-Asparagine
H1 L-Methionine	H2 L-Phenylalanine	H3 L-Serine	H4 L-Threonine	H5 L-Valine	H6 L-Valine plus L-Aspartic Acid

Figure 3.6a The left 6 columns of carbon source on AN MicroPlates™.

A7 D-Arabitol	A8 Arbutin	A9 D-Cellobiose	A10 α-Cyclodextrin	A11 β-Cyclodextrin	A12 Dextrin
B7 Gentiobiose	B8 D-Gluconic Acid	B9 D-Glucosaminic Acid	B10 α-D-Glucose	B11 α-D-Glucose-1-Phosphate	B12 D-Glucose-6-Phosphate
C7 Maltotriose	C8 D-Mannitol	C9 D-Mannose	C10 D-Melezitose	C11 D-Melibiose	C12 3-Methyl-D-Glucose
D7 L-Rhamnose	D8 Salicin	D9 D-Sorbitol	D10 Stachyose	D11 Sucrose	D12 D-Trehalose
E7 β- Hydroxybutyric Acid	E8 Itaconic Acid	E9 α-Ketobutyric Acid	E10 α-Ketovaleric Acid	E11 D,L-Lactic Acid	E12 L-Lactic Acid
F7 D-Saccharic Acid	F8 Succinamic Acid	F9 Succinic Acid	F10 Succinic Acid Mono-Methyl Ester	F11 m-Tartaric Acid	F12 Urocanic Acid
G7 L-Glutamic Acid	G8 L-Glutamine	G9 Glycyl-L-Aspartic Acid	G10 Glycyl-L-Glutamine	G11 Glycyl-L-Methionine	G12 Glycyl-L-Proline
H7 2'-Deoxy Adenosine	H8 Inosine	H9 Thymidine	H10 Uridine	H11 Thymidine-5'-Mono-phosphate	H12 Uridine-5'-Mono-phosphate

Figure 3.6b The right 6 columns of carbon source on AN MicroPlates™.

Chapter 4

4. Results and Discussions

This chapter discusses the results from the experiments and the analyses (using the methods discussed in Chapter 3).

4.1 APC Analysis Results

Using the methods discussed in **Section 3.2**, the amounts of total organics, substrate, AD intermediate and inhibitors in APC were determined.

4.1.1 COD, TS, VS, TSS, VSS, Analyses of APC

Table 4.1 lists the total COD, soluble COD, TS, VS, TSS, and VSS. The COD of APC is high and about 91 % of total COD are soluble COD. The VS of APC is 98 % of its TS; VSS of APC is 95 % of its TSS. The results show that APC is rich in organics.

Table 4.1 Organic and Inorganic Content of APC

Property	Value
COD (Chemical Oxygen Demand)	499 g/L (0.5 gCOD/g)*
sCOD (soluble COD)	454 g/L
TS	81.0 g/L
VS	79.4 g/L
TSS	6.1 g/L
VSS	5.8 g/L

*Calculated based on density of APC at 22 ℃ (1.05 g/mL).

Comparing the COD of APC to the theoretical oxygen demand (ThOD = 0.7 gCOD/g) of APL in Torri & Fabbri's study (2014), it can be seen that the APC (COD = 0.5 gCOD/g) has less organics than APL. The COD of APC used in this study is 10 times higher than the COD of the aqueous pyrolysis liquor (48.5 g/L) from pyrolysis of digestate at 530 ℃ in Hubner & Mumme's work in 2015. Before using the APC in AD, a dilution will be required to prevent organic overload. The percentages of volatile solids in total solids are high for both APC (98 % of TS) and aqueous pyrolysis liquor (92 % of TS).

4.1.2 pH and Elemental Analyses of APC

Table 4.2 lists the pH of APC and the elemental composition of APC. The pH of APC is 2.12 and this indicates that a neutralization step might be required before feeding APC to the inoculum.

Based on the elemental composition, the C:N molar ratio was calculated to be 44:1. This C:N ratio is higher than the recommended range for AD (20:1 to 35:1), therefore inhibition is expected. In comparison to the APL in Torri & Fabbri's (2014) work, which has a C:N ratio of 73:1, the C:N ratio of the APC in this research is much lower.

Table 4.2 pH and Elemental Composition of APC

Property	Value
pH	2.12
Elemental (%)	C: 15.2
	H: 9.8
	N: 0.4
	S: < 0.05
	O: 74.6

4.1.3 Glucose and VFA Quantification of APC

Table 4.3 shows the concentration of VFA's in APC. No significant amount of glucose was detected in APC. Given the high acetic acid concentration, APC has the potential as an AD substrate since acetic acid can be used directly by the methanogenic

microorganisms to produce methane. However, when feeding APC to the inoculum, dilution is required to prevent substrate overload. The amount of valeric and isovaleric acid in APC are under the detection limit.

The concentrations of VFA's in APC are higher than the APL from Torri & Fabbri (i.e. acetic acid: 26 g/kg, propionic acid: 1.6 g/kg); this can be explained by two possible reasons. First, the difference in type of feedstock used in pyrolysis, that is, hardwood vs. agricultural residue. As discussed in **Chapter 2.1**, hardwoods like Birch have higher cellulose and hemicellulose composition than agricultural residue like corn stalk. Pyrolysis of biomass with more cellulose and hemicellulose will contribute to higher amount of acids. Second, type of separation; separation by condensation might have collected more acids in comparison to phase separation. In comparison to Hubner & Mumme's aqueous pyrolysis liquor, the acetic acid and propionic acid in APC is much higher. Their APL from pyrolysis of digestate at 530 ℃ contains only 4.6 g/L of acetic acid and 0.6 g/L of propionic acid.

Table 4.3 VFA Quantification of APC

Property	Value
Acetic Acid Concentration	105 g/L (100 g/kg)*
Propionic Acid Concentration	9.9 g/L (9.5 g/kg)*
Butyric Acid Concentration	1.3 g/L (1.2 g/kg)*

*Calculated based on density of APC (1.05 g/mL).

4.1.4 Common Inhibitors Analyses of APC

Table 4.4 gives the results of ammonia-N content, light and heavy metal analyses in APC. It shows that the amounts of those inhibitors in APC are well below the inhibitory level recorded in literatures, except for iron and zinc. The result of analysis helps to eliminate ammonia, light and heavy metals (except for iron and zinc) as the possible inhibitors that causes failure in the AD of APC. From the analyses on the potential inhibitors, phenolics might be the most potent inhibitor for causing inhibition in AD of APC.

Table 4.4 Common AD Inhibitor Analyses on APC

Identity	Value	Inhibitory Level (References)
Ammonia-N	< 0.2 mg/L	1.7 ~ 14.0 g/L (Chen et al., 2008)
Aluminum	1.43 mg/L	1,000 mg/L (Cabirol et al., 2003)
Calcium	5.8 mg/L	Moderately inhibitory at 2,500 ~ 4,000 mg/L Strongly inhibitory at 8,000 mg/L (Kugelman & McCarty, 1964)
Cadmium	0.06 mg/L	180 mg/L (Bozym et al., 2015)
Chromium	0.51 mg/L	130 mg/L (Bozym et al., 2015)
Copper	0.27 mg/L	400 mg/L (Bozym et al., 2015)
Iron	381 mg/L	10.0 mg/L (Takashima et al., 1990)

Potassium	1.01 mg/L	Strongly inhibitory at 12,000 mg/L (McCarty, 1964)
Magnesium	0.52 mg/L	Growth cease at 400 mg/L (Schmidt & Ahring, 1993)
Sodium	7.98 mg/L	Moderately inhibitory at 3,500 ~ 5,500 mg/L Strongly inhibitory at 8,000 mg/L (McCarty, 1964)
Nickel	2.33 mg/L	10.0 mg/L (Bozym et al., 2015)
Lead	0.96 mg/L	200 mg/L (Schattauer et al., 2011)
Sulfur	81.2 mg/L	Dissolved sulfide: 100 ~ 800 mg/L (Parkin et al., 1990)
Zinc	11.0 mg/L	1.0 mg/L (Zhang et al., 2003)

The amount of total phenolics in APC was measured to be 24.4 g/kg (or 25,530 mg/L calculated using the density of APC). As expected, the total phenolics concentration is high due to degradation of lignin from the lignocellulosic biomass which was the feedstock used in pyrolysis. The amount of phenols in APC is higher than the APL (17

g/kg) from Torri & Fabbri (2014), which may cause severe inhibition. In 1997, Fang & Chan studied the toxicity of phenol towards different types of biogranules (cell aggregates); they found that the threshold level for: benzoate-degrading biogranules was 1,050 to 1,600 mg/L, propionate-degrading biogranules was 850 mg/L, and acetate-degrading biogranules was 1,100 to 1,700 mg/L.

