12-18-2020 10:00 AM

Bridging Thermochemical and Biochemical Conversion: Impact of Biochar Addition on the Anaerobic Digestion of Aqueous Pyrolysis Condensate

Neha Batta, *The University of Western Ontario*

Supervisor: Berruti, Franco, *The University of Western Ontario*
Co-Supervisor: Rehmann, Lars, *The University of Western Ontario*
Co-Supervisor: Moreira Valenzuela, Cesar M, *Escuela Superior Politécnica del Litoral (ESPOL)*
A thesis submitted in partial fulfillment of the requirements for the Master of Engineering Science degree in Chemical and Biochemical Engineering

Abstract

Profitable treatment of low value waste biomass is one of the biggest challenges of the industry. Where most of the current treatment strategies, such as pyrolysis, are efficient in complete breakdown of low value waste biomass, such as lignocellulosic wastes, it leads to the generation of secondary waste thereby compromising its efficiency. The aim of this research is to utilize the waste aqueous co-product of lignocellulosic pyrolysis (termed aqueous pyrolysis condensate, or APC) as a feedstock for anaerobic digestion, adapting the microbial consortia to the potential organic inhibitors (i.e. phenolics) present, to produce energy in the form of biogas, fostering a circular economy approach. Adaptation was found to be an effective strategy to increase the tolerance of the consortia to progressively higher APC concentrations. Adapted inoculum, in the presence of 2.14 g/L acetic acid equivalent (5% APC), gave biogas ratios of 2.5 as opposed to no biogas production in case of non-adapted inoculum. The use of biochar reduced total phenolic content by 80%, improving the biogas ratios by 88.8%. Higher biochar:APC ratios created a more favourable environment for the bacterial growth and propagation.

Keywords

Anaerobic Digestion; Aqueous Pyrolysis Condensate; Biogas; Biochar; Circular Economy; Waste-to-Resource; Microbial Adaptation; Energy Generation

Summary for Lay Audiences

With the ever-increasing population there comes the issue of continually increasing waste generation. The amount of waste generated is expected to increase by 70% in the coming years. Therefore, it is important to develop effective waste management strategies. Of the several methods available till date, most of the waste utilisation and management result in the generation of more waste, known as secondary waste. One such method is that of thermo-chemical conversion of waste. On the one hand, this method generates useful products which are environmentally friendly. However, it also leads to the generation of a complex aqueous waste which is high in acid content. Currently, there are no strict guidelines for the management of this aqueous stream.

Of the various methods available to deal with this aqueous waste stream, most are energy intensive and costly. Also, they produce more waste streams making it a cycle of never ending waste generation and energy consumption. This makes them an environmental liability.

The current work focuses on the utilisation of this aqueous waste stream to generate a renewable source of energy in the form of biogas. The methods used are energy conserving and environment friendly thereby mitigating the cost involved in the thermo-chemical process. Also, these methods promote the generation of renewable energy without the generation of any waste stream, thereby conforming to the standards of circular economy.

Co-authorship Statement

Chapter 2, part 2.1
Title: **Valorisation of aqueous condensate of biomass pyrolysis**
Authors: Neha Batta, Tahereh Sarchami and Franco Berruti
Current Status: Unpublished
This section of Chapter 2 is a part of a review paper on the valorisation of acetic acid. Neha Batta contributed to the writing and editing of the sections used in this thesis. Tahereh Sarchami contributed to the conception and writing of the review paper. Franco Berruti supervised and edited the document.

Chapter 3 and 4
Title: **Biochar-Enhanced Anaerobic Digestion of Aqueous Pyrolysis Condensate**
Authors: Neha Batta, Tahereh Sarchami, Cesar Miguel Moreira Valenzuela, Lars Rehmann and Franco Berruti
Current Status: Under preparation
The experimental plans were developed by Neha Batta with guidance from Dr. Cesar Moreira, Dr. Lars Rehmann and Dr. Franco Berruti. Neha Batta performed all the experiments, collected and analysed the data, and drafted the manuscript. Dr. Tahereh Sarchami assisted in and performed some analytical procedures. The manuscript was reviewed and edited by all co-authors.

Acknowledgements

Firstly, I would like to express my sincere gratitude to my supervisors, Prof. Franco Berruti, Prof. Lars Rehmann and Prof. Cesar Miguel Moreira Valenzuela, for their constant support and guidance. They have been most patient, supportive and encouraging at every step of the work, taught me to overcome hurdles and most of all helped me better my research abilities. I would especially like to thank Prof. Franco Berruti for the opportunity he gave me, to be a part of this project. It was the most pleasant and unexpected chance which has now given me a path to follow.

My sincere thanks to all the administrative staff in our department for their support.

This work would not have been possible without the wonderful support of my lab mates. I would like to give a special mention to Dr. Tahereh Sarchami for all her help and guidance, especially in helping me learn and understand several analytical techniques. My sincere thanks to Dr. Erin Johnson for being ever so patient with me, teaching me from my mistakes and always being there as a strong support. My lab mates and friends - Paavana Jayaram, Anuradha Krishnan, Garret Munch, Colin Couper, Ugur Gulmen, Yujie Zhang, and my ICFAR group. You all have been an integral part and like a family. I cannot thank you enough.

I must take this chance to thank NSERC and Titan Clean Energy Projects (Craik, Saskatchewan, Canada) for their funding support. This project would not have been possible without them.

Lastly, the most important person without whom none of this would have been a reality – my husband, Vivek Madan. It is because of his patience, unflinching support and confidence in me that I am here today and have been able to accomplish this work. My son, for being the best kid ever and being so understanding through all of this. I would also like to thank my family back home who has been my pillar, irrespective of the distances we are at. I have to mention my late grandfather, Mr. Raj Pal Batta, who passed away the day I got admission into this program, knowing and satisfied at the path I had undertaken. I hope I make you proud.

Table of Contents

List of Tables

List of Figures

List of Equations

List of Abbreviations

AD	Anaerobic Digestion
APC	Aqueous Pyrolysis Condensate
ATP	Adenosine triphosphate
BET	Brunauer-Emmett-Teller
CHNS	Carbon Hydrogen Nitrogen Sulphur
COD	Chemical Oxygen Demand
DIET	Direct Inter Species Electron Transfer
EI	Electron Ionisation
FC	Folin-Ciocalteu
FTIR	Fourier Transform Infrared spectroscopy
GAE	Gallic Acid Equivalent
GC-MS	Gas Chromatography – Mass spectrometry
5-HMF	Hydroxymethyl furfural
HPLC	High Performance Liquid Chromatography
Py-AD	Pyrolysis-Anaerobic Digestion
RID	Refractive Index Detector
SEM-EDX	Scanning Electron Microscopy – Energy Dispersive X-Ray
TCD	Thermal Conductivity Detector
TS	Total Solids
TSS	Total Suspended Solids
VFA	Volatile Fatty Acids
VS	Volatile Solids
VSS	Volatile Suspended Solids

1. Introduction and Context

1.1 Introduction

Waste generation is a constant consequence of human activity. It has been reported that nearly 7-9 billion tons of waste is generated every year [1] of which at least 2.01 billion tons of municipal solid waste is generated every year [2]. According to a World Bank report this figure is projected to increase by 70% in the next thirty years. At least 33% of this waste is managed in a manner that is toxic to the environment. Uncontrolled dumping, open incineration, and release into water bodies are some examples of such methods. Although the composition of this waste varies according to consumption patterns, it is usually a combination of green waste (food), wood, leather, paper, plastics, rubber, metal, glass, fibre and so on [2]. Amongst all other sources of renewable energy generation, biomass produced energy is the most dominant for future applications [3].

Biomass resources could vary from wood wastes, agricultural residues, waste paper, bio-solids, domestic waste, food processing waste, debris of living organisms, decomposing plant matter, etc. [4]. Biomass valorisation can be in one of or a combination of 3 ways, [3]

a. Physicochemical – including extraction, separation, trans esterification
b. Thermochemical – including pyrolysis, combustion, carbonisation, gasification
c. Biochemical – fermentation, anaerobic digestion

Of the numerous ways of biomass waste management, pyrolysis has been receiving a lot of attention [5]–[10]. It is the direct thermal decomposition in the absence of oxygen and is an irreversible [4] and energy intensive process that is effective in the treatment of ‘primary waste. In the process of treatment of primary waste, pyrolysis leads to the generation of three useful products – combustible gas, bio-oil and biochar. However, it

also leads to the generation of a ‘secondary waste’ in the form of aqueous pyrolysis condensate (APC), which is usually very high in acid content. Although the pyrolysis oil may be considered as a low grade fuel substitute, similar to fuel oil, or as a precursor for advanced fuels [4] and the biochar as a soil amendment strategy [11], the APC is a waste product of very low value. It is high in acid and phenolics, thereby making its disposal and treatment a challenging process. There is a lack of research in this area owing to the chemical complexity of the APC and the lack of standardisation of processes for its disposal [12]. Although different strategies have been explored in order to evaluate the APC for its conversion to resource [13], these are not always cost effective. There is a need to explore sustainable processes that do not have a negative effect on the environment and on the energy balance. Hence, in order to make the process a zero waste generation method, it is advisable to integrate the method with a renewable energy generation process in order to mitigate the cost involved in APC conversion and make the entire process sustainable [14]. The conversion process should consider the complexity of the APC, especially the fact that it changes according to the feedstock and pyrolysis conditions [15]. A process giving such flexibility could be anaerobic digestion [16], [17].

Anaerobic digestion is an energy conserving biochemical process which can be utilised to reduce the environmental impact and the costs of wet waste management [18]. It facilitates the conversion low energy aqueous liquids to high energy fuel, hence conserving energy [19]. There is no emission or leeching of hazardous pollutants into the environment, thereby making it one of the most reliable technologies for future considerations [20]. However, due to the complexity of the production process and the changing chemistry of the APC, there is not much work done in this area [15].

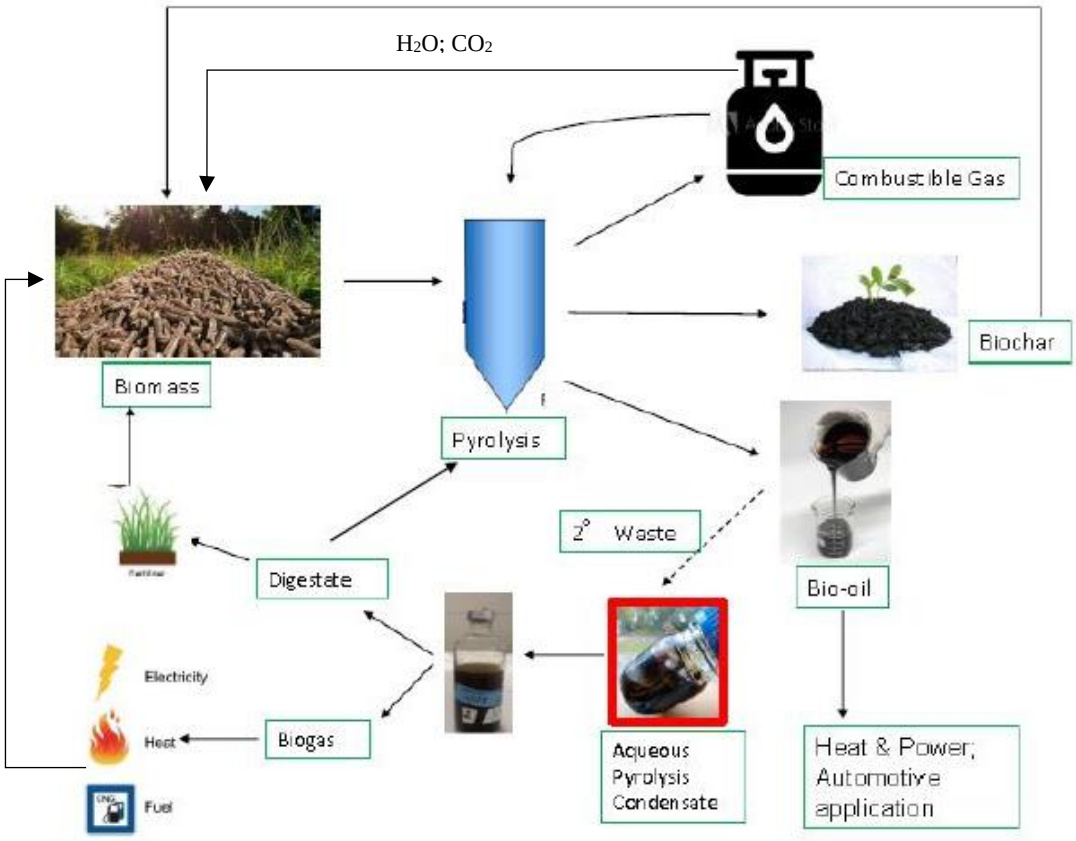

Figure 1.1- Premise of circular economy effect in the work proposed

The work done in this thesis attempts to integrate thermochemical and biochemical processes in order to achieve a positive energy balance (Figure 1.1). We attempt to use the APC as a feedstock for anaerobic digestion for the generation of biogas, with no additional nutrients. Parameters such as acclimatisation of inoculum, inoculum-substrate ratio and the use of biochar adsorbents as well as APC pre-treatment were investigated. In addition, detailed characterisation of the APC and biochar used was done in order to determine the feasibility of the process. The structure of the experiments conforms to the principles of circular economy in an attempt to generate zero-waste while developing a sustainable solution for the treatment of a high acid substrate.

1.2 Research Objectives

Main Objective:

Anaerobic digestion of aqueous pyrolysis condensate and assess the effect of biochar

Sub-objectives:

- To characterise the aqueous pyrolysis condensate derived from wood and biochar derived from pyrolysis of digestate.
- To assess the feasibility of biogas generation from aqueous pyrolysis condensate derived from wood.
- To compare the qualitative production of biogas using adapted and non-adapted inoculum.
- To assess the impact of APC and biochar loading on qualitative biogas production.

1.3 Originality

To the best of our knowledge, the work done in this thesis is the first to comply with the principles of circular economy. The anaerobic digestion of soft wood pyrolysis derived APC in the presence of biochar derived from digestate has been reported for the first time. Therefore, the process generates no secondary waste. The work also reports the consumption of 5 g/L acetic acid equivalent of APC (equivalent to 11.6% APC) with high biogas quality. In addition, this is the first time that biochar pre-treatment of APC for the removal of inhibitors and effect of biochar dosage on biogas quality has been assessed.

2. Literature Review

2.1 Pyrolysis and Aqueous Pyrolysis Condensate

Pyrolysis is a scalable thermochemical technology, which can depolymerize lignocellulosic biomass in a single oxygen-starved reactor into a liquid, a carbonaceous solid (biochar) and a gaseous product, consisting of a mixture of light hydrocarbon gases [21], [22]. The liquid product from pyrolysis, known as bio-oil, is composed of differently sized molecules derived primarily from three key biomass building blocks, i.e., cellulose, hemicellulose, and lignin, resulting in its composition and properties of considerable difference from those of petroleum-based fuel oils [23]. Pyrolysis bio-oils contain more than 400 compounds and these chemical functionalities in the bio-oil correlate strongly with the feed composition and the pyrolysis processing conditions. From a chemical point of view, bio-oil is an extremely complex mixture of organic components, including various types of oxygen-containing organic acids, esters, alcohols, aldehydes, ketones, furans, phenols, and dehydrated carbohydrates [4], [9]. Among these compounds only four molecules are reported in the literature with quantities sufficiently high (> 5 wt %). These molecules are glycoaldehyde (1.0-13.7 wt%), acetic acid (2.5-8.7 wt%), acetol (2.6-8.6 wt%), and levoglucosan (3.0-6.5 wt%) [24].

2.1.1 Effect of different pyrolysis parameters on acetic acid content

Pyrolysis is a complex process governed by various parameters that affect the heat and mass transfer mechanisms of the process which, in turn, affect the overall efficiency and product distribution. The end products are a result of primary and secondary reactions that occur during the decomposition of biomass [25]. The thermal decomposition processes of cellulose, hemicellulose and lignin are dependent on the process parameters and the optimization of these parameters could increase the yield

and composition of the desired end-products [25]–[27]. Therefore, it is important to review and analyse the effect of process parameters on industrially important end products, such as acetic acid. Table 1 shows a comparison of the most important pyrolysis parameters (temperature, holding time, and type of pyrolysis reactor) and their effects on acetic acid concentrations.

2.1.1.1 Temperature

The yields and quality of pyrolysis products depend on the operating temperature since it governs the decomposition of the biomass [28]. High temperatures, such as those higher than 550°C, cause massive fragmentation of the biomass, thereby increasing biomass conversion efficiency and causing primary and secondary reactions to alter product composition. However, low temperatures, such as lower than 300°C, cause decomposition of heteroatom sites. The major percentage (80%-90%) of biomass fragmentation occurs between 300°C-500°C. Higher temperatures promote secondary reactions which, in turn, decrease the liquid yield and increase gas yields. Bio-oils are a mixture of water with complex organic compounds , the composition of which is greatly dependent on operating temperatures [25], [29], [30] and they tend to separate into a light acidic aqueous phase (called aqueous pyrolysis condensate (APC) and a rich and viscous organic phase.

Various studies have reported that maximal bio-oil yield is obtained at temperatures between 350°C-600°C, whereas higher temperatures favour the production of gaseous components [5], [7], [28], [31], [32]. It has also been observed that carboxylic acids are the dominant functional groups in bio-oil at low temperatures [25]. Bio-oil obtained by flash pyrolysis is generally high in acid content [33]. The aqueous pyrolysis condensate (APC), however, has not been characterized in most studies. In the pyrolysis of cherry seed, it was observed that the bio-oil yield decreased with increase in temperature,

however, the aqueous phase yield seemed to increase with increasing the temperature [30]. In the pyrolysis of pine needles, it was found that the aqueous phase yield was highest (25 wt%) at 450°C and then reduced to 11 wt% at 500°C, while gradually increasing back to 20 wt% at temperatures highest than 600°C [28]. In another study on the slow pyrolysis of pomegranate seeds, the maximal liquid yields were reported between 500 °C and 600°C, in a fixed bed reactor, beyond which gasification was dominant [5]. Fixed bed slow pyrolysis of pistachio shells gave a very high concentration of acetic acid (9.98% peak area) in the liquid product at 500°C, above which gaseous product formation dominated due to the secondary reaction of volatiles [7]. However, a comparison of different operating temperatures showed that there was no major difference in the chemical composition of the liquid products with variations in operating temperature. This was attributed to the fact that the initial decomposition of cellulose and hemicellulose, which takes place at 350°C, is responsible for the formation of the liquid product by condensation of volatiles. Therefore, it is clear that, in slow pyrolysis, the temperature does not affect the composition of the liquid products beyond a certain point [7], [30]. In a study on the fractionation of bio-oil fractions from red-oak produced in a fluidized bed reactor at different temperatures, it was found that the acids were present in highest concentrations at a temperature of 350°C [34]. These results were similar to those found in pyrolysis of beech wood, spruce wood, hazelnut shell and olive husk, where maximum acetic acid (16.8, 15.6, 14.2 & 13.5 wt.%, respectively) was found at 350°C and consistently decreased until 600°C [35]. In another comparison of beech wood, spruce, iroko wood, albizia wood and corncob over a range of pyrolysis temperatures up to 700°C, all the samples showed a maximum acetic acid concentrations at 400°C [36], whereas Douglas fir wood and hybrid poplar wood generated the highest acetic acid yields at 450°C [37]. In another study, Douglas

fir wood gave maximum liquid yield of 55 wt% at 350°C with acetic acid up to 3 wt% [38]. Most studies focus only on the bio-oil or total liquid yield and there is a lack of literature data on the chemical characteristics of the APC. However, in one study on municipal solid waste pyrolysis in an auger reactor, the effect of temperature on the APC chemical composition was studied. It was observed that increasing the process temperature increases the pH and decreases carbon content, but has no effect on hydrogen content, thereby implying a high acid content at lower temperatures. However, no quantification of acetic acid was done in this study [6].

When direct cellulose was used to study the effects of fast pyrolysis temperatures, it was seen that, although cellulose pyrolysis began at 150°C, there were no major pyrolytic product formations up to 400°C. A direct gas analysis of the pyrolysis vapours showed that the maximum acetic acid yields were achieved at a temperature of 600°C, and at a pyrolysis time of 15 s. The breakdown of cellulose to acetic acid happens due to the ring scission of the cellulose at high temperatures [39]. Another possible explanation is the carbonylation of methanol through carbon monoxide, or oxidation of acetaldehyde, both of them being pyrolysis products of cellulose and hemicellulose [36]. This observation proves that there are multiple factors affecting the breakdown of cellulose and hemicellulose to generate acetic acid (and other products) in different permutations and combinations [26], [27], [34]–[36], [39]. However, acetic acid being a heat labile product is susceptible to decomposition at higher temperatures thereby lowering the yield at high temperatures [40].

2.1.1.2 Holding Time

Research has shown that, during fast pyrolysis carried out at short holding times (typically of the order of 1 to 3 seconds), vapours leave the reactor more rapidly, minimizing further decomposition taking place due to secondary cracking reactions,

thereby giving high liquid yields. However, operating at excessively short vapour residence times, although secondary reactions are minimized, could generate heat transfer limitations, thereby limiting the biomass conversion into bio-oil [25], [29], [30], [41]. One method to overcome this limitation is the use of small particle sizes of the biomass to achieve effective heat transfer within a short holding time [25], [29]. The optimization of holding time is a challenge due to the trade-off between bio-oil quality and quantity. It has been reported that longer residence times at higher temperatures give better quality bio-oil but decreases the total yields. Most of the research on holding time focuses on bio-oil yields rather than quality improvements [25], [27], [29]. The holding time, however, has been reported as one of the less critical parameters affecting bio-oil yields at temperatures below 450°C [38].