4.2 AD Inoculum Analysis Results

Using the methods discussed in **Section 3.2**, the organic content, substrate, intermediate and inhibitor level in AD inoculum can be determined.

4.2.1 COD, TS, VS, TSS, VSS Analyses of AD Inoculum

Table 4.5 shows the total COD, soluble COD, TS, VS, TSS, and VSS of AD inoculum. As noted in the table, the amount of VS in the inoculum is about 68 % of its TS; the VSS in the inoculum is 74 % of its TSS. However, it is worth noting that these values vary from one batch of inoculum to another and they change with time.

Comparing to the inoculums used in other studies, the percentage of VS in TS of the inoculum used in this study is typical. For instance, the inoculum used in Hubner & Mumme's study has VS of 67 % of its TS. Torri & Fabbri had the inoculum with VSS being 80 % of its TSS.

Table 4.5 Organic and Inorganic Content of Anaerobic Digestion Inoculum

Property	Value
COD	5.8 ~ 32.0 g/L
TS	17.4 ~ 33.8 g/L
VS	12.2 ~ 22.9 g/L
TSS	7.1 ~ 27.4 g/L
VSS	5.6 ~ 20.2 g/L

4.2.2 pH and Cell Density Analyses of AD Inoculum

The pH of the inoculum was 8.68, which is more basic than the inoculum used in Hubner & Mumme's study (the pH of inoculum being 7.70). This is very likely due to the nature of the feedstock used for AD by Storm Fisher (food waste that are rich in proteins and fats) at the biogas plant. However, the alkalinity of the inoculum might be an advantage in this research because APC is acidic; the alkalinity of inoculum and the acidity from APC will bring the system close to a neutral pH. This eliminates the need for additional pH adjustment, which sometimes could cause inhibition from alkali and alkaline earth metal ions.

The inoculum was determined to have cell density of approximately 8×10^7 cells/mL. The method for determining cell density of the inoculum was discussed in **Section 3.6** when referring to CLPP.

4.2.3 Glucose and VFA Quantification of AD Inoculum

In the beginning of each experiment, analyses were run to check the amounts of endogenous glucose and VFA's in the inoculum. Typically, since the inoculum has been degassed for a period of time to deplete the endogenous organics, the amount of glucose and VFA's are very small to the level that is not detected by the HPLC.

4.2.4 Common AD Inhibitors Analyses of AD Inoculum

The amounts of metals in the inoculum are well below the inhibitory level reported in the literatures (see **Table 4.6**). With such low amount of metals in both APC and AD inoculum, the possibility of light and heavy metal being the inhibitors in AD of APC can be eliminated. The small amount of those metals can be stimulatory to the AD process.

Table 4.6 Common AD Inhibitor Analyses on Anaerobic Digestion Inoculum

Identity	Value	Inhibitory Level (References)
Ammonia-N	2.0 mg/L	1.7 ~ 14.0 g/L (Chen et al., 2008)
Aluminum	0.17 mg/L	1,000 mg/L (Cabirol et al., 2003)
Calcium	4.6 mg/L	Moderately inhibitory at 2,500 ~ 4,000 mg/L Strongly inhibitory at 8,000 mg/L (Kugelman & McCarty, 1964)
Cadmium	< 0.005 mg/L	180 mg/L (Bozym et al., 2015)
Chromium	0.01 mg/L	130 mg/L (Bozym et al., 2015)
Copper	0.16 mg/L	400 mg/L (Bozym et al., 2015)
Iron	0.09 mg/L	10.0 mg/L (Takashima et al., 1990)

Potassium	1.12 mg/L	Strongly inhibitory at 12,000 mg/L (McCarty, 1964)
Magnesium	5.65 mg/L	Growth cease at 400 mg/L (Schmidt & Ahring, 1993)
Sodium	21.7 mg/L	Moderately inhibitory at 3,500 ~ 5,500 mg/L Strongly inhibitory at 8,000 mg/L (McCarty, 1964)
Nickel	< 0.005 mg/L	10.0 mg/L (Bozym et al., 2015)
Lead	< 0.05mg/L	200 mg/L (Schattauer et al., 2011)
Sulfur	1.96 mg/L	Dissolved sulfide: 100 ~ 800 mg/L (Parkin et al., 1990)
Zinc	0.09 mg/L	1.0 mg/L (Zhang et al., 2003)

4.3 Effects of Different Dilutions of APC on Biogas Composition

For details on experimental method, please see **Section 3.3.1**. As seen in the previous results, APC is rich in acetic acid, which is a substrate that can be used directly by the acetoclastic methanogens to produce methane. However, the amount of phenolics in APC is high. Before start studying AD of APC, it is necessary to know how the composition of the biogas produced changes with different dilutions of APC; changes in biogas composition may indicate to what extent AD is inhibited by APC. **Figure 4.1** shows the composition of biogas produced in first 200 hours after inoculation of APC diluted by 1:100, 1:200, 1:400 and 1:800. Results suggest that the biogas produced from the reactor inoculated with 1:100 diluted APC is solely carbon dioxide whereas the biogas produced from the reactor inoculated with 1:800 diluted APC has roughly 24 % of methane, suggesting that there is a direct relation between APC concentration and AD inhibition. This can be explained since APC is diluted by 1:800 times, the amount of inhibitory compounds present is less; therefore, methane production from endogenous organics was possible. It was observed that the biogas production stops at around 100 hours regardless of the dilution of APC.

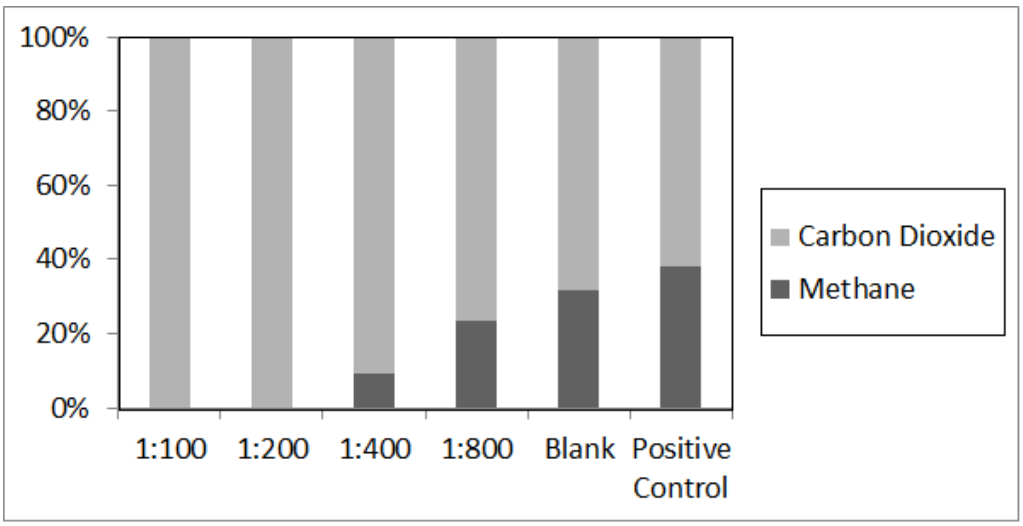

Figure 4.1 Composition of biogas produced in first 200 hours after inoculation of different dilutions of APC. (Positive Control = 1.5 g/L of Glucose; 1:100 = 1.0 g/L of acetic acid equivalent of APC; 1:200 = 0.5 g/L of acetic acid equivalent of APC; 1:400 = 0.2 g/L of acetic acid equivalent of APC; 1:800 = 0.1 g/L of acetic acid equivalent of APC).

4.4 Adaptation of AD Inoculum to APC

As seen from the previous section, APC inhibits AD resulting in poor methane production. The study carried by Torri & Fabbri (2014) showed adaptation of inoculum to APC is required in order for inoculum to be able to anaerobically digest APC.

4.4.1 Adaptation of Inoculum to APC with Glucose Addition on Day 27

For the materials and methods of this experiment, see **Section 3.3.2**. **Figure 4.2** and **Figure 4.3** show the methane and carbon dioxide production of inoculum inoculated with 1.0 g/L acetic acid equivalent of APC in comparison to inoculums inoculated with 1.0 g/L of glucose and 1.0 g/L of acetic acid until Day 27 after inoculation. As observed from the figures, the inoculum fed with glucose give the highest amount of methane and carbon dioxide production followed by the inoculum fed with acetic acid. The inoculum fed with APC did not have any methane production; the biogas produced is made solely of carbon dioxide. The result is in agreement with the APC dilution experiment, where after inoculation of 1.0 g/L (1:100 dilution) acetic acid equivalent of APC (see **Section 4.3**) only carbon dioxide was produced. The phenomenon was also observed in Torri & Fabbri's work (2014) where after each addition of their APL, the production of methane stopped and only carbon dioxide production occurred. The authors suggested that it might have due to a shift in carbonate equilibrium.

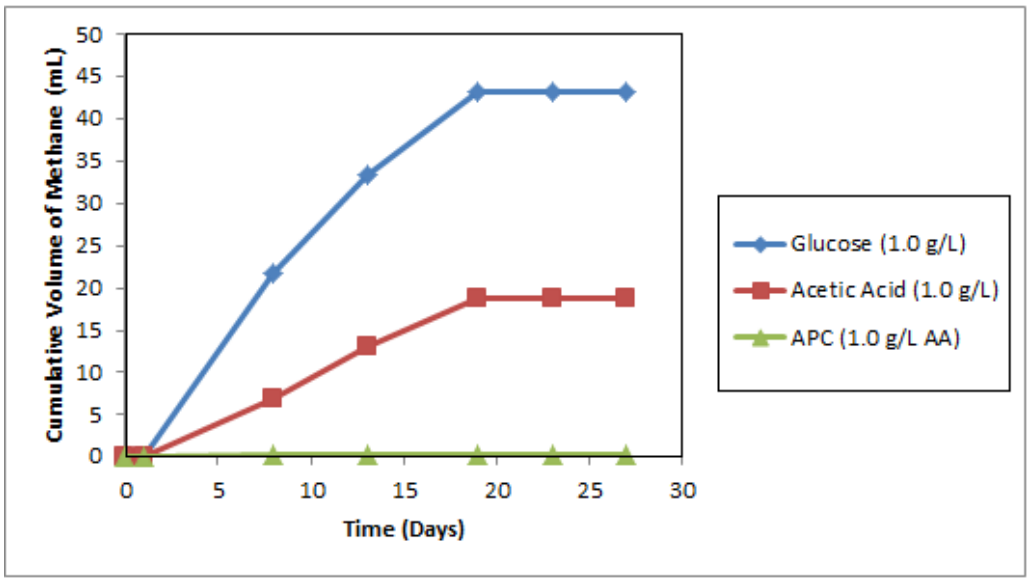

Figure 4.2 Cumulative volume of Methane production until Day 27 after inoculation. (Glucose = 1.0 g/L of glucose; Acetic Acid = 1.0 g/L of acetic acid; APC = 1.0 g/L acetic acid equivalent of APC).

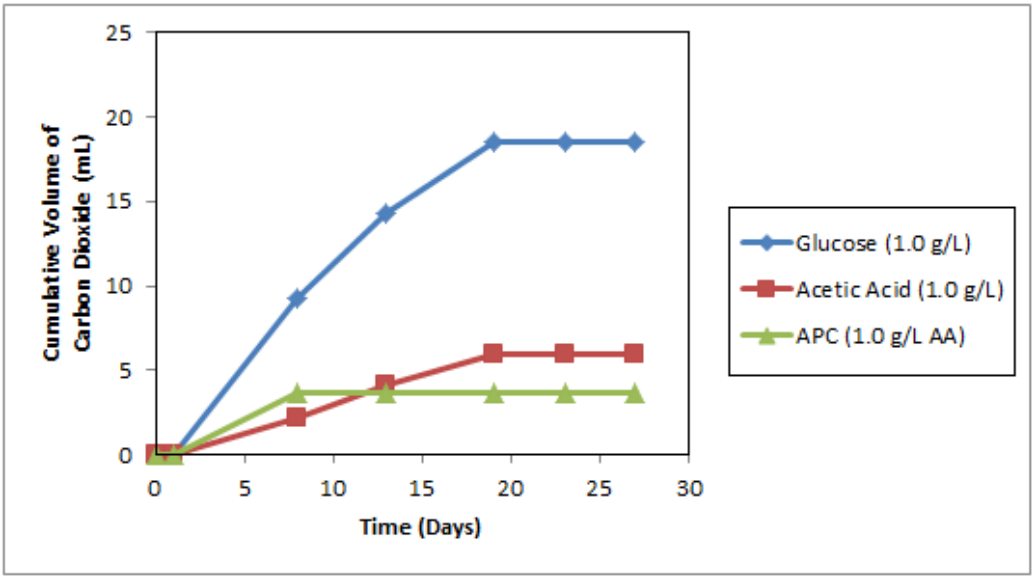

Figure 4.3 Cumulative volume of Carbon Dioxide production until Day 27 after inoculation. (Glucose = 1.0 g/L of glucose; Acetic Acid = 1.0 g/L of acetic acid; APC = 1.0 g/L acetic acid equivalent of APC).

The theoretical methane production from glucose and acetic acid can be calculated using the formula (see Equation 6) developed by Buswell and Hatfield (1936) based on the elemental composition.

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 \quad [6]$$

In total volume of 150 mL, 0.15 g of glucose and acetic acid were added (each has the concentration of 1.0 g/L). This gives 0.0008 moles of glucose and 0.0025 moles of acetic acid. By substituting the values of subscripts in the chemical formula (i.e. for glucose, $C_6H_{12}O_6$, n = 6, a = 12 and b = 6), the moles of methane from 0.15 g of glucose and acetic acid is calculated to be 0.0025. Using ideal gas law with temperature of 295 K (22 ℃), pressure of 1 atm and ideal gas constant of 0.082 atm×L/mol×K, the theoretical volume of methane produce from 0.15 g of glucose and acetic acid is 60 mL. The yield of methane of this AD from glucose is 75 % whereas the yield from acetic acid is 30 %.

When the chemical formula of the compound is unknown, which is the case for APC due to its complex composition, the theoretical methane production can be calculated based on the assumption that for one gram of COD stabilized, 350 mL of methane is produced at STP (McCarty, 1964). An APC diluted to 1.0 g/L acetic acid equivalent has COD of 4.99 g/L, which means in 150 mL of working volume, there is 0.75 g of COD. Assuming there is no inhibition and APC is completely digested, the theoretical methane production from 1.0 g/L acetic acid equivalent of APC is 262.5 mL.

Using the same method used to calculate the theoretical methane production, the theoretical carbon dioxide production of both 1.0 g/L of glucose and 1.0 g/L of acetic

acid are calculated to be 60 mL. The yield of carbon dioxide from glucose was 30 % and 8 % from acetic acid.

As there was no methane production from inoculum fed with APC, a model substrate (1.0 g/L of glucose) was supplied to the inoculum fed with glucose, acetic acid, and APC after 27 days of inoculation; the purpose was to study inhibition in the case of APC-inoculated inoculum. Results suggested that the methane production coming from glucose was inhibited by the presence of APC and the system had no gas production for a period of time (see **Figure 4.4**). Although methane production was inhibited, the addition of glucose showed that carbon dioxide production was not inhibited by APC at all since the amount of carbon dioxide produced from APC-inoculated inoculum was close to the amount of CO_2 that was observed from acetic acid-inoculated inoculum fed with glucose (**Figure 4.5**).