2.1.1.3 Heating Rate

Another significant factor that affects the decomposition of biomass is the heating rate, which determines whether the pyrolysis is fast or slow. It is a well-known fact that the faster is the heating rate, the higher is the liquid yield [42]. This happens due to the reduced time for secondary reactions (of tar cracking and re-polymerization). A faster heating rate thereby increases the amount of volatiles that are released. [25], [42], [43]. Fixed bed pyrolysis of chanar fruit endocarp, at a heating rate of 15 °C.min^{-1} at 550°C, gave bio-oil yields of 49.4 wt% and 34.9 wt% from the pyrolysis of the pericarp of white palm fruit. The acetic acid yield was 13.27 g.l^{-1} and 45.97 g.l^{-1} respectively [44]. In another study in the slow pyrolysis of cherry seed in a batch fixed bed reactor gave low liquid yields, yet the concentration of acetic was high in these liquid fractions. However, fast pyrolysis of the same feedstock in a continuous fluidized bed reactor did not necessarily give higher acetic acid concentrations in the liquid product [30]. Slow pyrolysis of pistachio shells in a fixed bed reactor at 40°C.min^{-1} gave a total liquid yield

of 47 wt% [7]. The above findings suggest that the heating rate is not the sole determinant of the liquid yield. This is interdependent on the feedstock and temperature of the reaction as well. However, the heating rate governs the effect of temperature on the pyrolysis yield [30].

2.1.1.4 Sweeping Gas

Studies have shown that low flow rates of sweeping gas results in low liquid yields. The low rate of secondary reactions at higher velocities increases bio-oil yield. However, at extremely high flow rates, the bio-oil yield was decreased due to the inefficient condensation of the very diluted vapours [7], [45]. In a study done on pine wood pyrolysis in a fixed bed reactor, with Argon as the sweeping gas at a flow of 150-600 $ml.min^{-1}$ and a residence time of 2.7 seconds, it was observed that there was not much effect on the liquid yield. Also, the acetic acid concentration in the liquid fraction varied with temperature and was found to be highest at low temperatures of 300°C, and lowered at high temperatures of 700°C [40] . Increasing the sweeping gas flow rates could influence product distribution in the liquid. This was proven when pine wood was pyrolyzed in an auger reactor to monitor the effect of sweeping gas flow rates at a constant temperature of 500°C with nitrogen as the sweeping gas at a residence time of 72 seconds. This study showed that higher sweeping gas flow rates of 40 $l\ N_2.min^{-1}$ gave marginally higher concentrations of acetic acid in the liquid phase. Increasing the sweeping gas flow rates minimizes secondary reactions thereby lowering the water content and increasing the organic acid content. In addition to this, the residence tie plays a significant role in determining he secondary reactions that take place thereby altering the composition of the liquid yield [7], [46]. However, there is a dearth of research to show the effects of sweeping gas on the acetic acid concentrations.

2.1.1.5 Type of pyrolysis reactor

The type of pyrolysis reactor can affect the mixing efficiency and the residence times of reacting biomass and vapours and, in turn, mass and heat transfer efficiency. Therefore, it is imperative to have the appropriate combination of the reactor type and pyrolysis conditions in order to achieve the required end product [38]. Bubbling fluid bed pyrolyzers, representing a relatively well-established technology for fast pyrolysis, have been shown to give consistently high liquid yields of up to 75 wt% from wood pyrolysis. However, several other factors, such as heating rate and type and particle size of the biomass determine the chemical composition of the final product. On the other hand, circulating fluid beds, though suitable for very short contact times as a result of the high gas velocities, require very small biomass particles and are proven to create higher char attrition in the bio-oil. Extensive char removal steps need to be included in order to get better quality of bio-oil [29]. In a comparison between continuous fluidized bed and batch fixed bed pyrolysis of cherry seed, it was observed that temperature variations had a major effect on the product distribution in the fluidized bed whereas temperatures above 500°C had no effect in case of fixed bed. [30]. On the other hand, the ablative pyrolysis processes do not use an inert heat carrier and they are limited by the rate of heat supply. This reactor leads to easier collection of the condensed vapours. Several reports in the literature confirm that short residence times are needed for high liquid yields [25], [29], [41]. This is a condition that is hard to achieve in mechanically driven systems, such as auger and mechanically fluidized reactors, thereby resulting in lower liquid yields. But these processes, due to the absence of an inert gas, yield concentrated vapours with higher partial pressures than bubbling and circulating fluidized bed reactors, corresponding to higher liquid collection efficiencies [29]. The above analysis clearly shows a trade-off between liquid yield and liquid collection efficiency in mechanical versus carrier gas-based systems. Even though here is a

comparison of the effect on product quality with respect to different types of reactors, there is a dearth of research on analysing the chemical composition of the secondary products, especially with respect to industrially important chemicals, such as acetic acid.

In a study done on auger pyrolysis of Douglas fir wood and fixed bed pyrolysis of beech wood, it was observed that though the liquid yield was higher in Douglas fir wood pyrolysis, the acetic acid yield was higher for beech wood pyrolyzed in a fixed bed reactor [38]. When compared for pure xylan based compound, O-acetyl-4-O-methylglucurono-xylan, it was observed that a high liquid yield of 60% was achieved in a tubular stainless steel reactor, at a temperature of 350°C whereas a vertical Pyrex reactor gave only 32% bio-oil at 450°C. In another attempt, an up-draft entrained flow reactor gave a liquid yield of 45% at 450°C. [47]. Fluidized bed fast pyrolysis of the same xylan based compound gave an acetic acid yield of 4.44 wt% at a temperature of 425°C [48].

Table 1- Effect of different pyrolysis parameters on liquid yield and acetic acid concentration

Feedstock	**Hemicellulose (wt %)**	**Reactor type**	**Temp (°C)**	**Holding Time (s)**	**Liquid Yield (wt %)**	**Acetic Acid (g/L)**	**Ref.**
Xylan (O-acetyl-4-O-methylglucurono-xylan)		Fluidized Bed	425	0.5	42	44.4	[47]

		Updraft entrained flow	475		45	-	[47]
		Vertical fixed bed	800	30	54.3	-	[47]
Douglas Fir Wood		Auger Reactor	350	8	59	30	[38]
Mallee		Fluidized bed	400		62	50	[38]
Chanar fruit endocarp	48.5	Fixed bed	550	1800	47	13.27	[44]
Palm fruit pericarp	58.5	Fixed bed	550	1800	31.8	45.97	[44]
White palm seed residue	72.7	Fixed bed	550		34.9	22.97	[44]
Straw	14.2	Fluidized bed	500	1-2	41 $\pm$ 1.3	4.83	[41]
Corn cob	3.4	Fluidized bed	500	1-2	47 $\pm$ 0.9	8.01	[41]
Oreganum stalk	9.3	Fluidized bed	500	1-2	45 $\pm$ 3.1	6.62	[41]

Pomegranate seed	25.52	Fixed bed	400	3600	51.08		[5]
			500	3600	76.02		[5]
			600	3600	76.4		[5]
			800	3600	73.6		[5]
Pistachio shells		Fixed bed	500	1800	47		[7]
Cherry Seed	28.59	Fixed bed	300	3600	12 (bio-oil) 30 (APC)	26.51 (bio-oil) 97.12 (APC)	[30]
			500	3600	20 (bio-oil) 32 (APC)	26.35 87.73 (APC)	
		Fluidized bed	400	1-2	35 (bio-oil) 10 (APC)	91.74 (bio-oil) 91.03 (APC)	
			600	1-2	17 (bio-oil)	5.63	

						32 (APC)	(bio-oil) 76.25 (APC)
Cherry shell	seed	31.93	Fixed bed	300	3600	7 (bio-oil) 35 (APC)	42.47 (bio-oil) 80.80 (APC)
				500	3600	15 (bio-oil) 36 (APC)	39.28 (bio-oil) 78.88 (APC)
			Fluidized bed	400	1-2	42 (bio-oil) 18 (APC)	74.5 (bio-oil) 73.32 (APC)
				500		41 (bio-oil) 12 (APC)	64.07 (bio-oil) 80.57 (APC)
				600		22 (bio-oil) 30 (APC)	36.25 (bio-oil)

						96.25 (APC)	
Hard wood		Fluidized bed	500	1.5		30.6 (bio-oil) 104.86 (SF-5)*	[49]
Olive Husk	23.6	Tubular	625	45-55	38	13.5	[35]
			725		46	11.5	
			825		46.5	7.01	
Hazelnut shell	29.9	Tubular	625	45-55	36	14.2	[35]
			725		44	12.4	
			825		43.7	8.26	
Spruce wood	21.5	Tubular	625	45-55	34.3	15.6	[35]
			725		40	14.1	
			825		39.7	8.34	
Beech wood	28.4	Tubular	625	45-55	32	16.8	[35]
			725		39.5	15.9	
			825		39.4	8.24	

Pine wood	25	Fixed-bed	300	2.7	28	10.4	[40]
			500		60	8.0	
			700		60	5.4	
Corn stalk pellets		Fixed Bed	400	600		26	[50]
Corn stover		Fluidised bed reactor	500			28.98	[51]
Birch bark		Fluidised bed (continuous)	500			104	[52]
Raw Digestate			330			9.2	[53]
			430			8.9	
			530			4.6	
Dried Biosolids (from anaerobically			800			29	[16]

digested sludge)

The temperature, holding time and reactor type all are the main factors influencing the pyrolysis end-product distribution. Studies suggest that pyrolysis at lower temperatures with faster heating rates increase the aqueous phase and carboxylic acid yield. Whereas the holding time is one of the less critical parameters, the type of reactor may affect the acid content of the liquid yield. It was found that fluidized bed reactors yielded maximal acid content. Of course, feedstock is another important consideration

2.2 Biochar

2.2.1 Biochar-Basic characteristics and production

Various thermochemical processes such as controlled combustion, gasification and pyrolysis can be used to produce 'pyrogenic carbonaceous material', each of these affecting the quality of the product. Biochar is a carbonaceous material, with a heterogeneous chemical composition [54], produced from the thermochemical conversion of biomass initially defined for agricultural use. It has also been used as adsorbent, food supplement for ruminants, biodegradable packing material, soil amendment, concrete additive, carbon sequestration, microbial fuel cell additive, catalyst, etc. [55], [56]. Biochar is a high surface area and porosity material, thereby making it a good choice for the removal of liquid phase inhibitors and contaminants [56]. It is also known to adsorb a large variety of compounds such as sulphur dioxide, ammonia, carbon dioxide, etc. by van der Waal forces for organic compounds and electrostatic interactions for inorganic compounds [57].

Pyrolysis conditions have a major effect on the properties of the biochar produced. Factors such as feedstock, temperature and additives define the characteristics of the biochar. Higher pyrolysis temperature gives lower biochar yield [56], [58] whereas fast pyrolysis leads to higher quality of biochar [11]. For instance, the carbon content of the biochar is dependent on the type of biomass used [55] as well as the temperature of pyrolysis [56], [58]. As the temperature increases, the carbon/nitrogen content of the biochar decreases. High temperatures also decrease the hydrogen/carbon content of the biochar thereby affecting its polarity [56].

In addition, the feedstock also affects the surface area of the biochar. One of the most important physicochemical properties of biochar is its adsorption capacity, which is dependent on the presence of acidic functional groups of the biochar. An increase in acidic functional groups means that the biochar will adsorb more ammonium ions (NH_4^+) [18]. The adsorption property of the biochar is also influenced by its specific surface area. An increase in the specific surface area, increases the adsorption capability for metal ions and organic compounds. The specific surface area of the biochar is determined by the reactor conditions. A recent comparison showed that the biochar derived from crop residue pyrolysis had a higher specific surface area than that of animal manure pyrolysis [56]. The function of biochar is also known to vary according to its particle size [59]. These parameters greatly affect the quality of the biochar and its handling characteristics and end applications. For optimum activity of the biochar, it is often activated physically at high temperatures or chemically, to increase the porosity and specific surface area. The pH of biochar is also affected by the reactor temperatures, where the higher the temperature, the higher the pH [55].

2.2.2 Biochar and Anaerobic Digestion

Although anaerobic digestion (AD) is one of the oldest and well established technologies, it is known for the process instability involved, especially when done in the presence of recalcitrant feedstocks [60], [61]. Low methane yields and process inhibition due to accumulation of toxic inhibitors are some of the main concerns in AD [18]. Although biochar is widely being explored as a soil amendment strategy [11], the properties of nutrient retention and enhancement of microbial growth make it a tempting additive for the improvement of anaerobic digestion processes. The morphology and porous structure of biochar may result in the immobilisation of the microbes by biofilm formation thus enhancing the digestion efficiency and biogas production in some cases [17], [61].

It was found that biochar enhanced the growth of methane producers *Methanosaeta* and *Methanosarcina* thereby improving methane content of the biogas produced [18], [62]–[64]. In addition, biochar assists DIET (direct inter species electron transfer) due to its conductive properties, hence enhancing methane generation [19], [56], [59].

Some studies have shown that the addition of biochar shortens the lag phase of methane production. Also, researchers report that biochar with high surface area enhance biofilm formation and carbon dioxide sequestration. However, the feedstock and pyrolysis parameters affect the physico-chemical properties of the biochar thereby making the biochar a positive or negative influence on the anaerobic digestion process [62]. Oxidised biochar functions as an electron acceptor thereby enhancing the VFA conversion process. On the other hand, reduced form of biochar facilitates nitrate reduction by becoming an electron donor. It is also known to mitigate inhibition effects of mild ammonia toxicity and other toxic inhibitors thereby promoting microbial growth [18], [50], [62].

The ash content of the biochar is composed of the minerals present in the original biomass, mainly insoluble calcium and magnesium carbonates, which contribute to its carbon dioxide sequestration activity and alkalinity of the biochar. This process is known as the mineral carbonation, wherein compounds containing Ca and Mg react with CO_2 to form stable carbonates thereby offering safe and permanent storage of CO_2 [62], [65], [66]. However, very high ash contents can be correlated to the low specific surface area and small pore size of the biochar.

On the other hand, a study reported that biochar derived from a pyrolysis mixture of paper sludge and wheat husks had no mitigation of ammonia inhibition at high ammonia concentrations. Also, that biochar did not have a significant effect on the total biogas production [67]. Another study investigating the role of biochar as a buffering agent, suggested that biochar had a positive effect on the buffering system during the anaerobic digestion of chicken manure and kitchen waste and considerably improved the biogas yield along with methane content [18]. Additionally, it has been reported that biochar derived from fruit woods increased the methane production rate by 47% and there was a 23% reduction in lag phase during anaerobic digestion of granular sludge. However, there was no change in the dissolved nitrogen content in the presence and absence of biochar, indicating that the physico-chemical characteristics of the biochar may affect its function.

Interestingly, researchers observed that fine particles of the biochar favoured fermentation and acidogenesis phase whereas coarse and medium sized biochar granules favoured methanogenesis [59]. Similarly, a study on the effects of fruitwood derived biochar, it was observed that biochar granules in the range of 0.5-1mm decreased in lag phase by 11% and increased in methane production rate by 86% on

addition of optimum amounts of fruitwood derived biochar. It was found that addition of biochar increases the amount of archaea in the microbial population [64].

In a comparison of biochars produced from the pyrolysis of corn stover and acid pre-treated corn stover, it was observed that the acid pre-treatment caused an increase in the sulphur content of the biochar, decreased its overall specific surface area and reduced pore size. The biochar derived for the pre-treated feedstock did not result in higher methane generation rather caused a decrease. This was attributed to the high sulphur content of the acid pre-treated biochar, due to the probable competition between the sulphate reducing bacteria and the methanogens [62]. A study on the effect of different types of biochar on anaerobic digestion highlights the importance of the right dosage of biochar during the process. It was observed that adding too much biochar had a negative effect on methanogenesis. A comparison of corn straw, coconut shell and sewage sludge derived biochar showed that the daily methane yield was lower in the biochar supplemented set-ups in the initial stages of AD. However, after a period of adaptation, the methane yields increased in case of coconut shell and corn straw derived biochar. Coconut shell biochar resulted in almost 87% increase in methane yield as compared to no biochar [58].

Although there are many studies reporting the effects of biochar on anaerobic digestion, there is a dearth of literature on the effect of biochar on the anaerobic digestion of APC. In batch tests, biochar and APC (derived from the pyrolysis of corn stalk pellets at 400°C) when added in a 1:1 ratio, improved methane production by almost double as compared to without biochar [50]. It is known that methanogens are more sensitive to the presence of APC. The addition of biochar mitigates the toxicity of the APC and promotes methanogen population. Similar effects were seen in continuous digestion experiments on APC derived from pine wood pellets. The biochar from the same

pyrolysis procedure was used to supplement the system, which yielded a biogas ratio of 1:1 when 8g biochar was added to 80mL of the reaction set-up [68]. In another study on the AD of APC derived from the pyrolysis of birch bark, where even nutrient addition did not increase the methane yield, biochar form the pyrolysis of *Miscanthus*, had a positive effect on the methane generation [52]. There is a large scope for detailed studies on the effects of biochar on APC AD, in order to better understand the process dynamics and hopefully stabilise the process.

2.3 Anaerobic Digestion

In the early 1800's, John Dalton and Humphrey Davy established the presence of methane as the combustible gas that is generated from rotting organic waste. It was then confirmed that this was a microbiological process where decarboxylation of acetic acid gives methane. Ever since, anaerobic digestion has been an area that is still being understood and explored. In fact, it took a very long time for the first major use of anaerobic digestion for pollution control, which was after the introduction of the concept of Solid Retention Time (SRT), by Stander, in 1950 [69]. The first major commercial application came in the early 1970's, when it was applied to industrial wastewater treatment. There have been major reactor and process improvements since the first commercial use of anaerobic digestion making it one of the most used technologies for organic waste treatment [70].

Whereas most developed countries used anaerobic digestion as a primary source of pollution control, the use of anaerobic digestion for the commercial production of biogas, was first done in 1897, in Mumbai, India. The coming years saw anaerobic digestion as a major source of energy production, especially for developing countries, with China accounting for the largest biogas program globally [69], [70]. Recently, there has been an increased focus on tapping anaerobic digestion as an energy source

world over [3] and is now considered as one of the most efficient solutions for valorisation of wastes to generate energy [18].

2.3.1 Biogas Production

As the name suggests, anaerobic digestion is a process that takes place in the absence of oxygen. It is the sequential biochemical breakdown of organic matter (mineralization), by symbiotic association of different groups of bacteria [3], [71]. The resultant is the generation of biogas, which is mainly composed of methane and carbon dioxide, and a digestate which can be utilised as a bio-fertilizer [71]. It is widely used as a method for the treatment of waste water, municipal solid waste and organic industrial waste [3], [72]. Unlike other waste management processes, this process allows for the treatment of biomass with less than 40% dry matter, without any pre-treatment requirements [3] thereby offering an efficient solution for waste treatment and energy generation. It is a dynamic process that allows for the utilisation of multiple substrates, such as organics and inorganics, for biogas production [69]. However, the efficacy of biogas production is governed by the structure and composition of the feedstock used. The biodegrability of a potential substrate is greatly dependent on its chemical composition and characteristics [20]. Some substrates might cause a nutrient imbalance or alter the microbial community dynamics of the system. One common proposed strategy to overcome these limitations is co-digestion (for instance in the presence of live-stock manure [73].

In addition, anaerobic digestion has major advantages of low energy requirement for operation and easy sludge recovery [60]. Some modes of anaerobic digestion offer higher degree of waste stabilisation along with easier sludge dewatering. The production of renewable energy in the form of biogas significantly reduces the overall costs for treatment of waste water and other organic wastes. It is now being tapped as a

major source dedicated for renewable energy production. [72]. In order to reduce environmental impact, a large number of governments are now providing incentives for the promotion of application of anaerobic digestion in agricultural practices [71], [72]. However, there is scope for enhancement of the anaerobic digestion process in order to improve operational stability, to make the process more feasible for wide-scale commercialisation [60].

Biogas, which is composed of approximately 65% methane, 35% carbon dioxide (with other trace gases like H_2S, H_2 and N_2, water vapour) is now being used as an energy source in many countries [70]. This is mainly due to the fact that its production is energy efficient and environmentally sustainable due to the low emission of hazardous pollutants. In addition, biomethane is classified as a 'super-low carbon fuel' and is also the greenest of all biofuels [69]. The most common mode of utilisation of biogas is by combined heat and power (CHP) for the simultaneous production of heat and electricity. Another method of value addition to the biogas is upgrading to natural gas. A large number of gas purification strategies are being adopted for improvement and better utilisation of biogas. The production of syngas (a mixture of H_2 and CO) is also now being targeted as a possible pathway of biogas valorisation, for the production of value-added chemicals [3].

2.3.2 Stages of biogas production – the microbial community involved

Anaerobic digestion is a multi-step process involving a large number microbes, in systematic stages, for the breakdown of complex organics to biogas (Figure 2.1.1). The coupled metabolism reactions are divided into four main stages of – hydrolysis, acidogenesis, acetogenesis and methanogenesis, where the products from one stage are used as substrates in the next stage until the final production of biogas. This mechanism is known as syntrophy [69], [71] .

Hydrolysis:

The first stage of anaerobic digestion is the breakdown of complex organic molecules that constitute biomass, such as proteins, starch, fats and cellulose, into basic monomeric units in the presence of water and enzymes. The groups of bacteria involved in the hydrolysis could be facultative and obligate anaerobes belonging mainly to *Enterobacteriaceae, Clostridium, Lactobacillus, Bacteriodes, Bifidobacterium and Bacillaceae.* Hydrolysis is also a slow step in the methane generation process thereby limiting the rate of methanogenesis [69], [71].