As seen in **Figure 4.4**, around Day 50 after inoculation, methane production from APC-inoculated inoculum was observed and the volume increased drastically until reaching the same level as the amount produced by acetic acid-inoculated inoculum. This shows that the inoculum requires some time to be adapted to the presence of APC and to start producing methane. From Day 47 to Day 118 after inoculation, the total COD in the inoculum was reduced from 12,650 mg/L to 8,840 mg/L, which is about 30% of total COD reduction. In Torri & Fabbri's work (2014), the methane production from APL did not occur until around 80 days after inoculation, the variation in the length of time required for adaptation might be due to the amount of inhibitors in APL or the robustness of the anaerobic inoculum used.

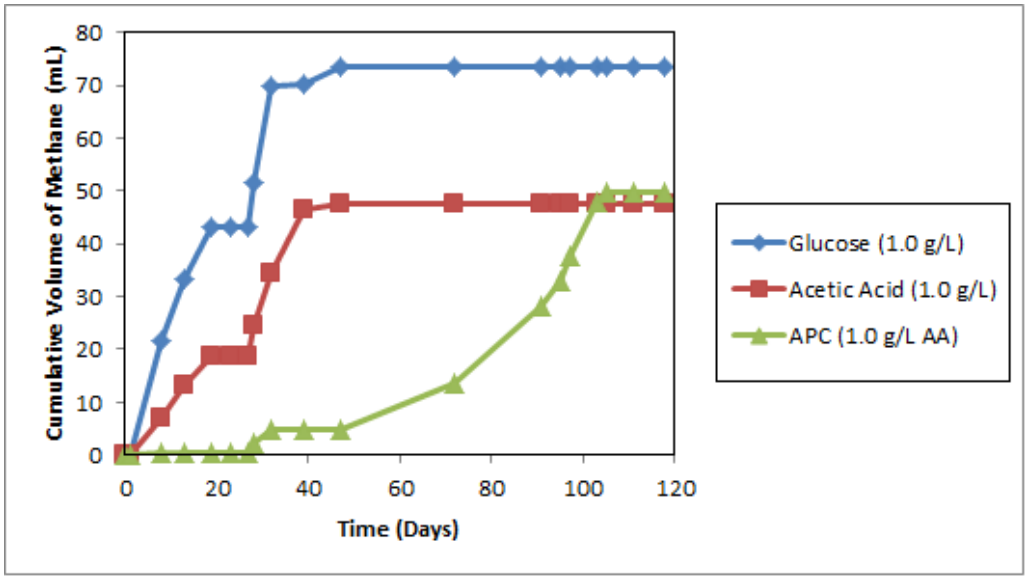

Figure 4.4 Cumulative volume of Methane production until Day 118 after inoculation; 1.0 g/L of glucose added to all three conditions on Day 27. (Glucose = 1.0 g/L of glucose; Acetic Acid = 1.0 g/L of acetic acid; APC = 1.0 g/L acetic acid equivalent of APC).

Since 1.0 g/L of glucose is added twice for inoculum with glucose as a control (on the day of inoculation and Day 27), the theoretical volume of methane production would be 120 mL. The results show the yield of methane from glucose positive control to be 62.5 %. The inoculum with acetic acid as a control also had 1.0 g/L of glucose added to it (on Day 27), which means the theoretical volume of methane production is 120 mL. The final yield of methane from acetic acid positive control is 37.5 %.

For inoculum that contained APC, the amount of COD reduced in 150 mL of working volume was 3,810 mg/L, which is equal to 0.57 g of COD stabilization. Since 350 mL of methane will be produced from one gram of COD stabilized, the theoretical methane production would be 200 mL. The methane yield from APC (1.0 g/L acetic acid equivalent) with 1.0 g/L of glucose added on Day 27 is 18.5 %. The final carbon

dioxide yields for the two controls are as follows: 26.7 % for glucose control and 20.8 % for acetic acid control.

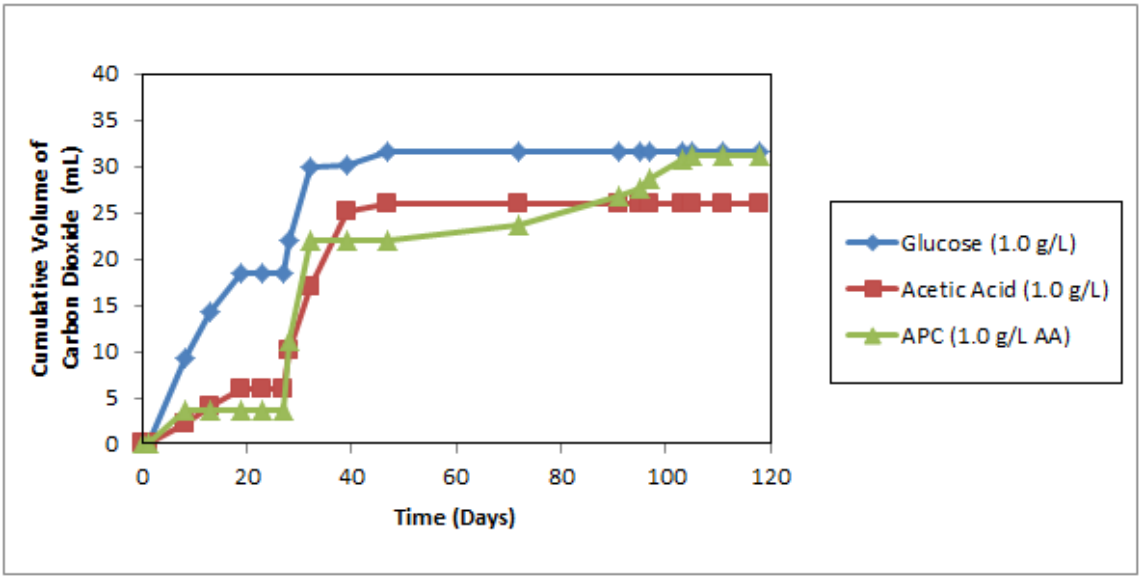

Figure 4.5 Cumulative volume of Carbon Dioxide production until Day 118 after inoculation; 1.0 g/L of glucose added to all three conditions on Day 27. (Glucose = 1.0 g/L of glucose; Acetic Acid = 1.0 g/L of acetic acid; APC = 1.0 g/L acetic acid equivalent of APC).

From the analysis of glucose concentration with time, glucose was completely used up by the inoculum even in the presence of APC, which indicates there is no inhibition in acidogenesis step of AD. For propionic acid, degradation was observed as time progresses (**Figure 4.6**). This shows the inhibition was not at the acetogenesis step. However, it seems there was an inhibition at the methanogenesis step as an accumulation of acetic acid was observed on Day 23 (**Figure 4.7**). The addition of glucose increased the acetic acid concentration (as seen on Day 47).

The accumulation of acetic acid might have resulted from two things: first, the inhibition in methanogenesis in which the methanogenic microorganisms were unable to convert acetic acid to methane; second, the degradation of glucose gave rise to more acetic acid, consequently, it accumulated onto the acetic acid that was already present before glucose addition. From Day 47 to Day 95, it can be seen that the acetic acid accumulated was reduced from more than 2.5 g/L to less than 0.5 g/L, this also shows that the AD consortia needed some time to be able to convert acetic acid to methane.

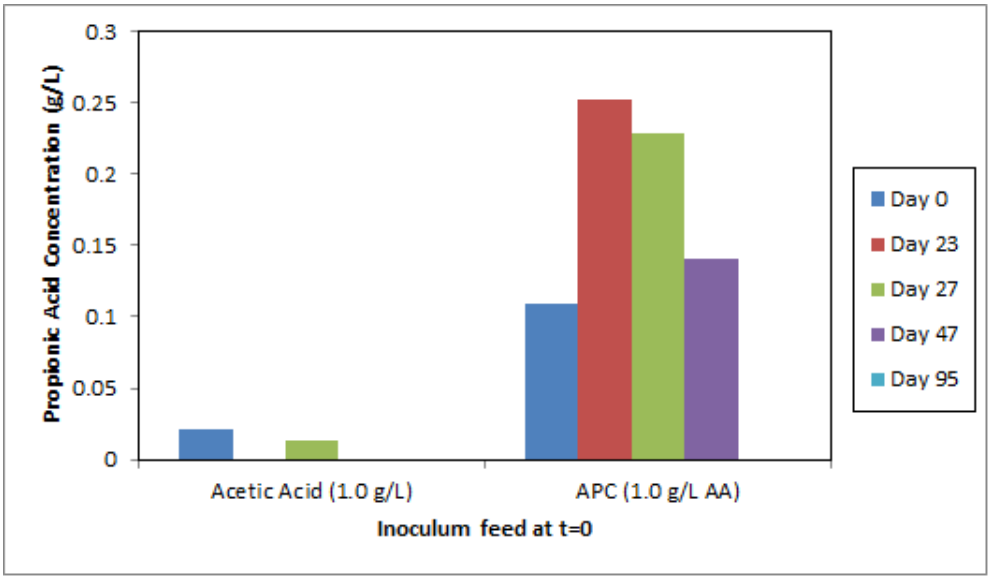

Figure 4.6 Propionic acid concentration of inoculum fed with glucose, acetic acid, and APC at different time points; 1.0 g/L of glucose added to all three conditions on Day 27. (Acetic Acid = 1.0 g/L of acetic acid; APC = 1.0 g/L acetic acid equivalent of APC).

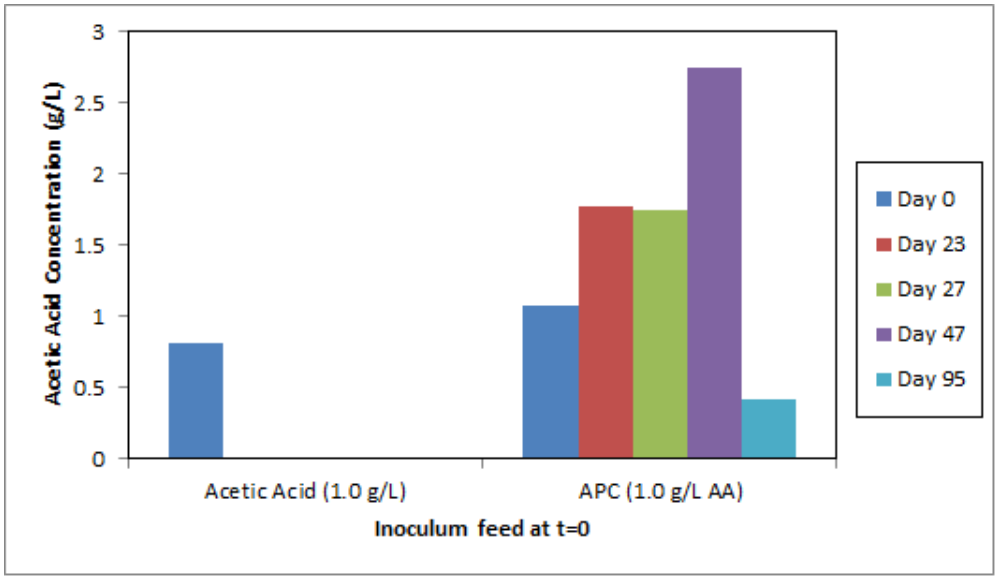

Figure 4.7 Acetic acid concentration of inoculum fed with glucose, acetic acid, and APC at different time points; 1.0 g/L of glucose added to all three conditions on Day 27. (Acetic Acid = 1.0 g/L of acetic acid; APC = 1.0 g/L acetic acid equivalent of APC).

From the gas production and the VFA concentration results, it can be concluded that the AD inoculum was capable of producing methane in the presence of APC; however, about 50 to 60 days was required for the adaptation to occur. During adaptation, this is what might have occurred: in the original inoculum, there was a group of microbes that is capable of resisting or degrading the toxic compounds from APC, but it was in minority, and grew to become the majority since other groups of microbes are eliminated by the toxicity of APC.

The amount of inhibitors present before and after adaptation will help in elucidating the adaptation process. The method for qualitatively measuring the amount of chromophoric inhibitors was discussed in **Section 3.2.4**. The normalized inhibitor value (IV/AA) was 13,954 before AD and the value after AD was 7,305. This result

suggested that the amount of inhibitor has decreased. This shows that methane production was able to occur because the amount of inhibitors has been reduced to the level that it no longer poses as a stress to the system.

Community Level Physiological Profiling (CLPP), or metabolic profiling, was used to check the difference between the un-adapted and adapted inoculum based on the carbon source utilization pattern. The carbon utilization appears to be equally rich when comparing the un-adapted (control) to the adapted inoculum (**Figure 4.8**). A closer look revealed that the adapted strain grew faster on some carbon sources (see **Figure 4.9**). This might apply a slight shift in the microbial community with an increase of organisms metabolizing certain carbon sources. Which group this might be cannot be concluded from this data and further studies with different carbon sources (e.g. ECO plates) is recommended.

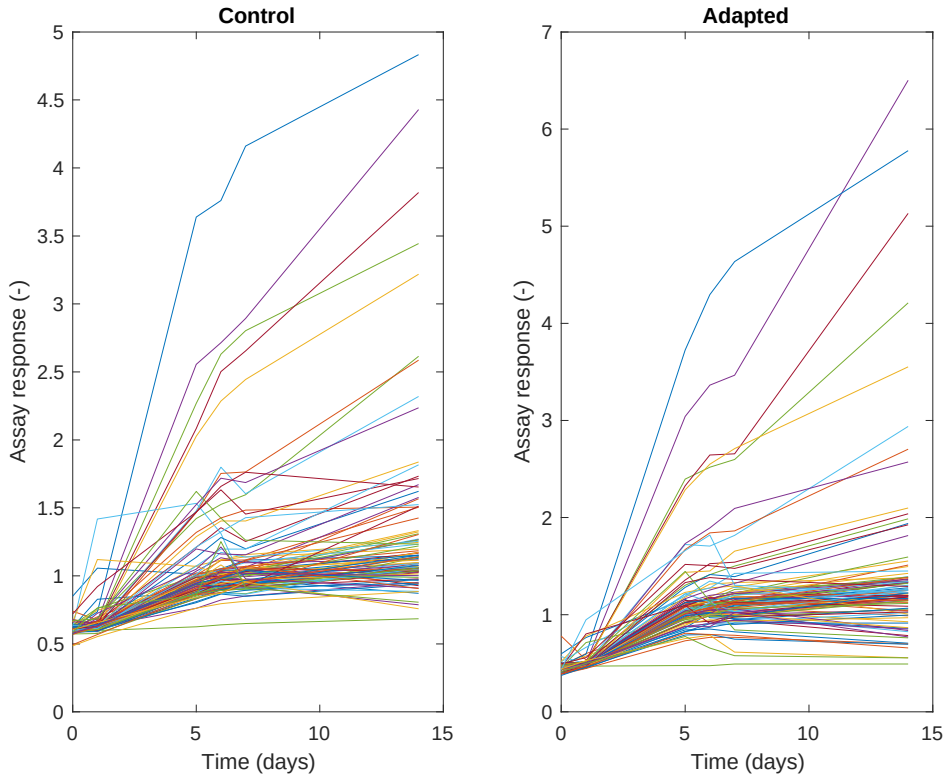

Figure 4.8 Carbon utilization pattern of un-adapted inoculum (control) vs. adapted inoculum. Each line represents the growth on a specific carbon source.