Acidogenesis:

Hydrolysis is succeeded by acidogenesis wherein the organics form the previous step are converted to propionic acid, butyric acid, valerate, formate, etc. accompanied by the production of acetate, carbon dioxide and hydrogen [69], [71]. Like methanogens, fermentative bacteria produce energy in the form of ATP but use substrate level phosphorylation [74].

Acetogenesis:

The acidogenic intermediates are then converted to carbon dioxide, hydrogen and acetate in this secondary fermentation process called acetogenesis. Accordingly, acetogens could be homoacetogens or obligate hydrogen producing acetogens (which are mainly responsible for converting fatty acids to acetate, carbon dioxide and hydrogen). For the obligate hydrogen producing acetogens to function, it is important for the partial pressure of hydrogen to be low (ideally below 10^{-4} atm) [71] since the accumulation of hydrogen causes the Gibbs free energy of the acetogenic reaction to be positive. The lowering of Gibbs free energy is achieved by a syntrophic relation with methanogens. The consumption of hydrogen by methanogens helps maintain a low partial pressure [69], [71].

On the other hand, autotrophic homoacetogens can convert carbon dioxide, carbon mono oxide and hydrogen to acetate. Therefore, they also contribute in maintaining the low partial pressure of hydrogen. The heterotrophic homoacetogens are responsible for the breakdown of formate and methanol to acetate without causing any accumulation of hydrogen [69], [71]. The heterotrophic homoacetogenesis is a fast and spontaneous reaction with a Gibbs free energy of -104.6kJ/mol. The acetogenic and acidogenic stages are not necessarily distinguishable in most cases and involve the following multiple groups: *Peptococcus, Lactobacillus, Desulfobacter, Bifidobacterium, Micrococcus, Veillonella, Desulfovibrio, Syntrophomonas, Clostridium, Suntrophobacter,, Staphylococcus, Streptococcus, Bacillus, Pseudomonas,* etc. [69].

Methanogenesis:

Methanogenesis is the process of production of methane, under anaerobic conditions. The group of bacteria responsible for methane production are known as methanogens. They are found in the environment in anaerobic conditions such as dumping grounds & water bodies, low sulphate environments such as and in animal rumen, producing large amounts of methane [69], [74]. Methanogens are the most crucial part of the entire process since they are extremely slow growing. They form the rate-limiting step of the entire anaerobic digestion process. Moreover, they need strict anaerobic conditions and are sensitive to environmental changes. Some of the most commonly studies methanogens include *Methanosarcina, Methanothrix, Methanobacterium, Methanogenium* and *Methanococcus* [71].

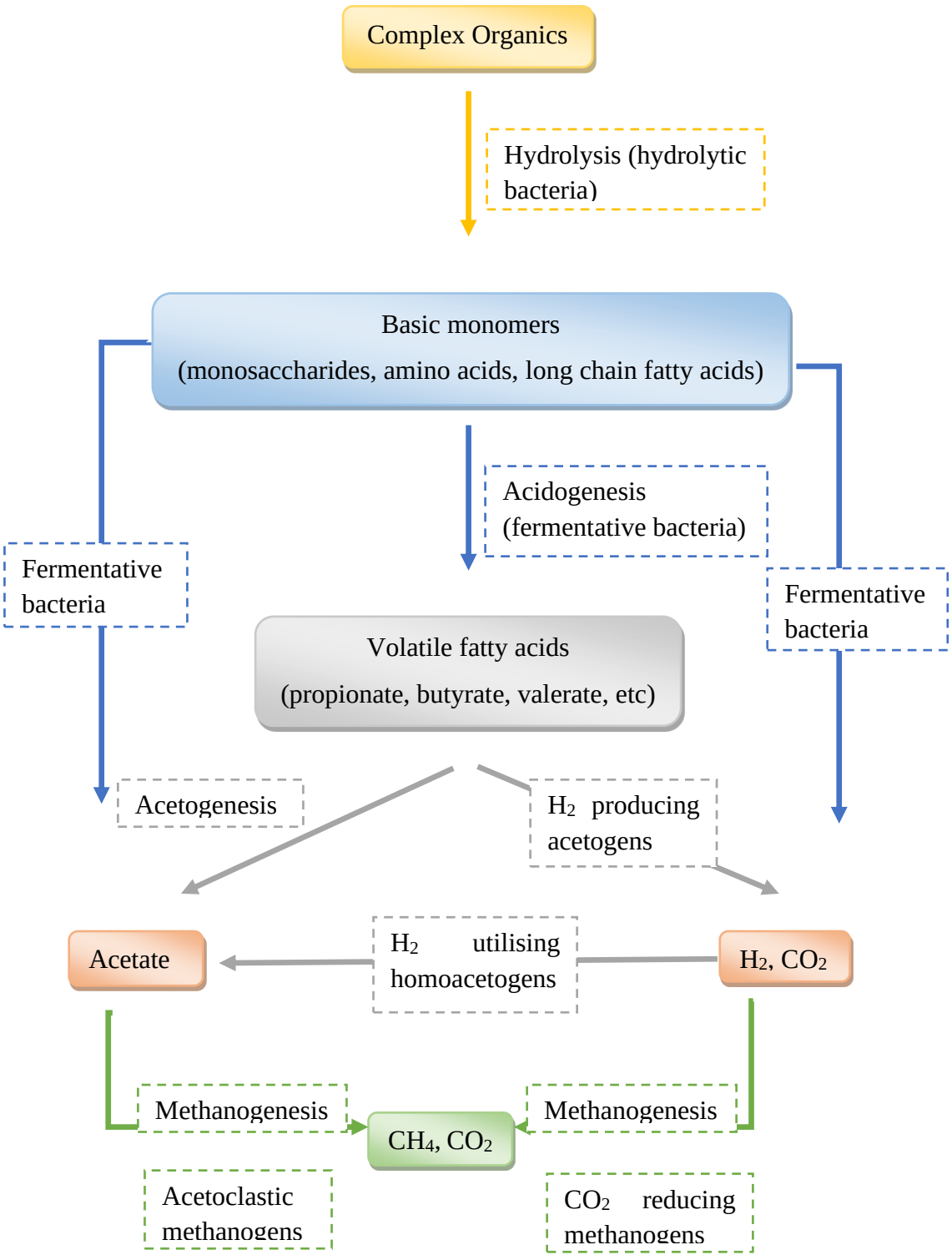
Complex Organics
Hydrolysis (hydrolytic bacteria)
Basic monomers
(monosaccharides, amino acids, long chain fatty acids)
Acidogenesis
(fermentative bacteria)
Fermentative bacteria
Fermentative bacteria
Volatile fatty acids
(propionate, butyrate, valerate, etc)
Acetogenesis
H2 producing acetogens
Acetate
H2 utilising homoacetogens
H2, CO2
Methanogenesis
Methanogenesis
CH4, CO2
Acetoclastic methanogens
CO2 reducing methanogens

Figure 2.1: Stages of anaerobic digestion for methane production

All methanogens are strict anaerobes and can metabolise a large range of C1 compounds (methanol, methyl amines, formic acid, carbon dioxide, carbon monoxide) and acetate to produce methane via complex biochemical pathway [71], [74]. Even though there is a range of substrates that the methanogens can metabolise, there are two main pathways by which methanogens operate – carbon dioxide reduction and acetate utilisation. Both the pathways involve several enzymes that are exclusive to methanogens. For instance, coenzymes such as tetrahydromethanopterin and methanofuran that are used in the carbon dioxide reduction pathway are found only in methanogens. The net Gibbs free energy for this pathway is -130.4 kJ/mol thereby giving them the unique property to convert the chemical energy to biomass. On the other hand, the Gibbs free energy for the breakdown of acetic acid is -31 kJ/mol indicative of a spontaneous reaction for the breakdown of acetic acid. The fact that they can conserve energy classifies the methanogens as autotrophs [74].

In the acetate pathway, the acetate is broken down to yield -CH3 which is then reduced to methane. Although there are other bacteria that could produce methane as a by-product, methanogens are obligate methane producers and are known to conserve energy by the Wolfe cycle. Unlike fermentative bacteria, methanogens generate energy using a transmembrane ion gradient using ATP synthase [69], [71], [74]. Another unique property of the methanogens is the reversible metabolism of converting methane to carbon dioxide. The exact mechanism of this remains unknown but research suggests variation in electron bifurcation patterns due to the change in availability of methanogenic substrates as a possible reason [74].

Methanogens can consume hydrogen and acetate fermentation by-products thereby preventing its toxic accumulation and making the system more conducive for the growth of fermenting bacteria. They are classified as acetoclastic, hydrogenotrophic, carboxydotrophic and methylotrophic according to the substrates they consume [74]. More than 70% of the methane is produced by the acetoclastic methanogens *Methanosarcina* and *Methanosaeta* [69].

2.3.3 Factors affecting Biogas production – Inhibitors and Enhancers

Anaerobic digestion is a complex process showing considerable variation in its progression. This also affects the inhibitors and enhancers of the AD process, which could vary according to the process parameters, inoculum conditions, feedstock and so on. The feedstocks used, especially, can influence the process due to the different chemical composition of each type depending on its origin [60]. Other factors like poor operational conditions [75] and environmental parameters play a major role in determining the effectiveness of methanogenesis. Some essential parameters include temperature, pH, metals, feed concentration, etc. [69], [71] whereas chemical compounds such as sulphur, organics, ammonia, heavy metals, above a threshold value can be inhibitory (Table 2) [75].

Temperature, for instance, impacts the maximum growth rate, decay rate and yield of the microbes involved. Methanogens can function over a huge rage of temperature, between 10°C-60°C. However, the efficiency of biogas production varies with temperature, with it being highest at thermophilic temperatures and lowest at psychrophilic temperatures. An exception is between the thermophilic ranges of 50°C-70°C, where methane production is not dependent on temperature [69]. Although methanogens show a wide tolerance to temperature, they are very sensitive to any

changes in pH. The pH changes after acidogenesis, when there is production of the volatile fatty acids (VFAs). A total VFA accumulation of 9 g/L-11.5 g/L has been reported to negatively affect methanogenesis [76]. An acetic acid concentration of above 2.4 g/L and propionic acid above 0.9 g/L is found to be inhibitory to the AD process [77]. The consumption of these VFAs during acetogenesis, further decreases the pH. The ideal pH for methanogens to function is in the range of 6.5-7.5 whereas that for the acidogens is 5.5-6.5 [69]. The ideal pH range for co-existence of all the AD species is 6.8-8.0 [71]. Another factor that affects the pH of the system is the presence of sulphates and sulphites. Also, the biogas hence produced has a high amount of H_2S. The sulphate reducing bacteria reduce the sulphates to sulphide by sulphate reducing bacteria (SRB) [69], [71]. It has been reported that dissolved sulphide concentrations of 100-800 mg/L are inhibitory to the AD process. The imbalance due to sulphides affects the methanogens and not the fermentative group of microbes thereby causing a reduction in methane concentrations. [60], [69], [78].

Several parameters depend on the characteristics of the feedstock. In case of organic wastes, the high amount of nitrogen is a major contributor to the alkalinity of the system [69]. Optimal C/N ratios of 20-30 are essential to the AD process [75], [79]. Of the different forms of nitrogen that are present, inorganic ammonia nitrogen in the form of free ammonia is the primary cause of toxicity to methanogens. This is mainly due to its ability to freely permeate the cell and cause an potassium imbalance [60]. It also reacts with carbon dioxide to form bicarbonate thereby increasing the pH of the system [69]. Higher C/N ratios can release small amounts of ammonia nitrogen into the system [79] thereby lowering biogas yields [80]. However, ammonia concentrations of up to 0.2 g/L have been found to be beneficial to the anaerobic digestion process. Where some strains of methanogens have been found to be more sensitive to high ammonia, some like

Methanosarcina have been found to be resistant to up to 10 g/L ammonia. This resistance is dependent on many factors, one of them being temperature. Higher temperatures result in high free ammonia concentrations thereby leading to higher chances of ammonia inhibition in thermophilic AD rather than in mesophilic AD process [81]. Another factor affecting the presence of free ammonia nitrogen is that of pH. A pH higher than 7.4 increases the amount of free ammonia nitrogen [75] It has been found that acclimatisation of the microbial population to the presence of ammonia and controlling the pH of the process can prevent ammonia toxicity [60]. Reports suggest that adaptation increased the tolerance of the microbial community up to 4 g/L total ammonia nitrogen [81].

Other factors such as the redox potential of the system is extremely important for the methanogens. Methanogens need a redox potential of -300 mV or lower in order to show optimal activity [69]. Another influencing parameter is the presence of metal ions that may affect the growth rate of the microbial population. Although some metals ions are needed for optimal cell growth processes, high amounts of these ions adversely affect the bacterial membranes and therefore are toxic [60], [69]. For instance, sodium, in the range of 0.1-0.2 g/L has a positive influence on the growth of methanogens. However, higher concentrations such as those in the range of 5.5 g/L have been reported to be toxic to the growth of methanogens [82]. One way of dealing with metal ion toxicity is the acclimatisation of the microbial community. Microbial growth is a complex amalgamation of several reactions involving the use of multiple metal ions at a time. Therefore, the effect of metal ions is also dependent on one another. It has been found that the Mg^{2+} concentration plays a vital role in determining the effect of Na^{+} at a certain threshold level [60]. On the other hand, heavy metals are severely toxic to the entire AD process [75].

Table 2: Inhibitory concentrations of components involved in the AD process

Component	**Optimal concentration**	**Inhibitory concentration**	**Reference**
C/N ratio	20-35		[50], [69]
Total VFA		9.0 g/L-11.5 g/L	[76]
Acetic Acid		>2.4 g/L	[77]
Propionic Acid		>0.9 g/L	[77]
Butyrate		>1.8 g/L	[82]
Sulphur	0.001 g/L - 0.025 g/L	0.1 g/L-0.8 g/L (dissolved sulphur) 0.05 g/L-0.4 g/L (undissociated H_2S)	[69]
Free ammonia	<0.2 g/L	>0.6 g/L	[69], [80]
Total Ammonia		4.1 g/L – 5.7 g/L	[80]
Sodium	0.1 g/L-0.2 g/L	5.5 g/L	[60]
Phenols		>1.2 g/L	[83]
Nitrophenols		1-5 mg/L	[84]
Chlorophenols		0.5 mg/L-10 mg/L	[60]
Furfural		>2 g/L	[85]
Catechol		>3 g/L	[82]

Another set of compounds that severely affect the AD process are the organics. Non-polar compounds accumulating in the system can cause membrane leakage and cell lysis. Phenolic compounds, especially, are major inhibitors due to their ability to interact with microbial membranes [86]. Compounds such as benzene derivatives, phenols, alkanes, surfactants, alcohols [60], furans and 5-HMF are microbial inhibitors and if present above the threshold limit, can conversely affect the conversion of sugars. High concentrations of phenols of more than 1.2 g/L have been shown to be detrimental to the methanogenic population [83] whereas in the case of nitrophenols the threshold is as low as 1-5 mg/L [84]. Furfural concentrations of 2 g/L and above have been shown to be inhibitory to methanogens [85]. Several methods have been explore for the detoxification of the inhibitors, including physico-chemical methods, solvent extraction and metabolic engineering [86].

2.3.4 Anaerobic Digestion of APC

APC or Aqueous pyrolysis condensate constitutes up to 50% of the pyrolysis product [87]. This low heating value liquid is high on various organic compounds like levoglucosan, organic acids phenols, furans, etc. [53]. However, the concentration of these compounds changes according to the pyrolysis parameters. The disposal of this liquid phase is a challenge due to its complex organic composition which might be toxic. This makes the APC a potential feedstock for anaerobic digestion and energy generation [15], [76], [87]. However, till date, reports of anaerobic digestion using APC as a feedstock are extremely limited, mainly due to the continuously changing parameters of the feedstock and inoculum that contribute to the complexity of the process involved.

As has been demonstrated by [50], batch experiments for the AD of corn stalk pyrolysis APC were unsuccessful due to inhibition of the biological process, with negligible methane production for over 20 days. This could not be overcome even by nutrient

addition. The final yield was found to be less than 20% of the theoretical biomethanation potential of the APC. The addition of biochar was shown to increase methane production. Another key takeaway from this study was that adaptation of the inoculum was an effective tool to mitigate APC toxicity. As opposed to the batch system, similar tests run on a continuous linked system, with biochar, achieved biogas ratios of 1:1 with a total yield near half the theoretical value [68]. In another study, APC generated from the pyrolysis of digestate, at different operating conditions, was taken and subjected to AD. It was found that high COD loadings (of 30 g/L) lowered the pH of the system thereby making AD conditions unfavourable. However, at low COD loading of 12 g/L, it was observed that most of the organic compounds had been degraded with methane yields of 220 L/gCOD was observed. This was done without any adaptation or nutrient addition [53]. The fact that adaptation is a successful strategy has been shown in other studies as well.

It was also observed that pre-treatment for the removal of toxic compounds enhances methane production [51], [52]. Strategies such as neutralisation by over-liming [51] and catalysed APC [16] have been explored. However, APC from catalytic pyrolysis did not yield high methane production. This was attributed to the formation of unknown organic compounds during the catalytic process [16]. In a batch study, it showed that the adaptation took 50-60 days in order for the process to result in the consumption of 1 g/L of acetic acid equivalent of APC. The study showed the increase in methane production when supplemented with nutrients and biochar separately, with biochar being most effective in increasing methane content. This clearly indicates that there supplementation in the form of nutrient or biochar is needed to overcome the toxicity of APC [52]. In a continuous linked process, the APC from pyrolysis of Douglas fir wood was used as a feedstock for bio-methane production using non-degassed feedstock

and activated carbon to reduce the phenolic content of the APC. Here, the APC was derived in two modes – one being the pyrolysis of biomass without any pre-treatment and the second being pyrolysis of biomass after acid wash pre-treatment. The acetic acid content of the APC varied with the temperature of the second condenser (between 36.9 g/L-45.3 g/L). An APC loading of 100 ppm gave considerable methane production. The APC resulting from the acid-wash pre-treatment biomass resulted in better bio-methane generation due to the high amount of levoglucosan content [12].

Some studies have reported that APC, at low concentrations, stimulates anaerobic microbial activity [16], [73]. This was demonstrated by utilization of the APC added as an additive for the anaerobic digestion of swine manure. The study showed that when diluted 50 times, the methane production was slightly higher than that of the control (without APC) suggesting that lower APC concentrations stimulated microbial activity whereas higher concentrations were inhibitory [73]. In another study, sewage sludge pyrolysis liquid was used for thermophilic anaerobic digestion in a cow dung matrix. Although there was clear inhibition of biogas production due to the toxicity of the APC, the addition of sewage sludge char/biochar helped overcome these effects and increase the cumulative methane yield [88]. Most studies report inhibition of biogas production at higher dosages of the APC. Even with pre-treatment processes, there is a lack of standard techniques to overcome possible inhibitors. However, one common conclusion that can be drawn is the positive effect of biochar and adaptation of the inoculum to the anaerobic digestion process [68].

3. Materials and Methods

3.1 APC characterisation

The aqueous pyrolysis condensate was produced by slow pyrolysis of soft wood at 600°C and was provided by Titan Clean Energy Projects (Craik, Saskatchewan, Canada).

3.1.1 COD and pH

The COD (chemical oxygen demand) of the APC was measured using colorimetric assay [52]. The APC samples where homogenised and diluted 1:100 for the analysis. 2mL of the diluted sample was added to the COD digestion vial (High Range, Hach, USA). The vial was mixed by inverting it gently and incubated at 150°C for 2 hours in the DRB200 reactor. After the incubation, the vial was inverted to allow proper mixing and allowed to cool completely. The resultant change in colour due to the conversion of dichromate ion to green chromic ion was recorded at 620nm in the DR 3900 reader.

The pH of the APC was measured using a pH meter (VWR Symphony SB70P).

3.1.2 CHNS Analysis

The C, H, N, S, and O content of the APC samples were determined using Thermo Flash EA 1112 series analyzer. The system was calibrated with 0.5, 1, 2 and 2.5 mg of BBOT (2, 5-Bis (5-tert-butyl-benzoxazol-2-yl) thiophene) (CE 36 Elantech, NJ, US) [89]. 1-2 mg of APC was placed with 8-10 mg of vanadium pentoxide and Chromosorb (CE Elantech, NJ, US) required to achieve complete conversion of sulphur. The analysis was performed in triplicates. Samples were combusted at 900°C in a stream of helium with a measured amount of oxygen. This produced N_2, CO_2, H_2O, and SO_2, which were then

separated and quantified by gas chromatography using a 5 mm diameter steel packed column with a length of 2 m, helium carrier gas with a flow rate of 140 mL min-1 detected with a Propack model thermal conductivity detector (TCD). The oxygen content was determined as shown in equation below, where the C, H, N, S, O and ash are mass percentages. The APC samples were dried before each analysis, thus the hydrogen and oxygen in the ultimate analysis doesn't contain the moisture. Equation 1 demonstrates the method by which oxygen % was calculated.

Equation 1- Oxygen Content

$$Oxygen\ \% = 1 - C - H - N - S - Ash \quad (1)$$

The molar ratios (H/C) and (O/C) were derived from the ultimate analysis.

3.1.3 Ammonia

The total ammonia-nitrogen was determined using a colorimetric test (MColortest™, EMD Millipore Corp., Billerica, MA, USA) based on the reaction of ammonium nitrogen to form monochloramine in the presence of a chlorinating agent [52]. This then reacts with thymol to give an indophenol derivate and form a yellow-green to green coloured solution (instead of blue; due to the reagent blank given). The sample was diluted 1:500 and the pH was adjusted to 5. The sample was then filtered using a 0.2 micron syringe filter. The assay was performed according to the test instructions.