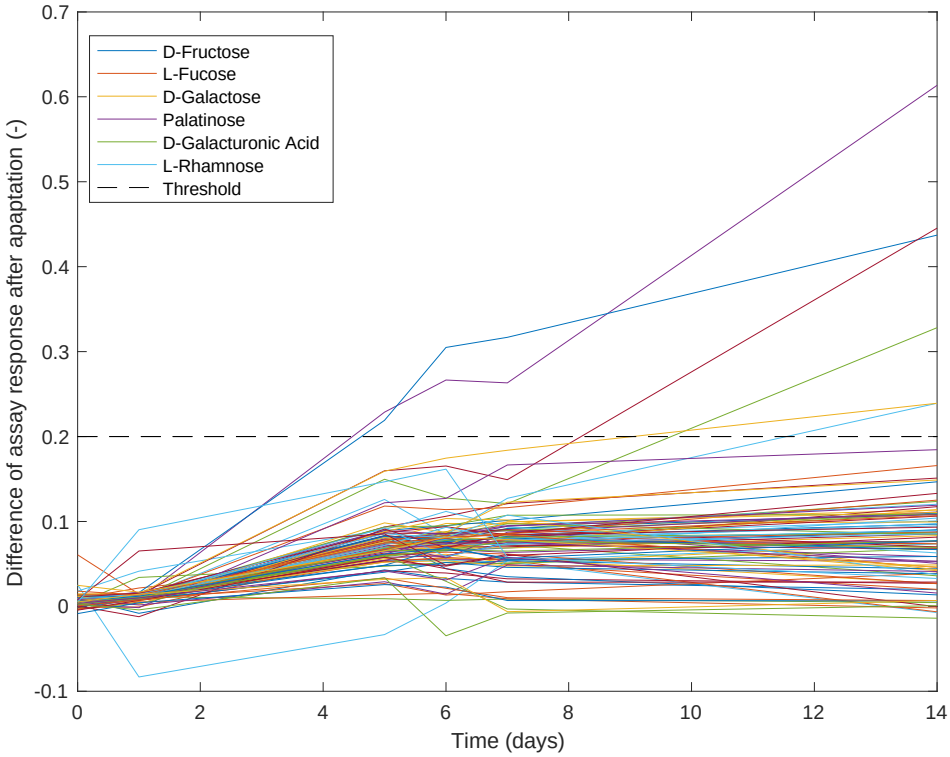

Figure 4.9: Increased rate of carbon utilization after adaptation. Only the carbon sources for which an increase > 0.2 was observed are listed.

4.4.2 Adaptation of Inoculum to APC with No Glucose Addition

From the previous experiment, it seemed that the inoculum required about 50 days of adaptation period to adapt to the toxicity of APC. To determine whether or not glucose addition has helped in the adaptation, a similar experiment was carried out but without adding any glucose. The details regarding this experiment are discussed in **Section 3.3.2**. As observed from **Figure 4.10**, very little methane production was observed for the first 40 to 50 days of inoculation. After 60 days, methane production was observed

to increase and the COD reduction of 30 % (from 26,200 mg/L to 18,150 mg/L) was achieved in the inoculum with 1.0 g/L acetic acid equivalent of APC. The initial carbon dioxide production (see **Figure 4.11**) from 1.0 g/L acetic acid equivalent of APC was again due to the shift in carbonates equilibrium, and then the carbon dioxide production started again after 60 days of inoculation. These results again suggested that methane production was inhibited by APC, and that after a period of adaptation, methane production started until reaching the same level as what was generated from acetic acid.

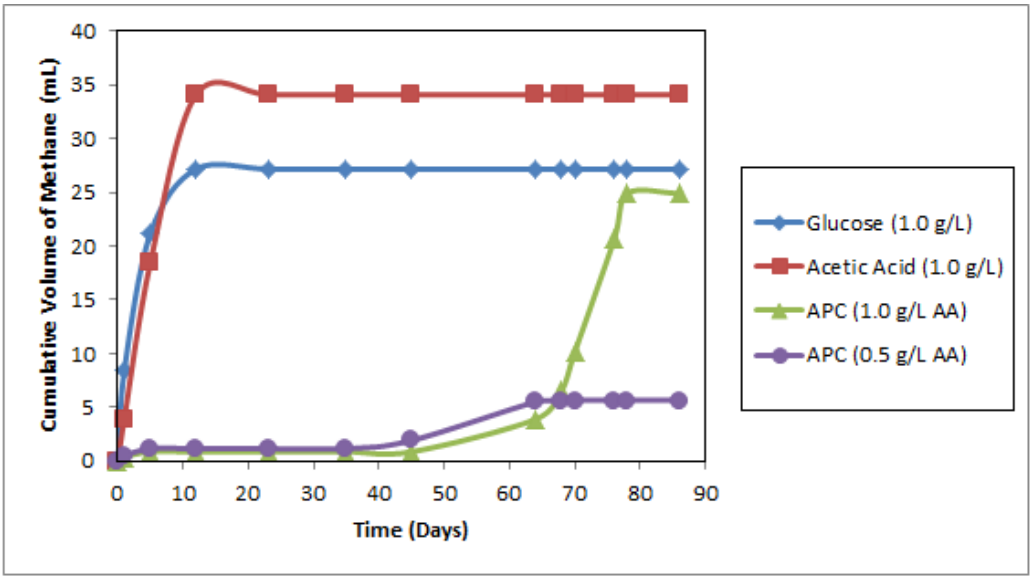

Figure 4.10 Total volume of Methane production until Day 86 after inoculation. (Glucose = 1.0 g/L of glucose; Acetic Acid = 1.0 g/L of acetic acid; APC (1.0 g/L AA) = 1.0 g/L acetic acid equivalent of APC; APC (0.5 g/L AA) = 0.5 g/L acetic acid equivalent of APC).

The theoretical volume of methane production for glucose and acetic acid control were calculated as before using chemical formula; for APC, the theoretical methane volume was calculated using the assumption of 350 mL methane per grams of COD stabilized.

The yields of methane for the three conditions are as follows: 47 % for glucose, 58 % for acetic acid and 6 % for APC.

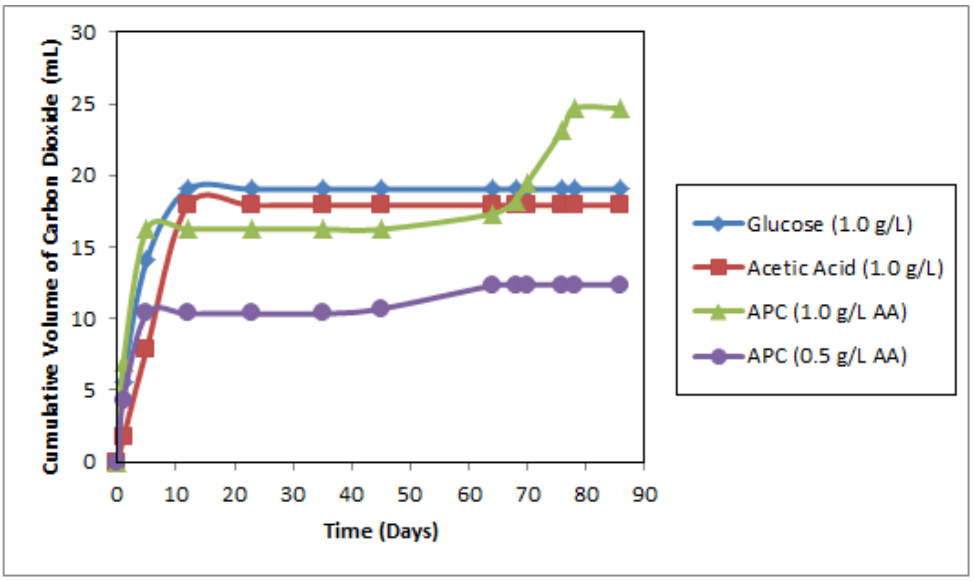

Figure 4.11 Total volume of Carbon Dioxide production until Day 86 after inoculation. (Glucose = 1.0 g/L of glucose; Acetic Acid = 1.0 g/L of acetic acid; APC (1.0 g/L AA) = 1.0 g/L acetic acid equivalent of APC; APC (0.5 g/L AA) = 0.5 g/L acetic acid equivalent of APC).