3.1.4 Total Solids (TS), Volatile Solids (VS), Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) determination.

One of the factors affecting biogas production in anaerobic digestion is the total and volatile solids of the feedstock. The methods used for the determination of these factors are adapted from standard procedures used to analyse waste water [90]. One of the

factors affecting biogas production in anaerobic digestion is the total and volatile solids of the feedstock. The total solids and volatile solids help determine the possible efficiency of the process. The total solids define the dry matter present in the substrate. High values of total solids have been reported to negatively affect biogas production efficiency. Total solids of 9% have been found to be optimum for maximal biogas production. On the other hand, the volatile solids are the organic fraction of the total solids which is measured by burning the samples from total solids at high temperatures. The residue after the burning is that of the inorganic fraction. The weight of the inorganic fraction, removed from the dried fraction, gives the weight of the organic fraction. The TS and VS can affect the microbial community balance in the AD system [91].

Total solids and Volatile solids:

The total solids were measured by drying a known amount of sample in a laboratory oven at 105°C for 1 hour. An empty aluminium evaporating dish was weighed and 5mL of sample was added to it. The sample was incubated for 1 hour and cooled in a desiccator. This process was repeated until the weight measured was constant. The total solids were calculated according to Equation 2:

Equation 2- Total Solids

$$TS\ (g/L) = \frac{W2-W1}{Sample\ volume\ (mL)} \times 1000 \quad (2)$$

where,

W2 is the weight (g) of the dish after drying

W1 is weight (g) of empty dish

The volatile solids were measured by placing the residue from total solids in a muffle oven at 550°C for 20 minutes and cooled in a desiccator. This process was repeated

until a constant weight was reached. The volatile solids were calculated using Equation 3:

Equation 3-Volatile solids

$$VS\ (g/L) = \frac{W2-W3}{Sample\ volume\ (mL)} \times 1000 \quad (3)$$

where,

W2 is the weight (g) of the dish after TS incubation

W3 is the weight (g) of the dish after incubation at 550°C

Total suspended solids and Volatile suspended solids:

For measuring the total suspended solids, the sample was filtered using a glass fibre filter in the presence of vacuum. The glass fibre filter was washed with distilled water and dewatered prior to use. The samples were subjected to the same process as that of the total solids. The total suspended solids were calculated according to Equation 4:

Equation 4 – Total Suspended Solids

$$TSS\ (g/L) = \frac{W5-W4}{Sample\ Volume} \times 1000 \quad (4)$$

where,

W5 is the weight (g) of dish with dried sample and filter

W4 is the weight (g) of dish with pre-dried filter

The volatile suspended solids were measured by incubating the samples from TSS at 550°C for 20 minutes and cooled in a desiccator until the weight was constant. The volatile suspended solids were calculated using Equation 5:

Equation 5 – Volatile Suspended Solids

$$VSS\ (g/L) = \frac{W5-W6}{Sample\ Volume\ (mL)} \times 1000 \quad (5)$$

where,

W5 is weight (g) of dish with filter after TSS incubation

W6 is the weight (g) of the dish with filter after incubation at 550°C

3.1.5 Total phenolics

The total phenolics assay was done colorimetrically using the Folin-Ciocalteu method as defined by [92]. The samples were diluted 100 times in ethanol and filtered using a 0.2 µm syringe filter. For the assay, 1.58mL of distilled water was taken in a cuvette. To this, 20µL of the dilute sample was added along with 100µL of F-C reagent (2N) and mixed properly. After 1-8 minutes, 300µL of sodium bicarbonate (20% solution) was added and the assay mixture was left to incubate for 2 hours, at room temperature. The absorbance was taken at 765nm against a blank in a UV-Visible spectrophotometer (Thermo Scientific., Illinois, USA). The total phenolic content was calculated based on a standard curve of gallic acid (Fig A1). The value was expressed in terms of GAE (gallic acid equivalent).

3.1.6 Volatile Fatty Acid (VFA) determination and Quantification

The volatile fatty acids were identified and quantified using GC-MS and HPLC (Agilent 1260 LC) [30], [52]. For the HPLC, Agilent HiPlex H column was used at a temperature of 50°C; the samples were diluted 100 times in mobile phase (5.0 mM H_2SO_4) and run at a flow rate of 0.5mL/min for 80 minutes. The compounds were detected using a Refractive Index Detector (RID) at a temperature of 50°C.

APC samples (50 mg) were dissolved in 1 ml of 2-Propanol to obtain a concentration of 50 mg/mL, then each sample was filtered through a 0.2-micrometer filter. The GC–MS system consists of a gas chromatograph coupled to a quadrupole mass spectrometer

(GC– MS QP 2010, Shimadzu) using a capillary column (DB5MS, 30 m × 0.25 mm i.d.; film thickness, 0.25 μm). Electron ionization (EI) was used with an ion source temperature of 200°C and the interface temperature of 250°C. In EI, the instrument was used in SCAN mode initially to confirm the identity of the compounds. The GC system was equipped with a split/splitless inlet. The injector temperature was 200 °C. AOC-20S autosampler with a 10 μL syringe was used for injections of 1 μL at a rate of 10 μL s−1. The carrier gas was helium (UHP) at a constant flow of 1.5 mL min-1. The oven temperature program had an initial temperature of 40°C held for 10.0 min, rising by 10°C/min to 200°C held for 10.0 min and rising by 10 °C min-1 to 300°C, which was held for 30 min, with a total run time of 75.0 min. This temperature program was selected to provide adequate separation of the compounds of interest.

3.1.7 Pre-treatment of APC with biochar

In an attempt to remove possible inhibitors, the APC was treated with biochar prior to subjecting it to anaerobic digestion. The procedure followed was based on a standardised method for the removal of total phenolics developed in our lab. 20mL APC was taken in a beaker and the pH was adjusted to 6.0 using 1N NaOH. After this, 5g biochar was added to the pH adjusted APC, while stirring continuously. Samples were taken every 15 minutes for 60 minutes after the addition of biochar. All the samples were filtered using a 0.2μm syringe filter. The filtered samples were then analysed for total phenolics using the F-C assay (as mentioned in a previous section of this thesis). The final sample was analysed for any changes in vfa concentration using HPLC.

The biochar used in the AD experiments was obtained from the pyrolysis of the digestate. This was then activated at 800°C. The activated biochar was analysed for specific properties that could affect its activity.

3.2 Biochar Characterisation

The biochar used in the AD experiments was obtained from the pyrolysis of the digestate produced by Char Technologies at 700°C. This was then activated at 900°C. The activated biochar was analysed for specific properties that could affect its activity.

3.2.1 pH

The pH of the biochar was analysed by making a 5% mixture in distilled water. This was stirred at 160rpm for 24 hours [58]. The pH was measured using a calibrated pH meter (VWR Symphony SB70P).

3.2.2 Proximate Analysis

The biochar was ground and sieved through a 0.25 mm sieve, so as to achieve a fine powder like consistency. The method followed was as described by [93]. The samples were analysed for moisture content, volatile matter and ash content. This data was used to calculate the fixed carbon content of the biochar.

For moisture content, the porcelain crucible was ignited at 750°C for 10 minutes and dried in a desiccator before use. 1g ground sample was added to a pre-weighed and dried crucible. It was allowed to incubate at 105°C for 2 hours and then cooled in a desiccator for an hour before taking the weight. The volatile matter was assessed by burning the samples from moisture analysis, at 950°C for 7 minutes in partially covered crucibles. The samples were cooled completely in a desiccator before weighing them. The residue from the volatile content was burned at 750°C for 6 hours in uncovered crucibles. The samples were dried in a desiccator before taking the weight. The values of the above parameters were calculated using Equation 6, Equation 7, Equation 8 and Equation 9:

Equation 6- Moisture content

$$Moisture\ (\%) = \frac{A-B}{A} \times 100 \quad (6)$$

Where,

A is the amount of sample used

B is the amount of sample after drying at 105°C

Equation 7 - Volatile Matter

$$Volatile\ Matter\ (\%) = \frac{B-C}{B} \times 100 \quad (7)$$

where,

C is the weight of the sample after drying at 950°C

Equation 8- Ash Content

$$Ash\ Content\ (\%) = \frac{D}{B} \times 100 \quad (8)$$

where,

D is the grams of residue left after burning at 750°C

Equation 9- Fixed Carbon

$$FC\ (\%) = 100 - [Moisture\ (\%) + Volatile\ matter\ (\%) + Ash\ (\%)] \quad (9)$$

3.2.3 Ultimate analysis

Done as mentioned in section 3.1.2 of this thesis.

3.2.4 Pore size and surface area

Biochar samples were tested for Brunauer-Emmett-Teller B.E.T. [94] with Nova 1200e Surface Area & Pore Size Analyzer (Quantachrome Instrument, Florida, US). The tests were performing using 0.3 g of samples by nitrogen gas sorption at 77.35 K. Samples

were degassed at 105°C for 1 hour to remove moisture, then the temperature was increased to 300°C and maintained for at least for 3 hours before analysis.

3.2.5 Functional group identification by FTIR

Small portions of the samples were analysed by FTIR spectroscopy [94] using a using the Platinum® attenuated total reflectance (Pt-ATR) attachment equipped with a diamond crystal in the main box of a Bruker Tensor II spectrometer. This experimental setup allows one to analyse an area of approximately 2mm x 2mm to a depth of 0.6 – 5 microns. The spectra were collected from 4000 – 400 cm-1 with a resolution of 4 cm-1 and 32 scans. The spectra were corrected for the contribution from water vapour and carbon dioxide. Some of the spectra were baseline corrected.

3.2.6 SEM-EDX

The morphology of the biochar was analysed by SEM-EDX [94] using a Hitachi SU3500 Scanning Electron Microscope (SEM) combined with an Oxford Aztec X-Max50 SDD energy dispersive X-ray (EDX) detector. Backscatter Electron (BSE) imaging was selected to better analyse the particles, with variations in greyscale based on the average atomic number of the material. EDX is a semi-quantitative technique that can detect all elements with a minimum detection limit of approximately 0.5 wt%. A 10 kV accelerating voltage was used for these analyses. The samples were coated with a thin layer of gold to minimize charging effects.

3.2.7 Total phenolics adsorption

The biochar samples were analysed for their efficacy to adsorb total phenolics using a model phenols solution. This method was developed in our lab. For this, a 35 g/L phenol solution was made. 20 mL of this solution was added to a beaker and kept on a magnetic stirrer. To this, 5 g of biochar was added while continuously stirring. Samples were

taken every 15 minutes for 2 hours. All the samples were filtered using a 0.2micron syringe filter. The samples were diluted 1:100 in ethanol and assayed for total phenolic content using the method described previously in this thesis.

3.3 Anaerobic Digestion Experiments

The anaerobic digestion experiments done in this study did not use any additional nutrients in an attempt to force the microbial culture to use the substrate of interest. Also, no buffer system was added since studies have found that the inoculum is enough to buffer the acidity of the APC [50].

3.3.1 AD Inoculum characterisation

The inoculum for anaerobic digestion was procured form Stormfisher, London, Ontario, where it is employed in a single stage mesophilic anaerobic digestion of organic food waste fractions. The pH of the inoculum was measured using a pH meter (VWR Symphony SB70P). The inoculum was degassed for a month before setting up experiments that needed degassed cultures (to rule out any background gas production). For some set ups, non-degassed inoculum was used as required (Table 3).

COD (Chemical Oxygen Demand)

The COD (chemical oxygen demand) of the degassed inoculum was measured using colorimetric assay [52]. The inoculum samples where homogenised and diluted 1:100 for the analysis. 2mL of the diluted sample was added to the COD digestion vial (High Range, Hach, USA). The vial was mixed by inverting it gently and incubated at 150°C for 2 hours in the DRB200 reactor. After the incubation, the vial was inverted to allow proper mixing and allowed to cool completely. The resultant change in colour due to the conversion of dichromate ion to green chromic ion was recorded at 620nm in the DR 3900 reader.

TS, VS, TSS, VSS

The total and volatile solids as well as the total suspended and volatile suspended solids were measured according to section 2.2.7 of this thesis.

3.3.2 Adaptation of inoculum

Several reports have suggested that adaptation is an effective method for increasing microbial tolerance to the presence of inhibitors, thereby improving biogas yields even at theoretically unfavourable conditions [60], [62]. The degassed AD inoculum was adapted to the presence of 3% APC (1.24 g/L acetic acid equivalent). The concentration was chosen on the basis of previous experiments done to establish optimum APC concentration tolerated by the inoculum with biogas production.

3.3.3 APC AD - Adapted vs non-adapted inoculum

The biogas production and substrate consumption of adapted and non-adapted inoculum was studied at different APC loadings. To set up the experiment, 1%, 3%, 5% & 7% APC was added to give final acetic acid concentration in the anaerobic digestion mixture of 0.43 g/L, 1.24 g/L, 2.14 g/L and 3.0 g/L respectively. The volume was made up using degassed inoculum leaving a headspace of 25% of actual bottle volume (on day 0). For non-degassed inoculum, APC equivalent to 1 g/L and 2 g/L was added to each bottle and the volume was made using non-degassed inoculum leaving a headspace of 25% of actual bottle volume (on day 0). In case of adapted inoculum (for degassed and non-degassed) the inoculum contained 10% (v/v) of adapted inoculum. The bottles were sparged with nitrogen until the oxygen was completely replaced. All the bottles were sealed with butyl rubber stoppers and aluminium crimps, and incubated at 37°C.

Gas analysis and acid consumption patterns were analysed once a week, by GC (Agilent) and HPLC (Agilent) respectively. Pressure was recorded using a pressure

meter (Keller Mano Leo 1) before extracting gas samples. After multiple sampling, the rubber stoppers and aluminium crimps were replaced to avoid any gas leaks. All set-ups were done in triplicates.

3.3.4 AD in the presence of biochar treated APC

Anaerobic digestion experiments were set-up using APC that was pre-treated with biochar. The APC pre-treatment was done as mentioned in previous section 2.2.8 of this thesis. The experimental set-up was done similar to the others where 1%, 3%, 5% & 7% APC was added to give final acetic acid equivalents of 0.428 g/L, 1.24 g/L, 2.14 g/L and 2.996 g/L respectively. The final volume was made up with non-adapted inoculum such that the headspace in each bottle was 25% of the actual bottle volume, on day 0. All the bottles were sparged with nitrogen, sealed with butyl rubber stoppers and aluminium crimps. The incubation was done at 37°C.

Gas analysis and acid consumption patterns were analysed once a week, by GC (Agilent 7820A) and HPLC (Agilent) respectively. Pressure was recorded using a pressure meter (Keller Leo 1) before extracting gas samples. After multiple sampling, the rubber stoppers and aluminium crimps were replaced to avoid any gas leaks. All set-ups were done in triplicates.

3.3.5 APC AD with in-situ Biochar

To analyse the effect of biochar on biogas production, the degassed inoculum was fed with 1 g/L, 2 g/L and 5.5 g/L acetic acid equivalent APC. Biochar obtained from the pyrolysis of the digestate was added to the inoculum in different loadings with 1:1 biochar:APC ratio, and 0.3:1 biochar:APC ratio -based on the total phenolic removal assay. The final volume was made up such that the headspace in each bottle was 25% of the actual bottle volume, on day 0. All the bottles were sparged with nitrogen, sealed

with butyl rubber stoppers and aluminium crimps. The incubation was performed at mesophilic temperature regime, 37°C. The samples were analysed for gas production and substrate utilisation at regular intervals. Also, the bottles were supplemented with respective acetic acid equivalents of APC after monitoring acetic acid consumption during AD.

Gas analysis and acid consumption patterns were analysed every seven days, by GC (Agilent) and HPLC (Agilent) respectively. Pressure was recorded using a pressure meter (Leo 1, Keller America) before extracting gas samples. After multiple sampling, the rubber stoppers and aluminium crimps were replaced to avoid any gas leaks. All the experiments were run in triplicates.

Table 3: General experimental set-ups used

Inoculum type	**APC (%)**	**APC (acetic acid equivalent) (g/L)**	**Biochar (g)**
Degassed; Non-adapted	1	0.428	-
Degassed; Non-adapted	3	1.28	-
Degassed; Non-adapted	5	2.14	-
Degassed; Non-adapted	7	2.996	-
Degassed; Adapted	1	0.428	-
Degassed; Adapted	3	1.28	-
Degassed; Adapted	5	2.14	-
Degassed; Adapted	7	2.996	-
Degassed; Non-adapted	7	1.495	Pre-treatment
Degassed; Non-adapted	9	1.926	Pre-treatment
Degassed; Non-adapted	11	2.35	Pre-treatment

Degassed; Non-adapted	13	2.78	Pre-treatment
Degassed; Non-adapted	Acetic acid	2.99	
Degassed; Non-adapted	Acetic acid	3.85	
Degassed; Non-adapted	Acetic acid	4.7	
Degassed; Non-adapted	Acetic acid	5.56	
Non-Degassed; Non-adapted		1	-
Non-Degassed; Non-adapted		1	0.3
Non-Degassed; Non-adapted		1	1
Non-Degassed; Non-adapted		2	-
Non-Degassed; Non-adapted		2	0.6
Non-Degassed; Non-adapted		2	2
Degassed; Non-adapted		5.56	1.6

3.3.6 Biogas measurements and quantification

Biogas samples were taken using a gas tight syringe (Dyna medical corporation, London, Ontario). Before sampling gas, the pressure was recorded for each bottle using a pressure metre (Leo 1, Keller America) as shown in Figure.1. The moles of gas were calculated using ideal gas law taking into consideration the changing headspace volume after each sampling. The gas samples (10mL) were injected manually into the GC. The method used was calibrated for the detection of nitrogen, methane and carbon dioxide using a TCD (Thermal Conductivity Detector). The front detector temperature was 300°C and back detector temperature was 250°C with a front inlet temperature of 250°C. The oven temperature was 185°C. Helium was used as a carrier gas [52].

Figure 3.1: Pressure Measurement

4. Results and Discussion

4.1.1 APC Organic Content Characterisation

Table 4 lists the chemical characteristics of the APC. The pH of the APC was 2.78, clearly indicating the acidic nature of the liquid. High COD (a measure of organic oxidizable content of waste samples) indicates that the liquid is high on organic matter and therefore not safe for disposal without adequate pre-treatments [16]. Due to the variability of the pyrolysis process and the feedstocks used, the COD content of APC is known to vary in the range of 30-300 g/L [16]. The organic content of the APC in this study was measured by the COD -141.2 g/L, TS which was 22.6 g/L of which 96.2% was found to be VS. The TSS was 9.1 g/L of which 82.9% was VSS fraction. The values of total and volatile solids indicate that bioremediation via anaerobic digestion is a possible treatment for energy generation from APC. The COD in this study is much higher than the COD of APC derived from digestate, which was 74.3 g/L [53] and similar to the COD of APC derived from commercial biosolids, which showed positive results for methane production capability [16]. On the other hand, the APC derived from birch bark pyrolysis and corn stover reportedly had extremely high COD of 499 g/L [52] and 486 g/L [51] respectively, and yet showed possibility of biogas generation at high dilutions.

However, there are other factors such as the high C/N ratio present a challenge towards efficient AD for the production of biogas [68]. The optimal C/N ratio for anaerobic digestion is known to be between 20-30 [75], [79]. The C/N molar ratio in this case was found to be 46.42, which is much higher than the optimal range for anaerobic digestion but lower than the C/N molar ratio of 73:1 as reported in another similar study [50]. This is close to that of the APC derived from corn stover [51] and the APC of birch bark pyrolysis [52], indicating a similarity in APC derived from wood. The ammonia-N

concentration was found to be 80 mg/L which is known to be inhibitory to most mesophilic anaerobic digestion systems [95]. However, some studies report that a similar amount of ammonia-N could also be beneficial to the anaerobic digestion process, especially in adapted systems [81].

Table 4: Chemical characterisation of APC

Property	**Quantification**
COD	141.2 g/L
pH	2.78
TS	22.6 g/L
VS	21.75 g/L
TSS	9.1 g/L
VSS	7.55 g/L
C (%)	3.9
H (%)	10.14
N (%)	0.1
S (%)	0.05
O (%)	85.81
C/N	46.4
Ammonia-N	80 mg/L
Total Phenolics	33.18 g/L GAE

4.1.2 VFA Identification and Quantification

The APC is a very complex liquid, with varying composition according to reaction conditions and feedstock [96]. Therefore, it is important to identify and quantify the compounds present in order to determine the possible inhibitors and substrate components of the APC. GC-MS and HPLC were performed to identify and quantify

the compounds present in the APC. Table 5 lists some of the main inhibitory compounds identified using GC-MS. It is clear that even though the APC is mostly aqueous, it has a complex organic composition including acids, alcohols, ketones, furans and phenols, most of which are AD inhibitors [60], [83]–[85]. Figure 4.1.1 shows the quantification of the main components of the APC. The amount of acetic acid in the APC was 42.8 g/L, which indicates a suitability for methane generation. However, the concentration of organic inhibitors is higher than the threshold (Table 2) and the acetic acid concentration needs to be suitably diluted for optimal anaerobic digestion.

The acetic acid concentration in this study was almost half of the amount reported for APC derived from bark pyrolysis which was about 100 g/L [52], higher than the reported for APC generated from pyrolysis of anaerobic digestate 9.2 g/L [53], commercial bio-solids 29 g/L [16], corn stalk pellets – 26 g/L [50] and corn stover – 28.98 g/L [51]. APC as a feedstock for anaerobic digestion, however, it may need a combination of pre-treatment processes such as major dilutions and biochar addition. In addition, the APC in this study consists of a considerable amount of levoglucosan of 27.62 g/L, a main product of cellulose degradation, thereby making it more suitable for AD process [86]. The feedstock in this study was woody biomass which is high on lingo-cellulosic compounds.

Table 5 - Some main inhibitor compounds identified by GC-MS

% Area	**Component**
36.31	Acetic Acid
7.73	7-keto Lithocholic Acid
6.69	Propionic Acid
4.55	Catechol

2.2	4-methyl Catechol
2.06	Phenol
1.93	Iso valeric acid
1.81	3-methyl phenol / meta Cresol
1.48	2-methyl phenol / Cresol
1.47	5-HMF
1.18	6-oxo Heptanoic Acid
1.07	Lactic Acid
0.7	Acetol
0.73	Butyric Acid
0.71	4-Ethyl catechol
0.69	gamma-Hydroxybutyric acid
0.58	3-methyl catechol
0.57	Hydroquinone
0.43	5-Methylfurfural
0.42	Hydroxy toluene
0.36	2-hydroxy tetrahydrofuran
0.33	Furfural
0.3	Iso valeric acid

The hydrolysis of pentoses followed by dehydration of the pentoses to furan-ring containing compounds [97] is the reason for relatively high amount of furans in the APC. However, sulphur reducing bacterial population is known to degrade the furans to acetic acid which is then consumed by the methanogens for the production of methane [97]. Studies have shown that anaerobic digestion is able to mineralize cresols,

catechol, 5-HMF, furfural and other furans, which are present in the APC. However, to avoid an inhibited AD the microbial consortia requires a process of adaptation [98] or the concentration of such substances should be under the threshold limit (Table 2) [83]. These compounds are known to inhibit the fermentation step of biogas production [99].

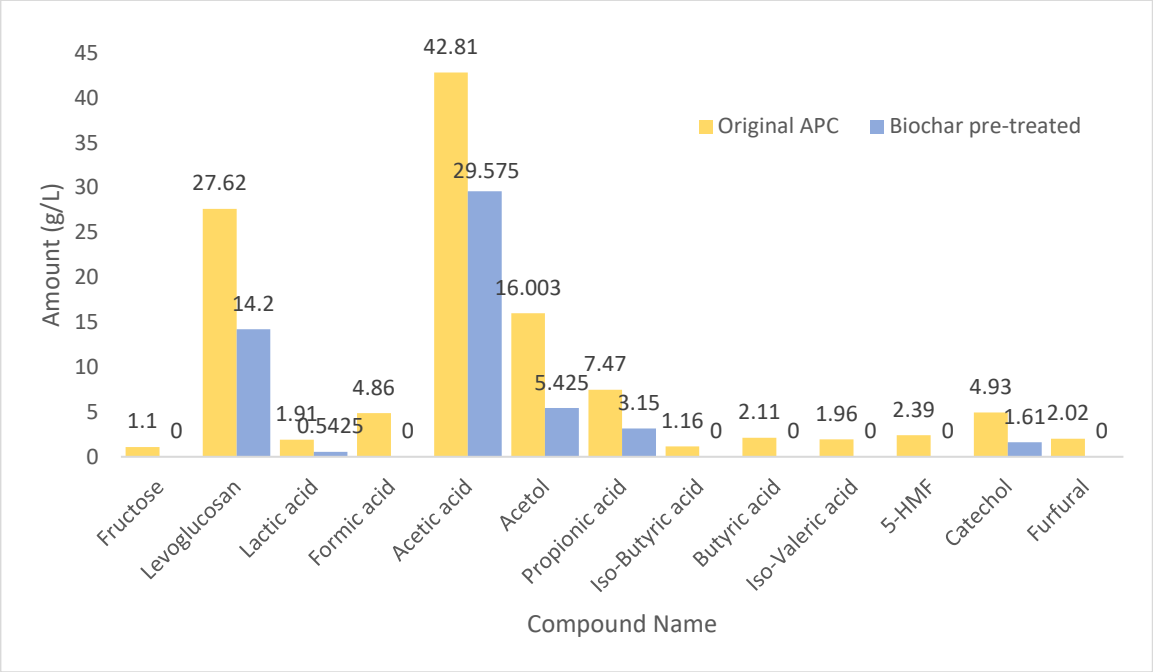

Figure 4.1: HPLC Quantification of selected compounds of APC

4.1.3 Total Phenolics Estimation

Phenols are compounds derived from the breakdown of lignin and are known inhibitors of anaerobic digestion process. A total phenol concentration of 1.2 g/L is known to be inhibitory to methanogens [83]. They inactivate enzyme systems and alter the permeability of the cell membrane [51]. The total phenolics were measured in terms of gallic acid equivalent by a spectorophotometric assay. For this a standard curve was generated (Figure A1) using gallic acid standard solutions (50 mg/L – 500 mg/L). The R^2 value of the standard curve was 0.9922. The total phenolic concentration of the APC was found to be 33.18 g/L GAE (gallic acid equivalent) (Table 4) which is high due to the lignin content of the feedstock used for APC generation. The phenolic content in

this study is higher in comparison with other studies – 25.5 g/L GAE [52] and 17g/kg [50] indicating a high chance of phenolic inhibition of the process.

4.1.4 Pre-treatment of APC with biochar

Literature reporting the pre-treatment of APC using biochar is unavailable. The biochar was used as a pre-treatment to observe the effects it has in the inhibitors, and also was used as a support for microorganism attachment. In this section we are discussing its use as a pre-treatment to lower the concentration of total phenolics and other organic inhibitors. The process followed was similar to that of removal of total phenolics. For the process to be efficacious, pH was adjusted using 1M NaOH. For 20mL of APC, almost 15mL of 1M NaOH was required to bring the pH to about 7. This gave an APC:NaOH ratio of 1:1.75 resulting in about 50% dilution of the APC. The diluted, pre-treated APC was analysed for change in concentration of major components (Figure 4.1.1). The process resulted in removal of almost 80% of the phenolics and other inhibitors. Although there was removal of inhibitors, the treatment also reduced the amount of levoglucosan and acetic acid which are important to the anaerobic digestion process.

4.2 Biochar Characterisation

Biochar properties are greatly affected by the origin of the pyrolysis feedstock [100] as well as pyrolysis conditions [101]. Some studies found that higher temperatures decrease the yield and affect the surface area properties of the biochar. The efficacy of a biochar is dependent on factors such as pore size, aromaticity, surface area, ash content, pH which in turn affect the impact of the biochar on the anaerobic digestion process [58].

4.2.1 pH

The pH of the biochar used in this study was found to be 7.2. Although this is not as alkaline as some reports of anaerobic digestate derived biochar [101], it is similar to that found in another study where biogas production residues were pyrolyzed at different temperatures. It was proposed that in some samples, the pH decreased after reaching a maximum at a temperature of 600°C, due to the reduction in content of alkali metals [100]. It is possible that the high production temperature of 750°C caused a similar effect in this case.

4.2.2 Proximate and Ultimate analysis

Proximate analysis was done to determine the moisture content, volatile matter and ash content of the biochar used (Table 6). It was found that the biochar had a very high ash content of 65% whereas the moisture content was just 0.608%. The biochar was found to have a low volatile matter content as well. This could be attributed to the high temperatures used of the production of the biochar. A similar effect was found in biochar produced from digestate. [100]. The high ash content of the biochar could make it a source of nutrients as well as liming effect (for maintaining the pH of the system) thereby facilitating methane production [102] if used in appropriate loading [58]. High ash content prevents the formation of aromatic structures thereby reducing considerably the fixed carbon.

The digestate biochar was reported to have a low fixed carbon of 24%. The fixed carbon however, cannot necessarily be considered as the sole reason since it is highly dependent on the ash content and volatile matter content which in turn may vary due to the heating rate [103]. This pattern was observed for digestate food waste biochar which had high ash content and low fixed carbon. Fixed carbon is higher in biochars derived

from feedstocks high on lignin content [104], [105]. Usually the fixed carbon is lower than the total carbon content. However, in this case the total carbon is slightly lower. This could be due to the presence of heteroatoms (sulphur, nitrogen, oxygen among others) which sometimes constitute the weight of the fixed carbon in proximate analysis methods [103]. The sulphur content of the biochar is also relatively high due to its origin.

The O/C molar ratio, which is dependent on temperature and indicates the stability of the biochar. O/C ratios of lower than 0.2 ascertains that the biochar will have a half-life of at least 1000 years [54]. However, the O/C molar ratio of the biochar in this study was found to be 0.411 indicating lower stability, high levels of oxidation and high polarity [106]. This is also confirmed by the low fixed carbon content of the biochar. The molar H/C and O/C ratio correlation is unique to each biochar due to the variation in the feedstocks and pyrolysis conditions [107]. The H/C ratio reflects the aromatic nature of the biochar and decreases with increasing temperature. This is because higher temperatures increase the loss of volatile hydrocarbons thereby reducing the ratio. It also helps determine the carbon storage value of the biochar and the level of fused aromatic ring structures. Low H/C and O/C ratios indicate high hydrophobicity. This property has been shown to improve the CO_2 sequestration capacity of the biochar [106]. A low H/C ratio is seen when the biochar is high on aromatics. The H/C ratio of the biochar in this study was found to be 0.32 which is relatively low due to the feedstock and high temperature of production used [58], [100], [105]. The H/C was in agreement to the ratio found in food waste digestate biochar which was produced at 700°C [104]. In biogas residue biochar made at 800°C, similar H/C molar ratios were found. However, biochar produced at lower temperatures showed higher H/C molar

ratios indicating the influence of temperature on the elemental ratios of a component [101].

Table 6: Properties of biochar

Property	Quantification
pH	7.2
Moisture (%)	0.608
Volatile Matter (%)	10.08
Ash Content (%)	65.23
Fixed Carbon (%)	24.097
C (%)	19.77
H (%)	0.53
N (%)	0.66
S (%)	2.96
O (%)	10.85
H/C	0.321
O/C	0.411

4.2.3 Pore size and surface area

During activation, the biochar is heated at extremely high temperature to vaporize the volatiles thereby increasing the pore size ad surface area [94]. The surface area is known to be affected by feedstock used and the pyrolysis conditions of biochar production. Feedstock high on lignin provides a better structural support thereby resulting in higher surface area [105]. The total pore volume of the biochar in this study was found to be 0.11cc/g which was smaller than that reported for biochar from food waste digestate [104]; the pore radius was $18.2e^{-10}$m. The BET surface area was found to be $128.18 m^2/g$,

which is in agreement with the findings of biochar produced from biogas production residues at high temperatures and the range found in literature.

The feedstock used for biogas production and the operating temperature are known to have an effect on the biochar surface properties [100]. The higher BET surface area can be attributed to the high temperatures of production [94]. The surface area of the biochar in this study is lower than the surface area of commercially available biochars but much higher than that reported for other biochars of similar origin [101], [104]. Relatively low surface areas (in comparison to commercial ones) may be due to the greater graphitisation of carbon in the presence of elements found in ash (Si, Fe, K). Furthermore, blocking and cracking of pores due to high temperatures during the biochar preparation process can result on lower surface area of the biochar. The relatively high surface area may provide a suitable environment for microbial communities to grow [100], [105].

4.2.4 FTIR

The FTIR spectra (Figure 4.2) is in agreement to that found in other biochars produced from pyrolysis of digestate at high temperatures [100], [101]. Peaks at ~775.4 cm^{-1} shows C=C bending and the presence of alkene compounds. It also suggests the presence of benzene derivatives or aromatic compounds. Peaks between ~1200 and ~1370 indicate the presence of aromatic amines due to C-N stretching and aromatic esters due to C-O stretching. Bands between ~1050 and ~1000 are indicative of silica and $CaCO_3$ which in agreement with the high ash content of the biochar. Bands at ~1110 suggest the presence of phosphates [100]. Peak at ~1479.2 and ~1515 shows C-H bending which corresponds to various aromatic ring mode and alkanes [94], [104]. These are similar to the peaks reported for biochar derived from gasification of switch grass and corn stover at 760°C [94]. Strong peaks between ~1021 and ~1118 show C-

O stretching and ~1440 to ~1395 indicate O-H stretching that corresponds to carboxylic acid. Peaks between ~1085 and ~1050 indicate primary alcohols, and ~1079 indicate secondary alcohols.

The high temperatures used, for the production of the biochars from biogas production digestate, is known to increase the aromaticity and lower the H/C ratio of the biochar [100]. This is now confirmed by the FTIR spectra as well as the ash content analysis of the biochar in this study. However, the variability of feedstocks and pyrolysis conditions used prevents having a standardised output for all biochars.

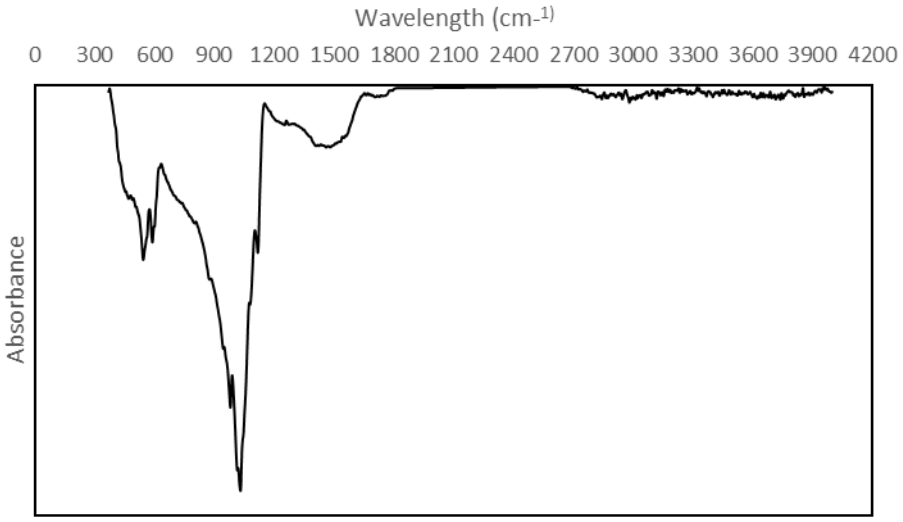

Figure 4.2: FTIR spectra for digestate biochar

4.2.5 SEM

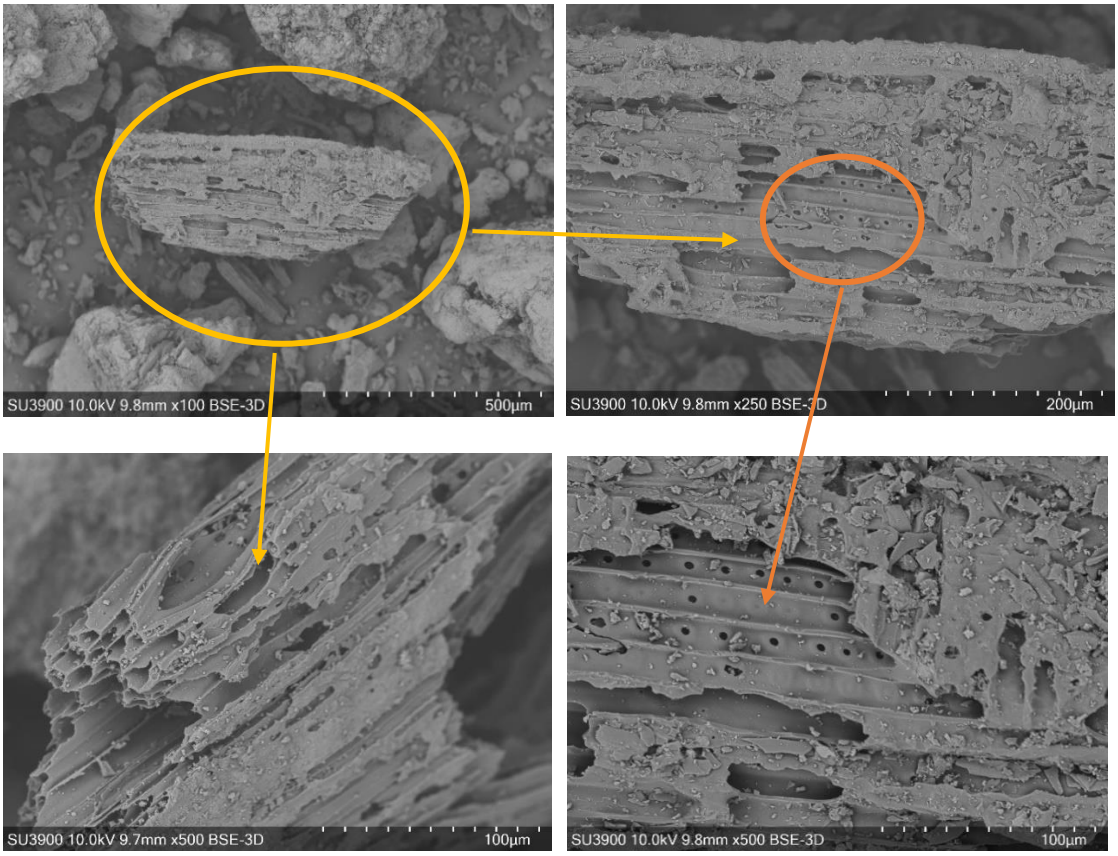

Figure 4.3: SEM analysis of digestate biochar. Images are taken at 100X, 200X and 500X magnification.

SEM was used to study the surface morphological characteristics of the biochar, Figure 4.3 shows images of different magnifications of a biochar sample. As it can be seen, the biochar has pores of different sizes. An increase in in pore size and surface area happens due to the loss of volatile matter [106].

4.2.6 Total Phenolics absorption

The biochar was used for the absorption of phenolic inhibitors. As can be seen in Figure 4.4, 82% of the total phenolics were absorbed after a treatment of 60 minutes. The porous structure of the biochar contributes to the absorption mechanism. The phenolic molecular size and diameter is much smaller, in the range of 0.5-0.4 nm [108], than the

pore size of the biochar used here. This could be a possible explanation for the absorption of phenolic molecules into the pores of the biochar. Other methods such as over-liming and use of commercially activated carbon have been used where over-liming was shown to be effective in removal of phenolic inhibitors. However, the activated carbon removed less than 50% of the phenolic inhibitors [62], [86].

In another attempt, lab generated activated carbon was used to study the mechanism of absorption. It was stated that phenolic absorption on activated chars can take place through electron donor-acceptor complexes, Van der Waals interactions or π-π interactions of the phenol ring and graphene layer of the biochar [108]. Although porosity is an important factor, there is a lack of research on the correlation between pore size and adsorption capacity. The exact mode of phenolics removal in this case needs to be further investigated.

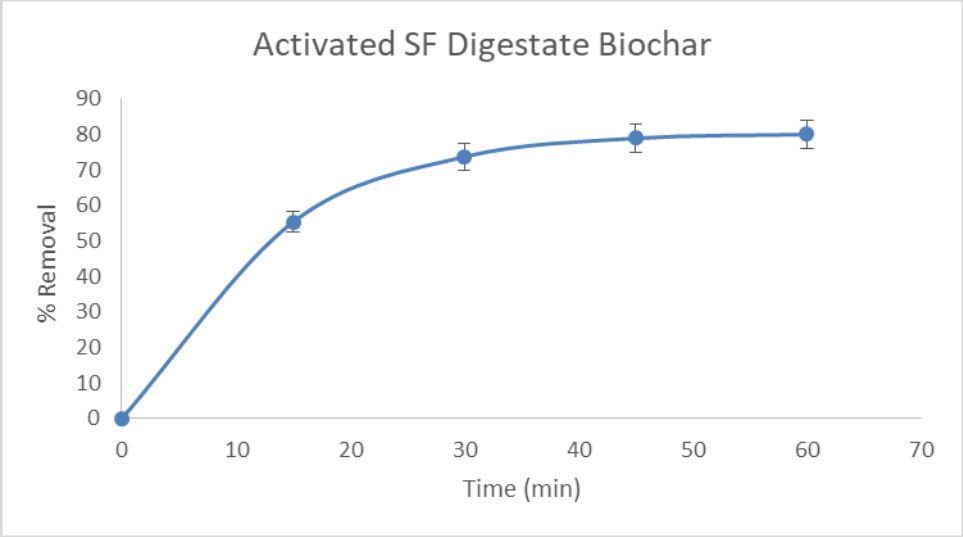

Figure 4.4 - Absorption of phenolics using biochar

4.3 Anaerobic Digestion of APC

4.3.1 AD inoculum Characterisation

The AD inoculum was characterised before setting up the digestion experiments (Table 7). The VS of the inoculum was found to be 55% of the TS whereas the VSS was 84.4% of the TSS. This is similar to the values observed in other studies [50], [52], [53] but slightly lower than the one found for inoculum used in AD of APC derived from corn stover pyrolysis [51] . However, it is important to note that the AD inoculum is dynamic in nature thereby changing these values with time. The pH of the inoculum was ideal for methanogenesis [71] and similar to the pH of the inoculum used for AD of APC derived from digestate pyrolysis and corn stover pyrolysis [51], [53] .

Table 7: AD Inoculum characteristics

Property	Quantification
COD	20.4 g/L
pH	7.58
TS	14.9 g/L
VS	8.2 g/L
TSS	9.3 g/L
VSS	7.85 g/L

4.3.2 AD with degassed inoculum: Adapted vs Non-adapted

Studies have shown that methanogens have very slow growth rates. In addition, acetic acid concentrations higher than 1mM are known to favour the growth of *Methanosarcina* which has a faster doubling time of 1-2 days. Lower concentrations favour *Methanoseata* which has a doubling time of 7-9 days [69]. The acetic acid

concentrations used in this study are in the range of 8mM to 47.5 mM, which is much higher than the optimal concentrations that favour the growth of methanogens.