When looking at VFA concentration (**Figure 4.12** and **Figure 4.13**), there was an accumulation in acetic acid at the beginning of digestion. On Day 68 of APC inoculation, a reduction in the amount of acetic acid can be observed and propionic acid was completely consumed. Analysis on the inhibitor level shows that the normalized inhibitor level was reduced after 86 days of digestion from a normalized inhibitor value of 13,954 to 5,920. These results show that after adaptation period, the inoculum was able to degrade inhibitors and use acetic acid produce methane.

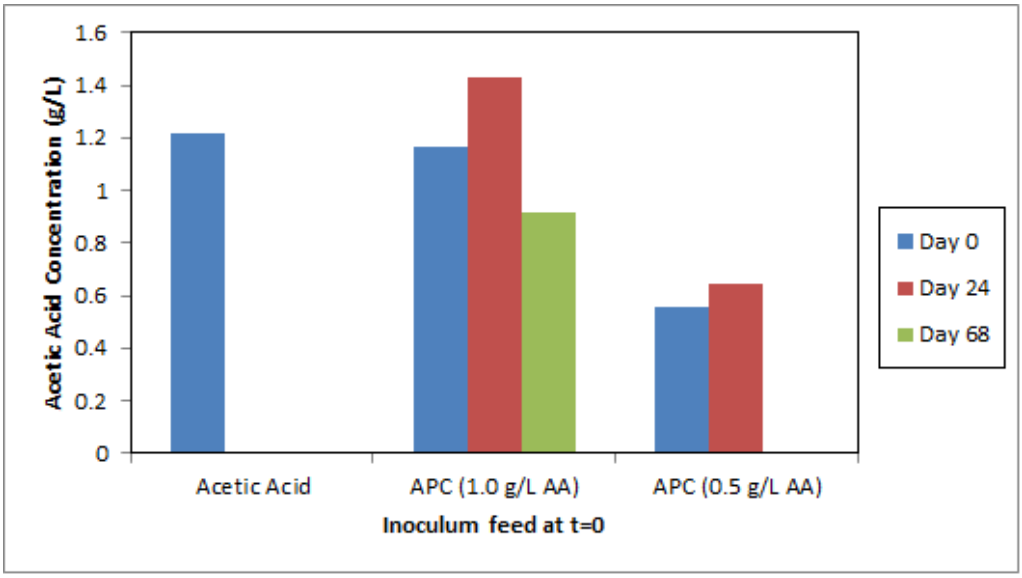

Figure 4.12 Acetic acid concentration of inoculum fed with glucose, acetic acid, and APC, at different time points. (Glucose = 1.0 g/L of glucose; Acetic Acid = 1.0 g/L of acetic acid; APC (1.0 g/L AA) = 1.0 g/L acetic acid equivalent of APC; APC (0.5 g/L AA) = 0.5 g/L acetic acid equivalent of APC).

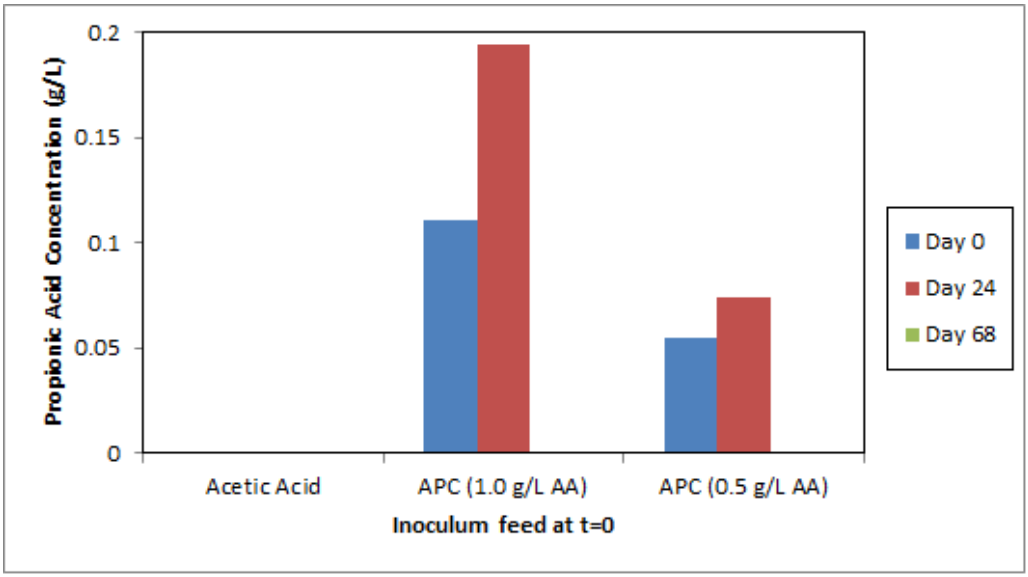

Figure 4.13 Propionic acid concentration of inoculum fed with glucose, acetic acid, and APC, at different time points. (Glucose = 1.0 g/L of glucose; Acetic Acid = 1.0 g/L of acetic acid; APC (1.0 g/L AA) = 1.0 g/L acetic acid equivalent of APC; APC (0.5 g/L AA) = 0.5 g/L acetic acid equivalent of APC).

It seems that regardless of whether glucose is added to the AD of APC or not, the maximum COD reduction that can be achieved was only about 30 %. This result is comparable to Hubner & Mumme's work in 2015, in which the total COD reduction from 530℃ pyrolysis liquor was about 37 %; however, their pyrolysis liquor contained less phenolic compounds (203 mg/L) than the APC used in this study, hence higher COD reduction in the AD of pyrolysis liquor was observed.

The COD at the end of both digestions are 8.84 g/L and 15.2 g/L. Although there was a reduction in COD, the amount is still higher than the regulatory level set by the government. There is a possibility for further COD reduction once the inoculum becomes more adapted to digest APC.

4.5 Effects of Addition of Biochar and Nutrient on Biogas Production

The method used for this study can be found in **Section 3.3.3**. The experimental setup was similar to Torri & Fabbri's work in 2014. As observed in **Figure 4.14**, no methane production was observed from the inoculum containing only APC. Initially, the inoculum containing APC and biochar (APC + Biochar) had the highest methane production out of the three conditions containing APC (does not include glucose-only and blank). Results suggested that biochar has reduced the toxicity effect coming from APC. Previous studies found that char-like materials have the ability to partially absorb inhibitors and decrease the toxicity of inhibitors such as phenols, ammonia, etc. (Hanaki et al., 1997; Mumme et al., 2014). Biochar can also buffer the pH and allow biofilm formation. This is why methane production was still able to occur in the beginning even in the presence of APC. Around Day 50, methane production from the inoculum that contains APC with nutrient (APC + Nutrient) started to increase. It can be deduced that nutrient did not help to reduce the toxicity initially; however, the nutrients supplied the energy required by the microbes to overcome the toxicity from APC. Once the toxicity was reduced, methane production starts occurring. The inoculum without any other additives except APC had no methane production until around 60 days after inoculation. Based on these results, it can be concluded that the presence of biochar helps to relieve the toxicity, which resulted in a fair amount of methane production at the start of inoculation, but the presence of nutrient enables the microbial population to cope with the toxicity and consequently resulted in an increase in methane production.

For carbon dioxide production (**Figure 4.15**), APC + Nutrient had the highest production followed by APC + Biochar. This could also explain why methane production was slightly higher in APC + Nutrient in the end. Although acetoclastic methanogenesis contributes to two-thirds of the methane production, one-third of methane production comes from hydrogenotrophic methanogenesis, in which carbon dioxides are used with hydrogen to produce methane. The carbon dioxide produced in the earlier stages was used by the hydrogenotrophic methanogens to produce methane.