Whereas most studies focus on the quanititive production of biogas, this study focuses on the qualitative aspect of biogas production. The anaerobic digestion for biogas prodution was assessed for different parameters. The degassed inoculum was acclimatised to the substrate and the biogas production pattern was studied. Figure 4.5 shows a comparison of the qualitative biogas production for adapted and non-adapted in the presence of different APC loadings. The results have been compared to the biogas produced by degassed inoculum in the absence of APC. For concentrations of 0.5 g/L acetic acid equivalent and 1.24 g/L acetic acid equivalent, the adapted inoculum had higher methane concentrations than the non-adapted. In case of 0.5 g/L, the methane concentration decreases for adapted inoculum after 14 days due to the complete consumption of the acetic acid (Figure). A similar trend can be oserved at higher concentration of 1.24 g/L acetic acid equivalent for adapted and non-adapted inoculum. Adapted inoculum had a 106% more methane content after 21 days of incubation when fed with 1.24 g/L acetic acid equivalent of APC. At 2.14 g/L acetic acid equivalent, there is almost no biogas production with non-adapted inoculum. In comparison, the adapted inoculum biogas ratios were high at 2.5 with a 92% more in methane content. A continuous linked Py-AD system for the degradation of aqueous phase derived from the slow pyrolysis of pine wood reported an approximate pyrobiogasratio of 1 after 120 days of AD, in the presence of 2% vfa [68]. In comparison, the biogas ratios obtained here are higher.

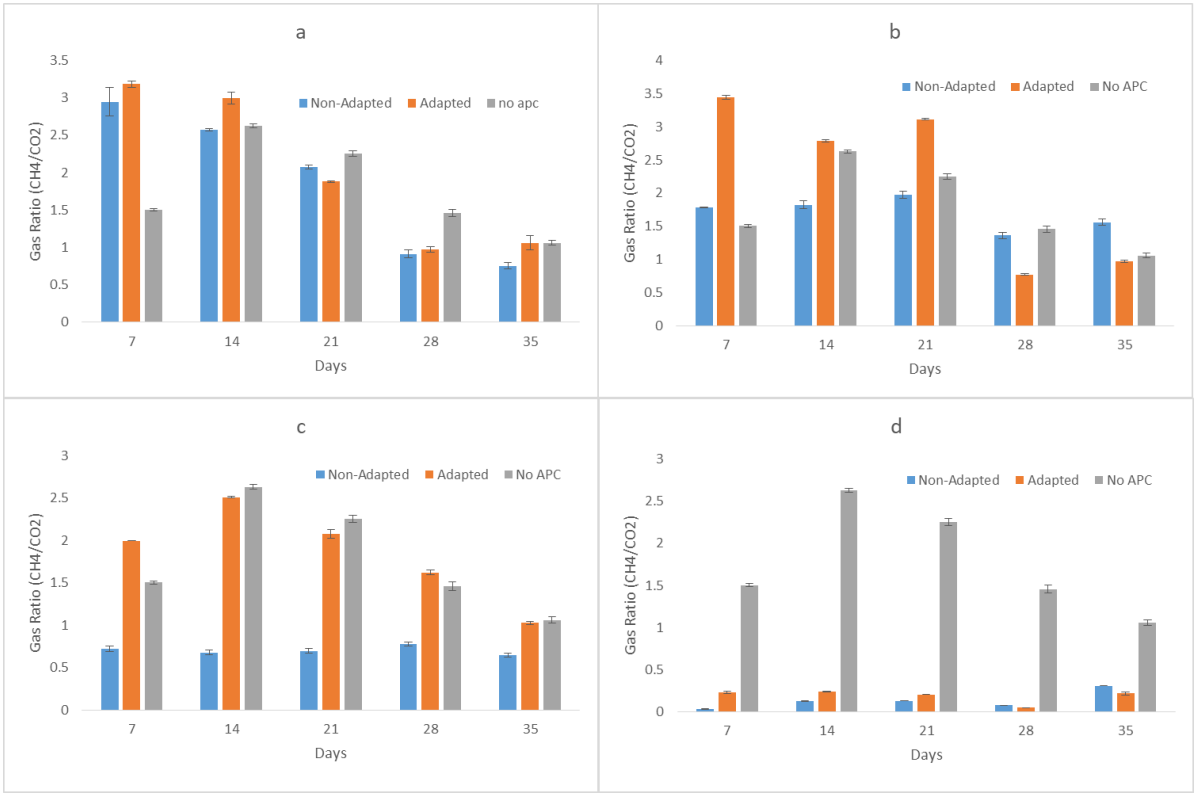

Figure 4.5: AD performance with degassed inoculum - adapted vs non-adapted. (a)1%APC - 0.5 g/L acetic acid equivalent; (b) 3% APC - 1.24 g/L acetic acid equivalent; (c) 5% APC - 2.14 g/L acetic acid equivalent; (d) 7% APC - 3.0 g/L acetic acid equivalent.

When the acetic acid equivalent was increased to almost 3 g/L, the biogas production nearly ceased for adapted and non-adapted cultures indicating inhibition due to other APC components. The gas production patterns at lower APC concentrations suggest that the APC actually stimulates the methanogen population when fed at low concentrations. This is in agreement with previous studies where APC is used as an additive for the anaerobic digestion of swine manure [73] However, at higher concentrations, the gas ratio for adapted and non-adapted inoculum are lower than the inoculum with no substrate. For adapted inoculum, this could be because the concentration was more than double of that used for adaptation of the inoculum. This

might have lead to the accumulation of AD inhibitors at concentrations for which the inoculum was not acclimatised.

Whereas 95% of the acetic acid is consumed within the first 15 days in case where the APC loading is 0.5 g/L acetic acid equivalent with a methane content of 74.6% in the first 7 days, concentrations higher than 1.24 g/L acetic acid equivalent almost cease biogas production with the methane content dropping to 40% with 2.14 g/L acetic acid equivalent. The patterns suggest an accumulation of acetic acid and propionic acid indicating the inhibitory effect on methanogens and acetogens respectively. Acetogens are the group of bacteria that breakdown the vfa's such as propionate to acetate for methanogenesis to take place [69], [71]. An accumulation of vfa's is a clear indication of the concentrations being too high for the non-adapted inoculum thereby inhibiting the entire process.

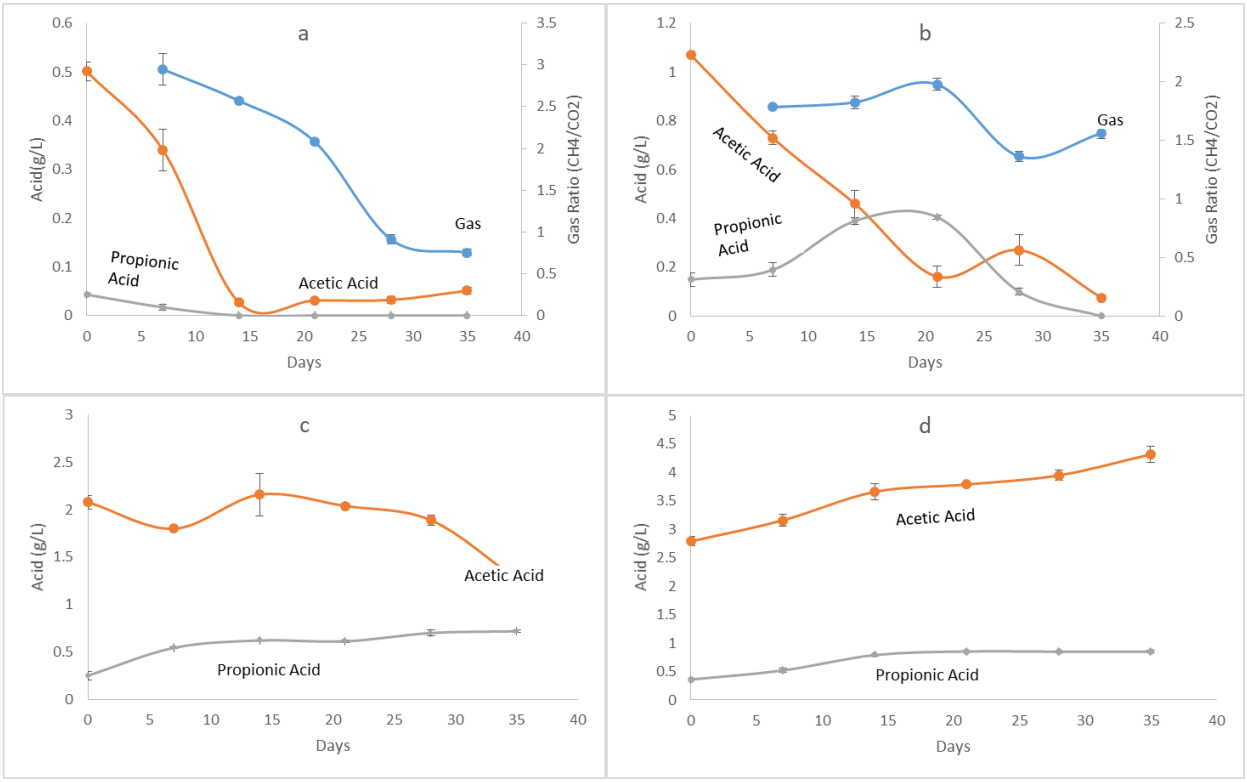

Figure 4.6: AD performance with degassed inoculum in the presence of non-adapted inoculum. (a)1%APC - 0.5 g/L acetic acid equivalent; (b) 3% APC - 1.24 g/L acetic acid equivalent; (c) 5% APC - 2.14 g/L acetic acid equivalent; (d) 7% APC - 3.0 g/L acetic acid equivalent.

On the other hand, adaptation of the inoculum to the presence of APC has shown to have a positive effect on the AD process. Figure shows the biogas production and acid consumption patterns of adapted inoculum. The adapted inoculum utilised all the acetic acid within the first 15 days at lower concentrations of 0.5 g/L acetic acid equivalent, resulting in 76% methane content in the first 7 days of biogas production. This was comparable to the concentrations with non-adapted inoculum. However, at higher concentrations of 2.14 g/L acetic acid equivalent, adapted inoculum had the highest methane content of 71.4% within the first 15 days of incubation.

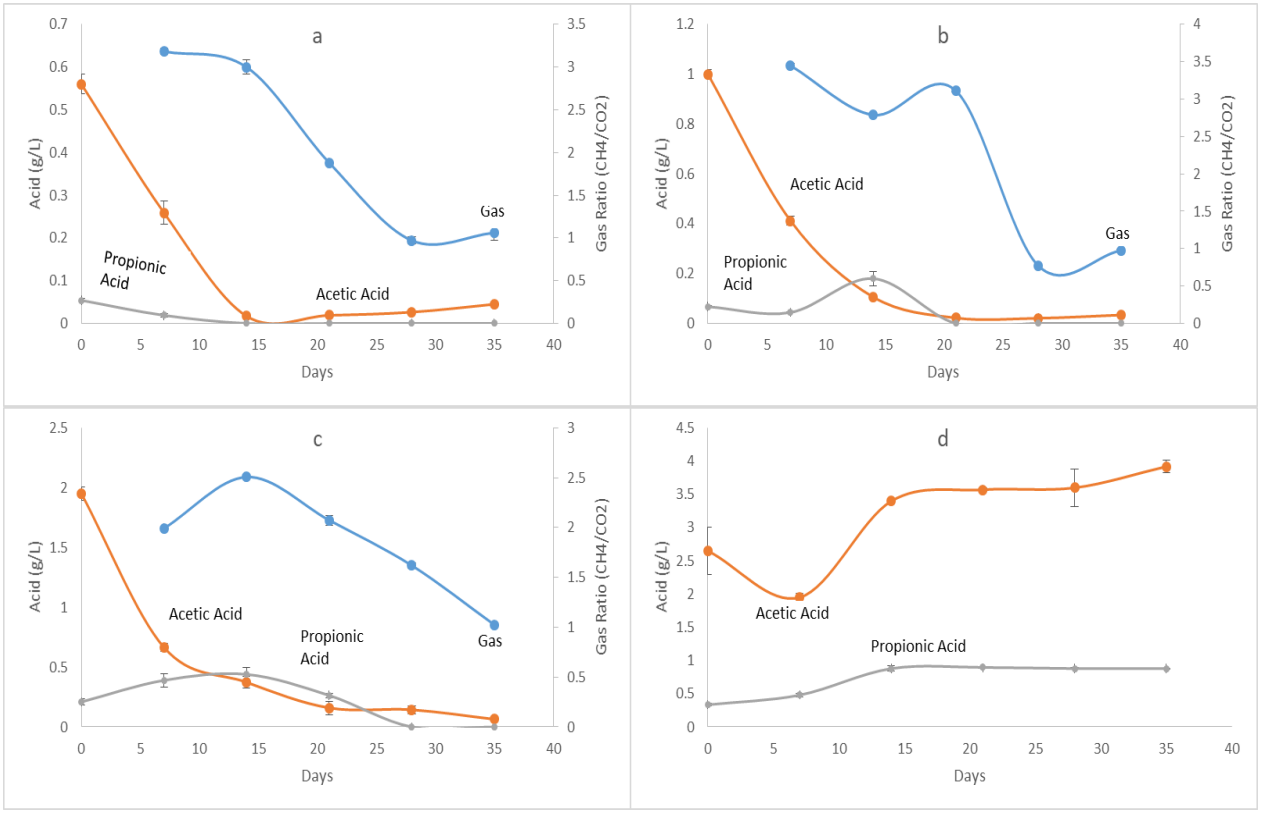

Figure 4.7: AD performance with degassed inoculum in the presence of adapted inoculum. (a)1%APC - 0.5 g/L acetic acid equivalent; (b) 3% APC - 1.24 g/L acetic acid equivalent; (c) 5% APC - 2.14 g/L acetic acid equivalent; (d) 7% APC - 3.0 g/L acetic acid equivalent.

Subsequent decrease in methane production after a peak shows might be due to the accumulation of propionic acid indicating loss of acetogenic activity. The accumulation of acids thereafter might have altered the pH of the system along with accumulation of various inhibitors which were not broken down [75], [76]. Although reports suggest that acetic acid concentrations of above 2.4 g/L completely inhibit the AD process [77], adaptation of the inoculum might be an effective method to overcome this inhibition. The results observed here are in agreement with literature findings [51], [60].

4.3.3 AD with degassed non-adapted inoculum in the presence of biochar pre-treated APC

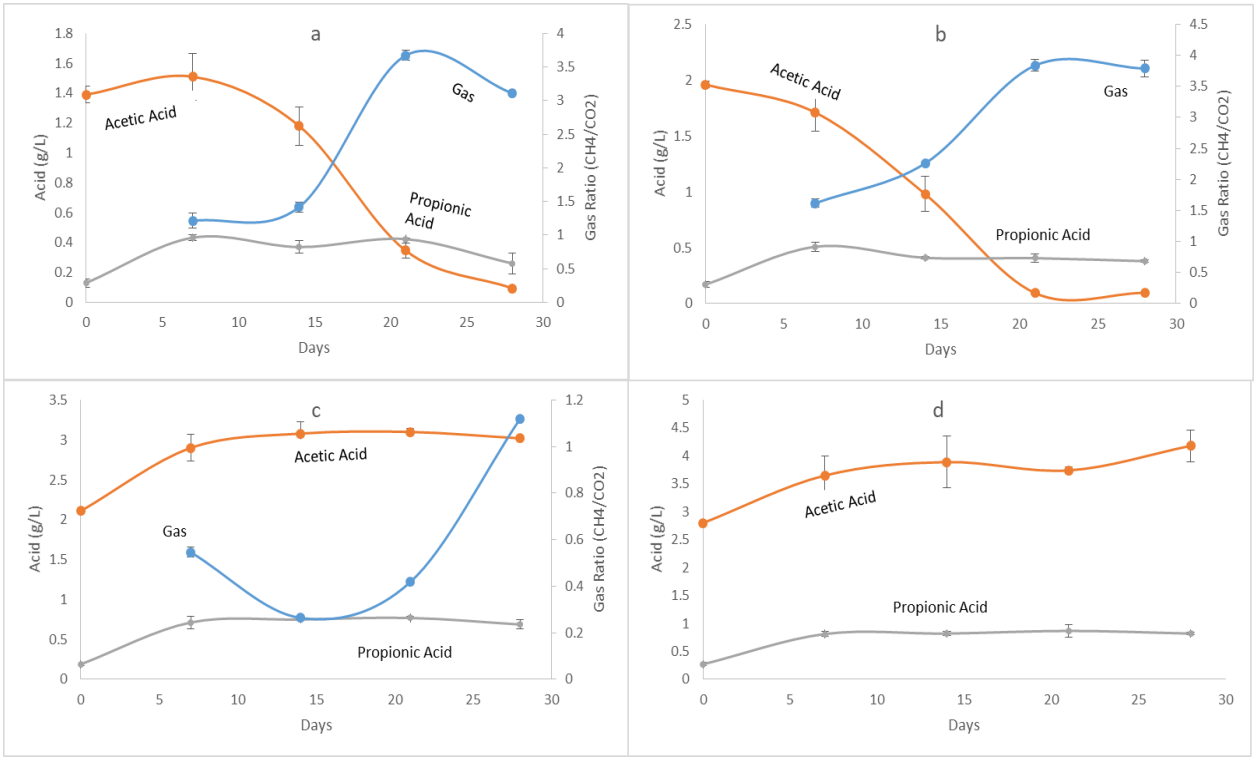

Figure 4.8: AD performance with degassed non-adapted inoculum in the presence of biochar treated APC (a) 7% APC- 1.5 g/L acetic acid equivalent (b) 9% APC - 1.92 g/L acetic acid equivalent (c) 11% APC - 2.3 g/L acetic acid equivalent (d) 13% APC - 2.78 g/L acetic acid equivalent.

This was the first attempt at pre-treating the APC using biochar for the removal of phenolics. Other common methods that have been explored for pre-treatment of APC, for AD, are that of over-liming, activated carbon [51] and air-stripping for removal of ammonia-nitrogen [16]. Over-liming was shown to be extremely effective in the partial removal of phenolic and furan compounds. It was observed that in a batch process with over-limed APC concentration at 5% (equivalent to 1.59 g/L acetate), the methane content was at nearly 80% [51]. However, over liming can cause the biogas to be high on H_2S content, which is not desirable. In this study the methane content at 2 g/L acetic

acid equivalent of biochar pre-treated APC was 80%. In this study, the digestate biochar was used to reduce the toxicity of the APC by the removal of phenolics, which are a major class of inhibitors of AD [83], [84]. Figure shows the AD performance in the presence of biochar treated APC. Biogas ratios for APC loading of up to 2 g/L acetic acid equivalent reach a maximum of 3.8, with the methane content reaching 80% of the biogas composition after 21 days of incubation and a complete consumption of the acetic acid. This is much higher when compared to the AD of un-treated APC using non-adapted inoculum where a maximum ratio of 2.5 was observed with incomplete utilisation of acetic acid. Also, there is a build-up of propionic acid at all concentrations. This propionate is used up by the sulphate reducing bacterial group for sulphogenic oxidation which is a part of incomplete conversion to acetate [60]. The high concentrations of sodium used for the pre-treatment process which could also be inhibitory to the acetogenic and methanogenic population [82]. At higher concentrations of 2.3 g/L acetic acid equivalent, biogas ratios of 1 are achieved after a month of incubation. Concentrations higher than that had no biogas production at all.

4.3.4 AD with degassed non-adapted inoculum in the presence of pure acetic acid

The AD performance in the presence of pure acetic acid was observed as a positive control in order to better understand the probable reason for inhibition when using APC. This method has been used as a standard method for assessing the methane production potential of the inoculum [109] Clearly shows that even in the presence of extremely high concentrations of 5.56 g/L of acetic acid, biogas ratios of as high as 3.5 and a methane content of 78% after 7 days of incubation (Figure). For all the concentrations used, the acid was completely consumed within the first 15 days of incubation after which gas ratios decreased due to the lack of substrate. Also, there was no evident build-

up of propionic acid indicating that at higher concentrations of APC, it is the accumulation of inhibitors present in the APC that cause the inhibition of AD process. It also shows that high concentrations of acetic acid are not inhibitory to the AD process and the inoculum has a buffering capacity of its own. The self-buffering of AD inoculum has been observed in other studies as well [50]

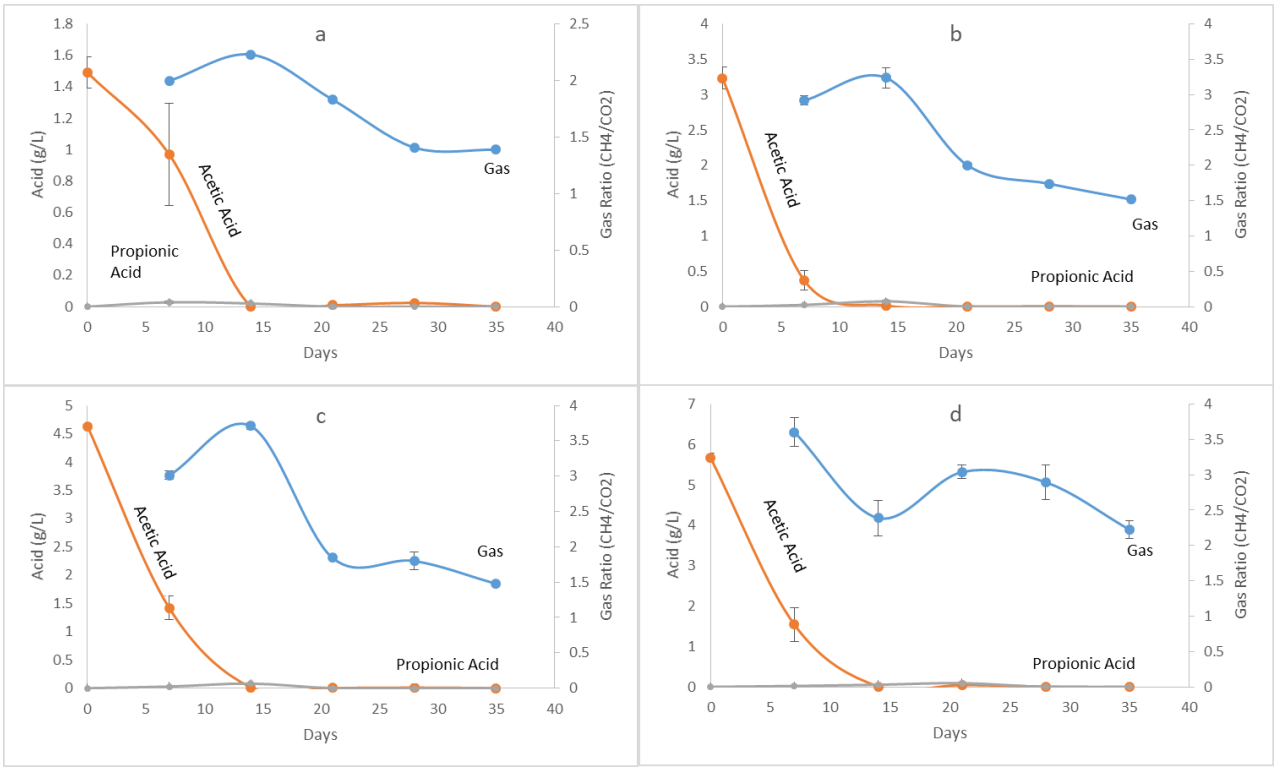

Figure 4.9 : AD performance with degassed non-adapted inoculum in the presence of pure acetic acid. (a) 1.5 g/L acetic acid; (b) 3.2 g/L acetic acid; (c) 4.7 g/L acetic acid; (d) 5.6 g/L acetic acid

4.3.5 AD with degassed non-adapted inoculum and in-situ biochar

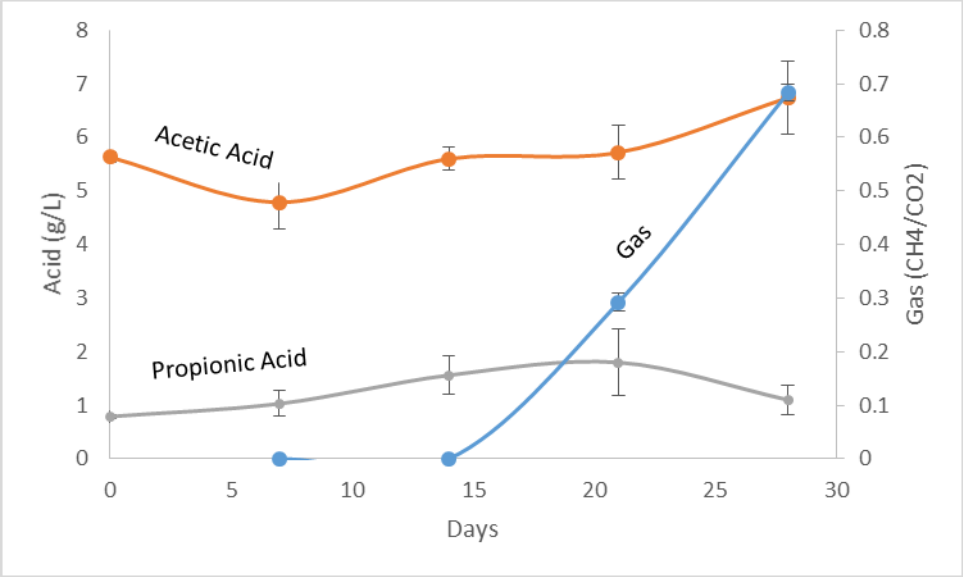

Figure 4.10: AD performance with degassed non-adapted inoculum with in-situ biochar

The AD of APC at higher concentrations of 5.56 g/L was attempted by adding biochar in-situ. As can be observed in Figure , there was no biogas production at such high concentrations as opposed to the patterns observed with pure acetic acid at similar concentrations. It is clear that the high concentration of APC has accumulated the inhibitors as well thereby ceasing biogas production. There is an accumulation of propionic acid and acetic acid to 1.8 g/L and acetic acid to 6.4 g/L respectively. Studies show that a propionic acid concentration of above 0.9 g/L and acetic acid above 2.4 g/L is inhibitory to methanogenesis [77]. This indicates loss of acetogenic and methanogenic activity. However, it can be seen that after 30 days of incubation, gas ratios reach closer to 1. This could be indicative of the fact that longer incubation period, in the presence of biochar, can stimulate the acclimatisation of the inoculum to higher APC concentrations and achieve better biogas ratios.

4.3.6 AD with non-degassed non-adapted inoculum in fed-batch mode with 2 g/L acetic acid equivalent

AD performance of non degassed and non-adapted inoculum was observed for two APC loadings based on previous results. At 2 g/L acetic acid equivalent, the biogas production was consistent and qualitatively better in the presence of biochar.

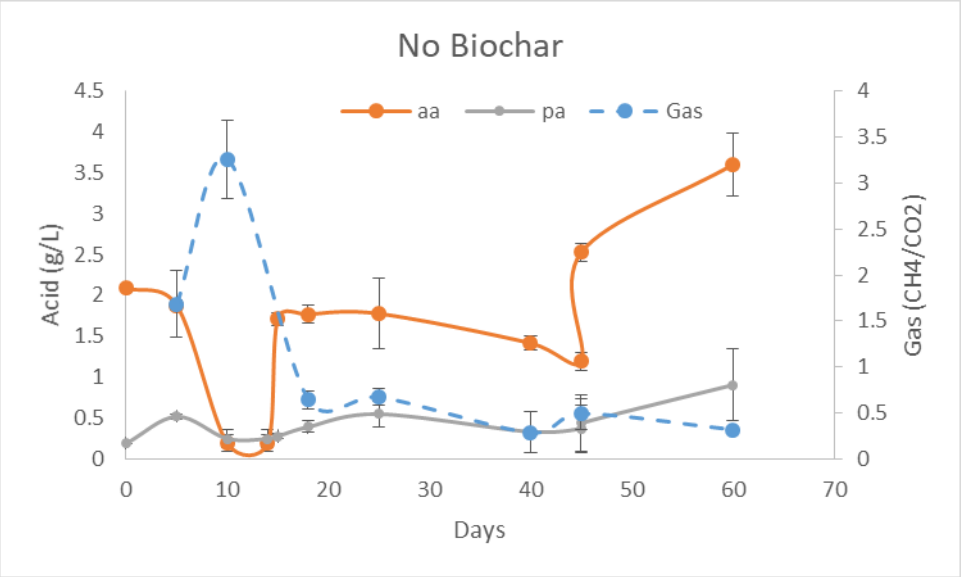

Figure 4.11: AD performance of non-degassed inoculum with 2 g/L acetic acid equivalent without biochar

It can be observed that even in the absence of biochar, 90% of the acetic acid is consumed within the first 10 days of incubation. This is also the period of maximum methane production consituting 76.3% of the biogas composition, as can be seen in Figure . However, after subsequent additions of APC at day 15 and day 45, it can be observed that there is a sharp decrease in methane and overall biogas production. This can be attributed to the accumulation of inhibitors such as phenolics and acids at high APC loading [19], [73]. Also, there is a build up of propionic acid indicating that the acetogenic activity was slow. Towards the end of the digestion, the acetic and propionic acids accumulate to 3.5 g/L and 0.9.8 g/L respectively. These concentrations are known

to be inhibitory to methanogenesis [77] and hence cause complete ceasure of biogas production.

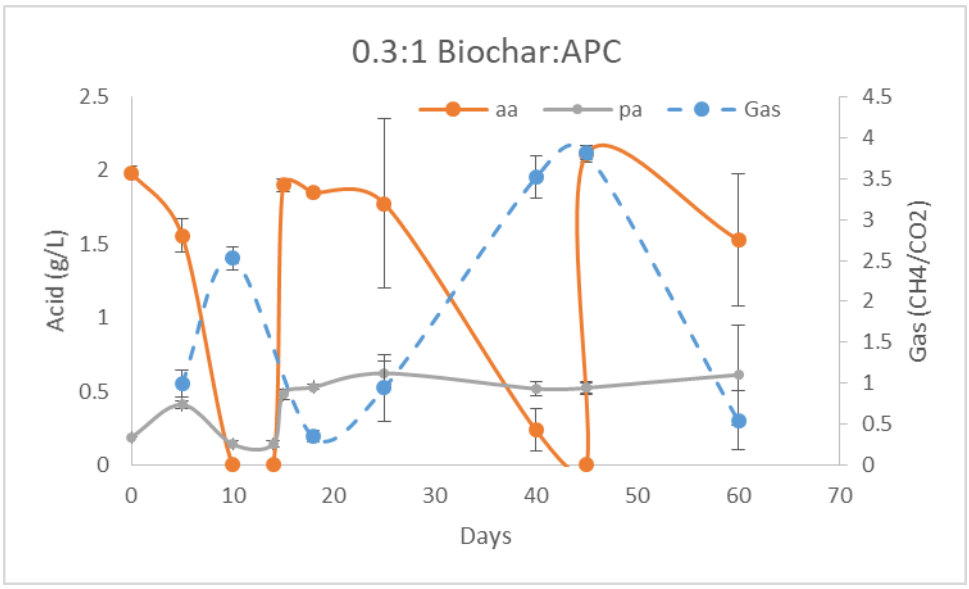

Figure 4.12: AD performance of non-degassed inoculum with 2 g/L acetic acid equivalent with Biochar:APC ratio of 0.3:1

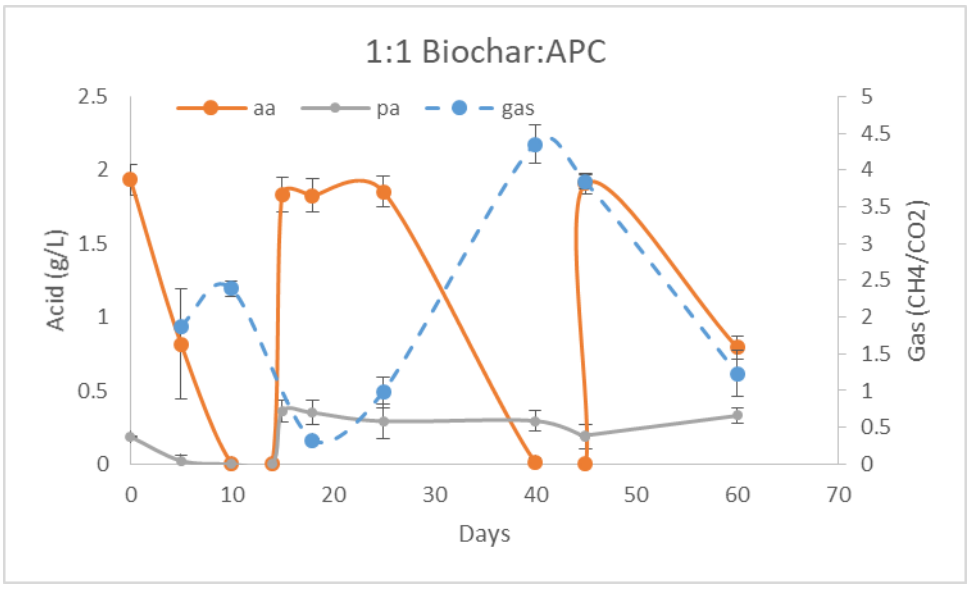

Figure 4.13: AD performance of non-degassed inoculum with 2 g/L acetic acid equivalent with Biochar:APC ratio of 1:1

In the presence of biochar, however, the biogas ratios are consistent even with subsequent additions of the APC. As can be seen in Figure and Figure , all the acetic acid is consumed within the first 10 days of incubation. The methane content after the second addition of APC was nearly 81% of the biogas composition at day 40, with a biochar:APC ratio of 1:1 (Figure). It is also interesting to note that accumulation of propionic acid had little effect on the biogas ratios in the presence of biochar. Although it takes a little longer for the consumption of acetic acid after subsequent additions, the methane content is seen to increase and reach a maximum at both the biochar loadings. The acetic acid consumption is faster with higher biochar loading. This is in agreement with a previous study sewage sludge pyrolysis liquor was subject to anaerobic digestion with no daily methane production unless there was supplementation with biochar [88].

It is also seen that there is lesser amount of propionic acid build-up at higher concentrations of biochar. This could be since biochar promotes DIET (directinter-species electron transfer) thereby increasing the rate of propionate utilisation and improving methane yield [56], [58], [59].

The use of biochar has allowed the consumption of a cumulative amount of nearly 5 g/L acetic acid equivalent APC, with no accumulation of phenolic compounds. This confirms that the AD process in fed batch in the presence of biochar, is an effective mechanism to valorise the APC.

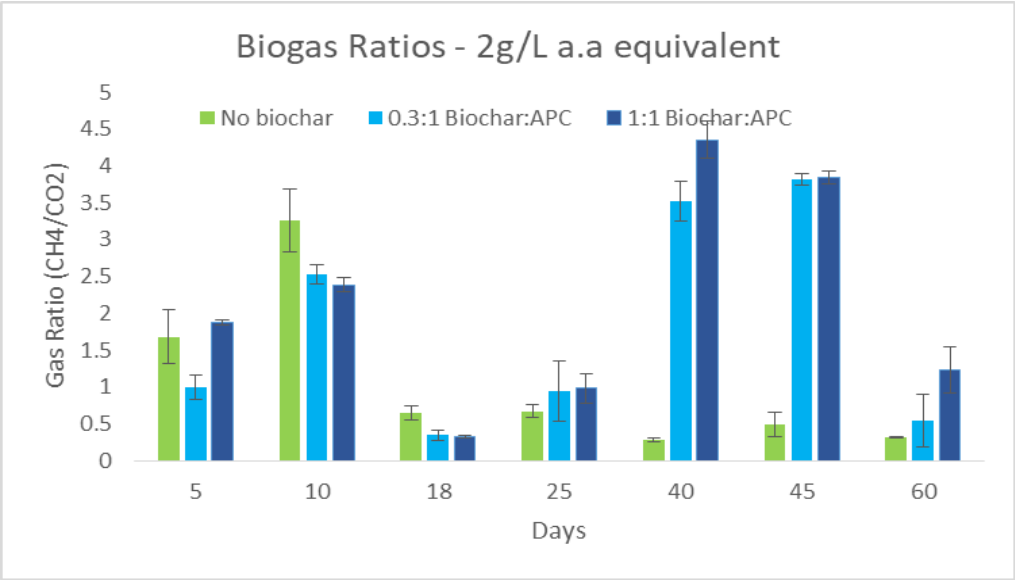

Figure 4.14: Comparison of Biogas ratio in the presence of 2 g/L acetic acid equivalent

A comparison of the biochar ratios (Figure) shows that though initially the set-up with no biochar had higher methane production, the subsequent addition of APC caused inhibition due to accumulation of components such as propionic acid and phenolic or furan compounds. A similar pattern was observed for AD in the presence of coconut shell and corn straw derived biochar [58]. In the presence of biochar, the methane content is much higher at higher biochar loadings and remains consistent even after 2 additions of APC. This indicates that the microbes needed a period of adaptation the presence of biochar after which there is consistent high methane production. At 2 g/L acetic acid equivalent, a biochar:APC ratio of 1:1 improved the biogas ratios by 88.8% after 40 days of incubation. This can be due to the buffering capacity of the biochar [18] as well as carbon dioxide sequestration activity, which in turn increases methane content [65]. These results are in agreement with literature where a 1:1 ratio of biochar:APC was effective in increasing the methane yield by 60% after 200 days of incubation when fed with 1.3 g/L APC, in comparison to no biochar [50].

4.3.7 AD with non-degassed non-adapted inoculum in fed-batch mode with 1 g/L acetic acid equivalent

Biogas production at 1 g/L acetic acid equivalent was compared in the presence an absence of biochar.

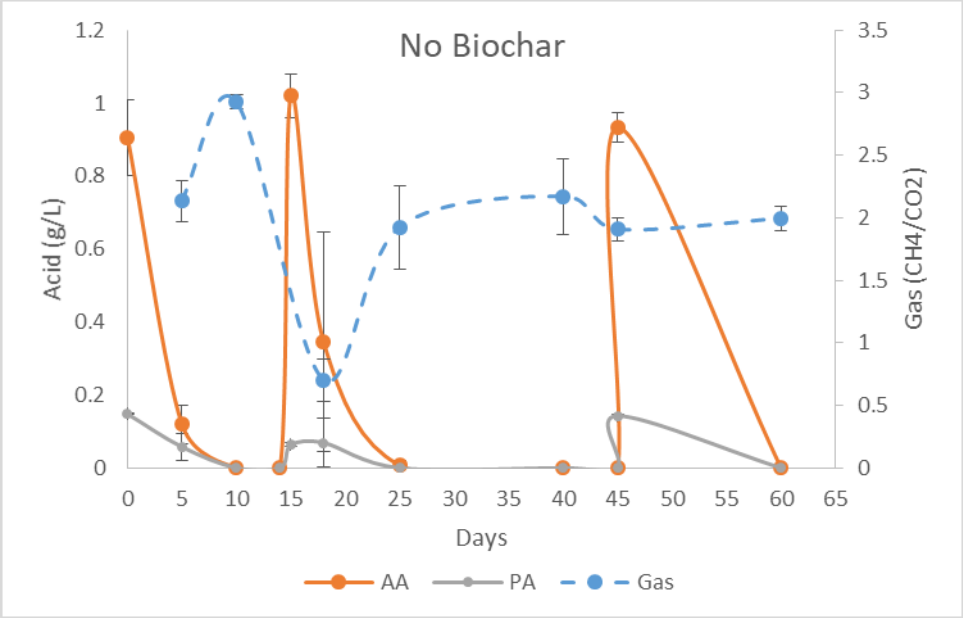

Figure 4.15: AD performance of non-degassed inoculum with 1 g/L acetic acid equivalent without biochar

There was a consistent methane production even in the absence of biochar (Figure). As was observed form earlier experiments, higher dilutions of the APC actually stimulated ethane production. A similar pattern is observed here. There is 100% consumption of acetic acid in the first 10 days of incubation which coincides with the highest biogas ratio observed. However, when APC was added on day 15, there is a sharp decrease in biogas production, with methane content being lower than the carbon-dioxide content. It can be seen that 3 days after the addition of biochar (on day 18), the inoculum is able to consume the acid effectively and biogas production reaches a ratio of 2.2 with no accumulation of propionic acid. By day 25 again, all of the acid is effectively consumed.

In the first two additions, the acid is consumed completely within 10 days of APC addition. However, after the third addition, the methane content decreases slightly. This could be due to the accumulation of phenolic inhibitors and change in pH due to consequent APC additions.

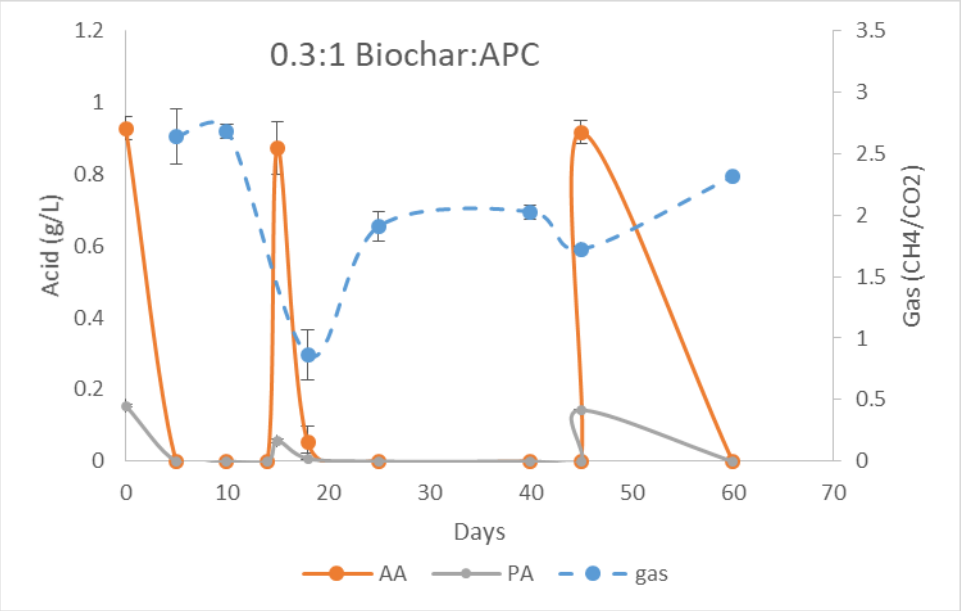

Figure 4.16: AD performance of non-degassed inoculum with 1 g/L acetic acid equivalent with Biochar:APC ratio of 0.3:1

In the presence of biochar, the acid consumption is much faster (Figure and Figure). It can be seen that all the acid is consumed within the first 5 days of incubation. This clearly indicates that biochar enables methanogenesis and reduces the lag phase of methanogens. This is in agreement with the results found in a similar study [62]. Interestingly, at higher biochar loading, we observe an accumulation of propionc acid near day 25 (Figure). Studies have shown that methane production is affected by biochar loadings as well. High biochar concetrations are known to increase the propionic acid build up whereas optimal amounts reduced vfa accumulation [58]. Whereas the consumption of acetic acid is a thermodynamically favorable process [74], the conversion of propionate is not [58] thereby becoming the rate-limiting step in

methanogenesis. Even though there is accumulation of propionic acid, the biogas ratios remeins consistent. This could be due to the conversion of propionic acid by sulphur reducing bacteria, to acetate [60]. Also, the acetic acid is readily consumed in less than 10 days after the second addition. The biogas ratio is seen to decrease a bit on day 45. This recovers after addition of APC on day 45. The initial drop can be due to substrate limiting conditions.