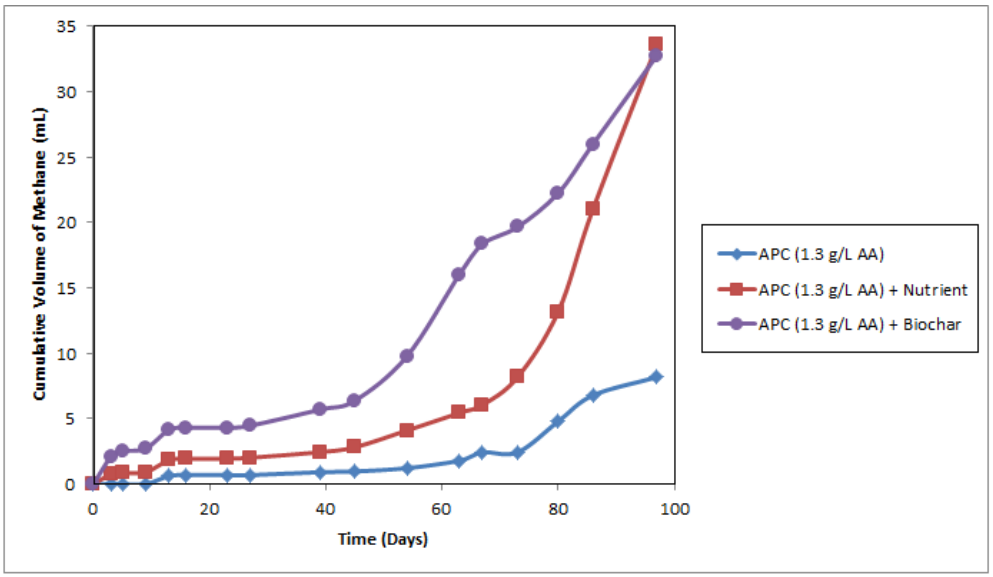

Figure 4.14 Cumulative volume of Methane production until Day 97 after inoculation. (APC = 1.3 g/L acetic acid eq. APC; APC + Nutrient = 1.3 g/L acetic acid eq. APC + ammonium chloride + potassium phosphate; APC + Biochar = 1.3 g/L acetic acid eq. APC + Biochar from pyrolysis of Miscanthus at 600 ℃).

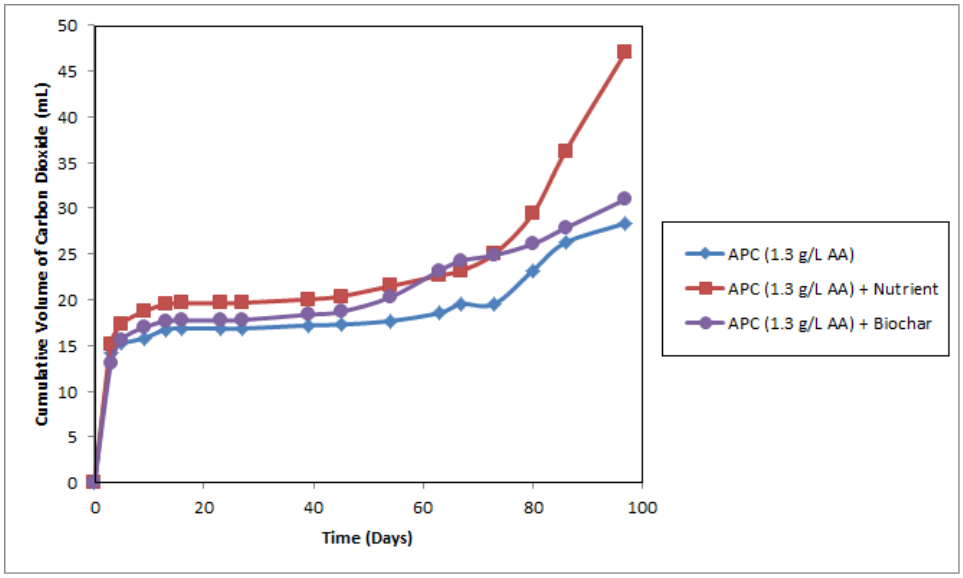

Figure 4.15 Total volume of Carbon Dioxide production until Day 97 after inoculation. (APC = 1.3 g/L acetic acid eq. APC; APC + Nutrient = 1.3 g/L acetic acid eq. APC + ammonium chloride + potassium phosphate; APC + Biochar = 1.3 g/L acetic acid eq. APC + Biochar from pyrolysis of Miscanthus at 600 ℃).

Torri & Fabbri reached a different conclusion based on the work they did in 2014. They found that the addition of biochar gave the highest methane production whereas the addition of nutrient did not have a significant effect on the methane production. This discrepancy of results might be due to some reasons. First, the origins of the two inoculums used for anaerobic digestion are different. The inoculum used in this study was robust since it was capable of degrading a variety of complex polymers that are rich in fatty acids and amino acids, therefore, addition of nutrient may be enough to overcome the toxicity. It has been shown that the inoculum used in this study is relatively basic and the addition of biochar, which has pH of 9.53 in solution, is going to cause the inoculum to become more basic. An increase in pH could lower the efficiency of anaerobic digestion. Second, the feedstock that was used in pyrolysis to generate the

aqueous pyrolysis liquid/condensate is different; this resulted in different types and concentration of inhibitors present in the aqueous liquid/condensate. The feedstock used in pyrolysis (Miscanthus vs. Cornstalk pellets), along with the pyrolysis temperature (600 ℃ vs. 400 ℃), can also affect the property of biochar. Lastly, the methods used to separate the organic and aqueous portion of the pyrolysis volatiles are different; this creates a difference in the chemical composition of the aqueous pyrolysis product.

Chapter 5

5. Conclusion

This research showed the feasibility of anaerobic digestion of aqueous pyrolysis condensate (APC) from pyrolysis of Birchwood at 500 ℃ using inoculum from food waste biogas plant.

The analysis of APC revealed its potential as a substrate for anaerobic digestion, especially for the step of methanogenesis, due to its high organic and acetic acid content. The analyses on total ammonia nitrogen, light metal ions and heavy metal in APC has eliminated ammonia and most metals as potential inhibitors in anaerobic digestion of APC. However, the analyses found high amount of phenolic compound which have been identified as inhibitors of methanogenesis in many research studies.

APC with 1.0 g/L of acetic acid equivalent was subjected to anaerobic digestion without any treatment other than dilution to prevent substrate overload and compared its biogas production with the biogas production from AD of 1.0 g/L of acetic acid and 1.0 g/L of glucose. The result showed that methane production was inhibited by APC initially and the addition of glucose on Day 27 showed that only methane production was inhibited but not carbon dioxide production. After 50 days of adaptation period, the inoculum was able to produce methane until the production reaches the amount from the AD of 1.0 g/L of acetic acid. From Day 47 to Day 118 of AD, 30 % of total COD has been reduced. A qualitative analysis on chromophoric inhibitor using DAD of HPLC

showed the concentration of inhibitors has been reduced to about half of its initial amount after 113 days of AD.

In the experiment (Effects of Addition of Biochar and Nutrient on Biogas Production) that aimed to compare our results with those of Torri & Fabbri's work (2014), a different conclusion from what Torri & Fabbri reached was found. The results showed that APC digestion was effective with nutrient present as well as with biochar present. Multiple reasons that could have caused this discrepancy in conclusion include the difference in pyrolysis feedstock, origin of AD inoculum, and separation of aqueous from organic pyrolysis volatile.

Chapter 6

6. Recommendations

An alternative source of AD inoculum, for instance, an inoculum that is already converting the feedstocks with similar properties and compositions as APC, can be used to achieve better conversion efficiency in future studies.

In the analysis of APC, it is recommended that the concentrations of some other possible inhibitors resulting from the pyrolysis of lignocellulosic biomass (such as ketones, aldehydes, furans, etc.) in APC should be determined. Quantitative analysis of those compounds should also be done throughout the entire process of anaerobic digestion.

Once the main inhibitors have been identified, in order to make APC more digestible, some pretreatment can be done on APC prior to AD to remove those inhibitors. Methods such as distillation or absorption onto matrix/resin can be used to remove inhibitors.

It would be interesting to identify which species have become dominant after adaptation or has the ability to degrade inhibitors and increase the number of those species. To prevent the imbalance in the microbial population, instead of increasing the number of certain microbial species, one can identify the enzyme utilized by the microbe to perform inhibitor degradation and increase the production of those enzymes by metabolic engineering.

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