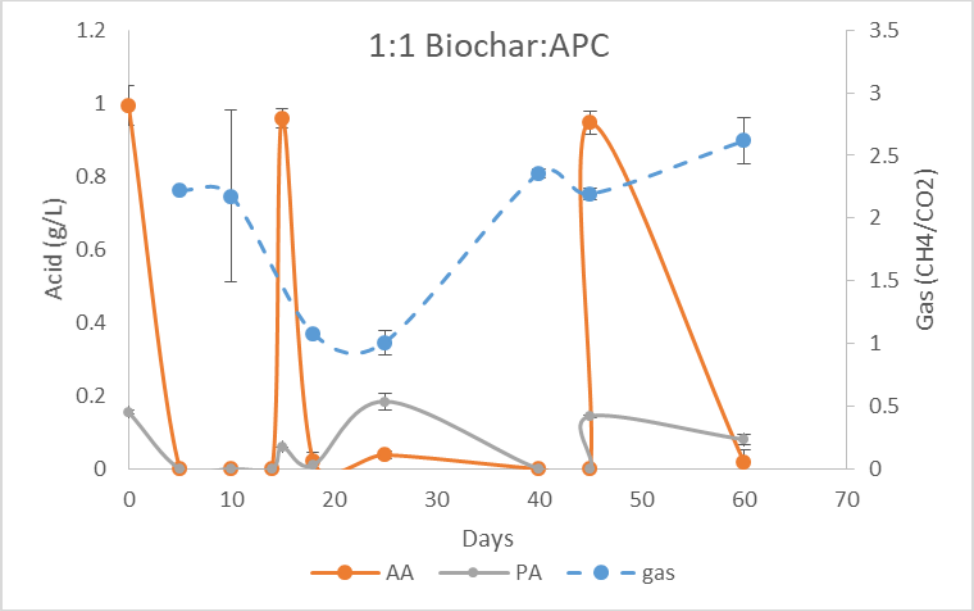

Figure 4.17: AD performance of non-degassed inoculum with 1 g/L acetic acid equivalent with Biochar:APC ratio of 1:1

In a comparison of AD performance in the presence and absence of biochar, it can be seen that biochar has a positive effect on methane production. Figure shows that biochar helps stabilize the methane generation process. The slight decrease in methane content on day 10, in the presence of biochar could be because of starvation due to the absence of acetic acid which was consumed within the first 5 days [50]. This also proves that appropriate amounts of APC stimulates methane production. The biogas ratios show that a higher loading of biochar improves methane production.

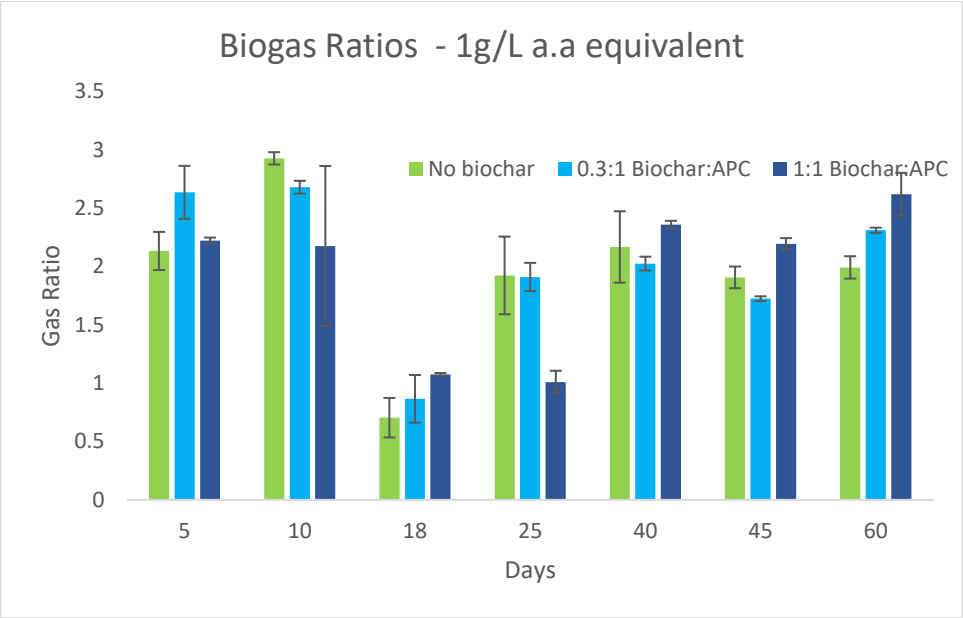

Figure 4.18: Comparison of Biogas ratio in the presence of 1 g/L acetic acid equivalent

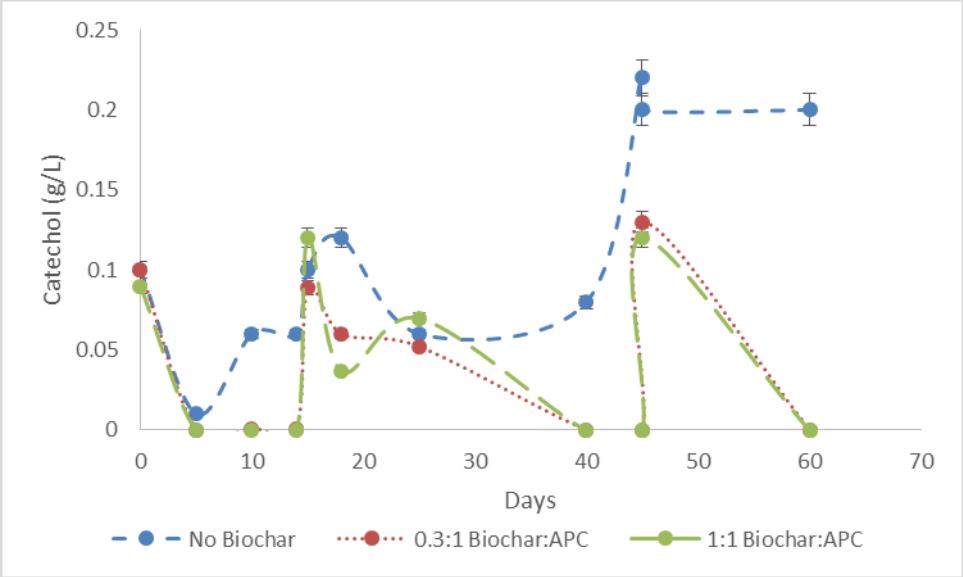

Figure 4.19: Effect of Biochar on catechol consumption

The effect of biochar addition on the phenol derivatives that were predominantly found in the APC was also recorded. Catechol, a hydroxyl-phenol, is known to be more toxic than phenol, in the AD process [110]. Figure shows the consumption pattern of catechol throughout the AD process. Although the amount of catechol present at this dilution of the APC was not inhibitory to methanogenesis, it is clear that the addition of biochar

prevents the accumulation of the accumulation of inhibitory compounds. In the absence of biochar, there is an accumulation of catechol after subsequent additions of APC, with a 50% increase in catechol concentration. However, in the presence of biochar, the supplementation of APC does not lead to an accumulation of catechol. Moreover, with 1:1 ratio of biochar:APC, the catechol consumption is more efficient after the second addition of APC. This result is in agreement with our study on the absorption of total phenolics using biochar.

4.3.8 Comparison of different biochar loading

A comparison of AD performance at different substrate and biochar loading showed that, in this study, higher biochar loading when substrate concentration was high yielded higher biogas ratios (Figure).

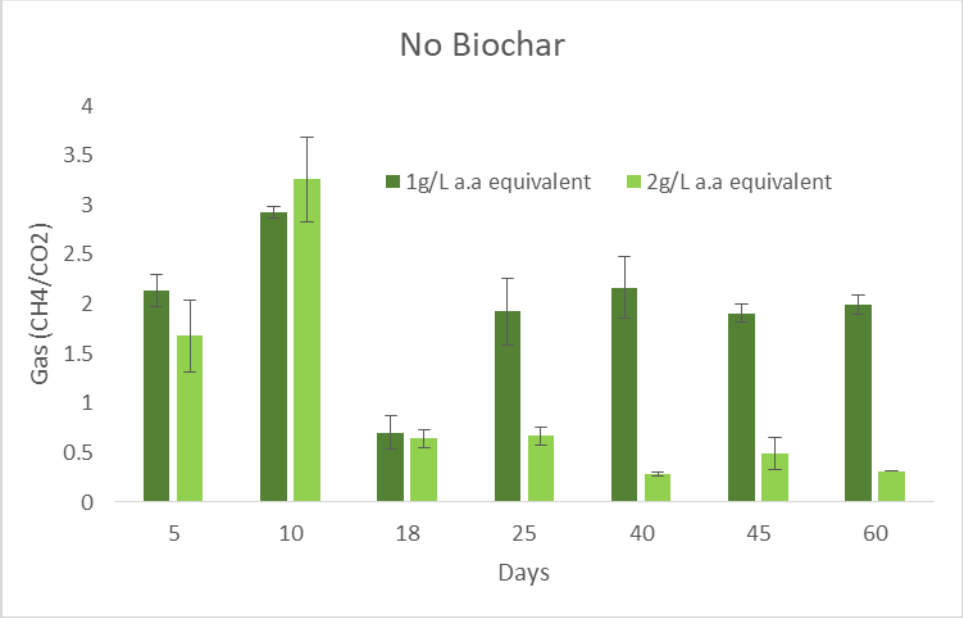

Figure 4.20: AD performance comparison in the absence of biochar

The biochar loading has been shown to affect methane production in an AD process. In a study, corn stover biochar at 26 g/L was found to decrease the methane production by almost 44% [58]. In the absence of biochar, however, the biogas quality was better at

lower APC loading (Figure). This is clearly because of the lower amount of inhibitors present in the system at lower APC loading.

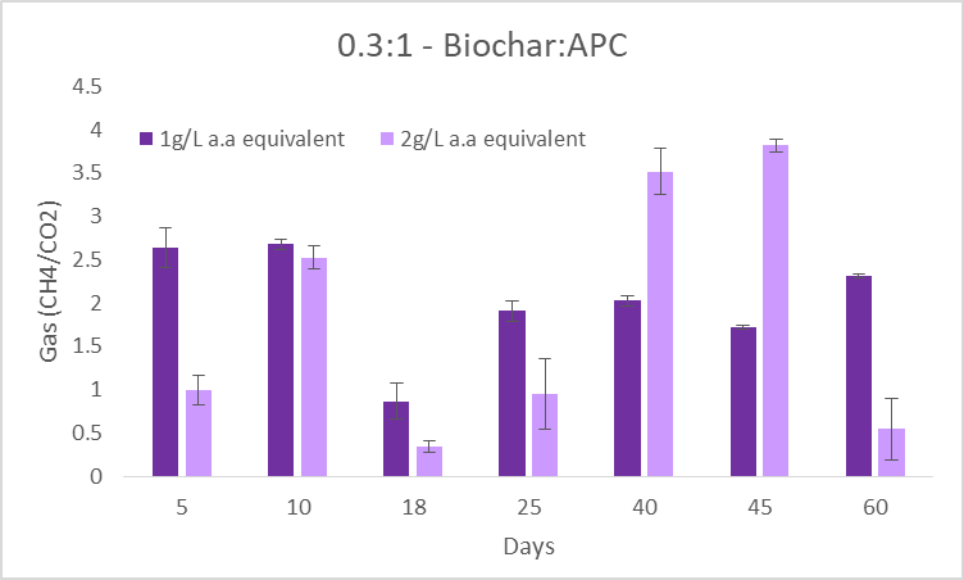

Figure 4.21: AD performance comparison with Biochar:APC ratio 0.3:1

It can be seen that biochar:APC ratios of 1:1 are more effective in improving methane generation at higher APC concentrations giving biogas ratios of 4.35 after the second addition of APC (Figure). Whereas for 1 g/L acetic acid equivalent, a biochar:APC ratio of 0.3:1 was more efficient during the AD process (Figure). This also suggestes that at higher concentrations of APC, the biochar loading needs to be higher is order to mitigate the toxicvity of the APC. A similar result was observed in the continuos fermentation of APC in the presence of biochar [68]. Also, it is important to determine ideal biochar loading in order to avoid any negative effects on methane production due to excess biochar addition [61]. The higher methane content in the presence of biochar could also be attributed to the high ash content of the biochar which is known to have carbon dioxide sequestration activity. This high ash content is also known to contribute to the buffering capacity by exhibiting an overliming effect [62], [65]. The ash content could also be a nutrient supply (such as that of phosphorus) thereby enabling

methanogen activity [50]. The high surface area of biochars allows the vfa utilising microbial population to grow on the surface thereby enhancing the rate of biogas produtcion [58].

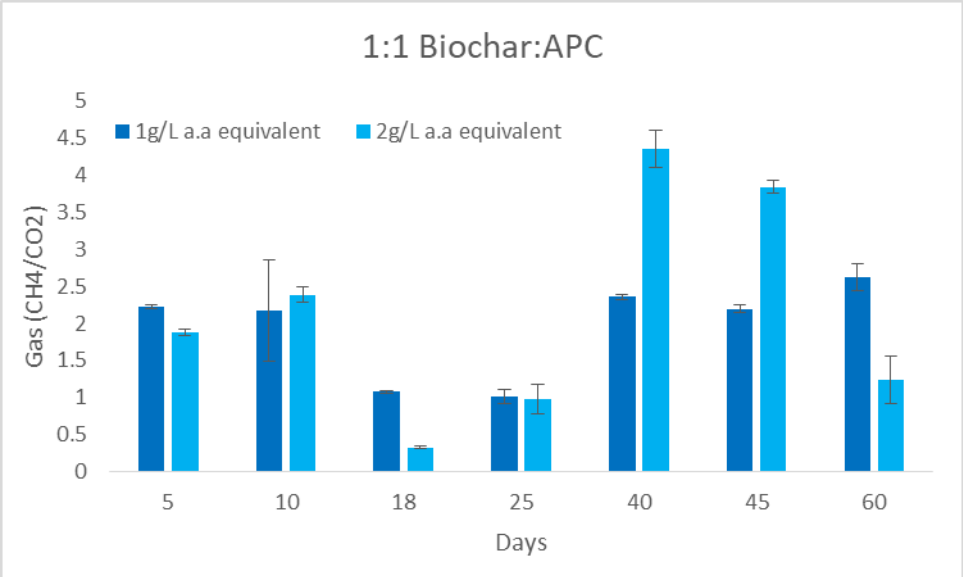

Figure 4.22: AD performance comparison with Biochar:APC ratio 1:1

5. Conclusions and Recommendations

5.1 Conclusions

The APC was found to be rich in organics. The acetic acid and levoglucosan act a carbon source for anaerobic digestion. However, there is a high amount of organic inhibitors such as phenols and furans. The slightly high C/N ratio and COD can be fixed by dilution or pre-treatment. The pre-treatment of APC was successful in removal of major inhibitors. However, there was also a considerable reduction of major substrates for methanogenesis. Also, the dilution with 1M NaOH increased the concentration of sodium in the APC to potentially toxic levels. Hence, another approach that may be adopted is the acclimatisation of the inoculum or the use of a pre-treatment i.e., biochar.

The biochar generated after the digestate pyrolysis presented higher surface area compared with other reported results. The pH of the biochar was 7.2 which is optimal for methane production. Although it has a high ash content, which makes it unsuitable for most other applications, it may be advantageous for methane generation due to carbon dioxide sequestration activity. However, some studies reported a reverse effect. It is evident that the effect of ash content on methanogenesis needs more investigation. The high ash content may also increase the liming potential of the biochar thereby maintaining pH stability of the process. The mechanism of biochar effect on biogas production has been of a lot of interest lately. The adsorption of total phenolics also shows promise for the potential use of this biochar as an enhancer of anaerobic digestion.

The anaerobic digestion experiments indicate that the valorisation of the APC for energy generation is possible at lower concentrations. For higher concentrations, adaptation of the microbial consortia is an effective strategy. Adaptation done in the

presence of 3% APC (1.24 g/L acetic acid equivalent) was effective for improving biogas production up to 5% APC loading (2.14 g/L acetic acid equivalent). The AD in the presence of pure acetic acid shows that high concentrations of up to 5.56 g/L did not inhibit methanogenesis. This also indicates that AD inhibition in the presence of APC was due to the accumulation of propionic acid and phenolic compounds. In order to overcome the possible inhibition due to the presence of phenolic compounds, incorporation of biochar in-situ can be done to increase methane generation. Experiments done in this study demonstrated an increase biogas ratios by 88% at higher APC loadings. However, it is important to analyse and estimate appropriate biochar loading for each APC loading. In this study, in the presence of biochar, we have been able to convert up to 5 g/L acetic acid equivalent of APC which corresponds to almost 11.6% APC.

5.2 Recommendations

The utilisation of APC derived from wood in AD process was shown to be an effective strategy in the present study. Based on the results observed in this study, future recommendations could include the assessment of quantitative biogas production for the conditions that demonstrated highest biogas ratios. Also, adaptation at higher APC concentrations may be explored as a strategy for AD of higher APC concentrations. Biochar addition in to adapted inoculum could also be explored to increase the qualitative and quantitative production of biogas at higher APC concentrations.

In addition, since anaerobic digestion is a dynamic process, analysis on the effect of APC addition and adaptation, as well as biochar addition on the microbial consortia might be an effective tool to better understand the biochemical pathways involved in the AD process.

6. Bibliography

[1] D. M. C. Chen, B. L. Bodirsky, T. Krueger, A. Mishra, and A. Popp, "The world's growing municipal solid waste: trends and impacts," *Environ. Res. Lett.*, vol. 15, no. 7, 2020.

[2] S. Kaza, L. Yao, P. Bhada-Tata, and F. Van Woerden, *What a Waste 2.0: A Global Snapshot of Solid Waste Management to 2050*. The World Bank, 2018.

[3] L. Appels *et al.*, "Anaerobic digestion in global bio-energy production: Potential and research challenges," *Renew. Sustain. Energy Rev.*, vol. 15, no. 9, pp. 4295–4301, 2011.

[4] S. Yaman, "Pyrolysis of biomass to produce fuels and chemical feedstocks," *Energy Convers. Manag.*, vol. 45, no. 5, pp. 651–671, 2004.

[5] S. Uçar and S. Karagöz, "The slow pyrolysis of pomegranate seeds: The effect of temperature on the product yields and bio-oil properties," *J. Anal. Appl. Pyrolysis*, vol. 84, no. 2, pp. 151–156, 2009.

[6] Y. Yang, S. Heaven, N. Venetsaneas, C. J. Banks, and A. V. Bridgwater, "Slow pyrolysis of organic fraction of municipal solid waste (OFMSW): Characterisation of products and screening of the aqueous liquid product for anaerobic digestion," *Appl. Energy*, vol. 213, no. January, pp. 158–168, 2018.

[7] K. Açikalin, F. Karaca, and E. Bolat, "Pyrolysis of pistachio shell: Effects of pyrolysis conditions and analysis of products," *Fuel*, vol. 95, pp. 169–177, 2012.

[8] E. Henrich, N. Dahmen, F. Weirich, R. Reimert, and C. Kornmayer, "Fast pyrolysis of lignocellulosics in a twin screw mixer reactor," *Fuel Process. Technol.*, vol. 143, pp. 151–161, 2016.

[9] A. Demirbas, “Biorefineries: Current activities and future developments,” *Energy Convers. Manag.*, vol. 50, no. 11, pp. 2782–2801, 2009.

[10] S. Kersten and M. Garcia-Perez, “Recent developments in fast pyrolysis of ligno-cellulosic materials,” *Curr. Opin. Biotechnol.*, vol. 24, no. 3, pp. 414–420, 2013.

[11] B. H. M. Bowden-green, “Granulation of Biochar for Soil Amendment,” no. January, 2016.

[12] S. S. Liaw, V. H. Perez, R. J. M. Westerhof, G. F. David, C. Frear, and M. Garcia-Perez, “Biomethane Production from Pyrolytic Aqueous Phase: Biomass Acid Washing and Condensation Temperature Effect on the Bio-oil and Aqueous Phase Composition,” *Bioenergy Res.*, 2020.

[13] P. H. Hall, “Recovering valuable products from the aqueous streams of fast pyrolysis,” p. 154, 2017.

[14] A. Rafiee and K. R. Khalilpour, “Chapter 11 - Renewable Hybridization of Oil and Gas Supply Chains,” K. R. B. T.-P. with P. for C. and E. H. Khalilpour, Ed. Academic Press, 2019, pp. 331–372.

[15] M. Pecchi and M. Baratieri, “Coupling anaerobic digestion with gasification, pyrolysis or hydrothermal carbonization: A review,” *Renew. Sustain. Energy Rev.*, vol. 105, pp. 462–475, 2019.

[16] S. Seyedi, K. Venkiteshwaran, N. Benn, and D. Zitomer, “Inhibition during anaerobic co-digestion of aqueous pyrolysis liquid from wastewater solids and synthetic primary sludge,” *Sustain.*, vol. 12, no. 8, pp. 8–11, 2020.

[17] D. Fabbri and C. Torri, “Linking pyrolysis and anaerobic digestion (Py-AD) for the conversion of lignocellulosic biomass,” *Curr. Opin. Biotechnol.*, vol. 38, no.

March, pp. 167–173, 2016.

[18] F. C. Luz *et al.*, “Ampelodesmos mauritanicus pyrolysis biochar in anaerobic digestion process: Evaluation of the biogas yield,” *Energy*, vol. 161, pp. 663–669, 2018.

[19] S. R. Shanmugam, S. Adhikari, H. Nam, and S. K. Sajib, “Biomass and Bioenergy E ff ect of bio-char on methane generation from glucose and aqueous phase of algae liquefaction using mixed anaerobic cultures,” vol. 108, no. November 2017, pp. 479–486, 2018.

[20] K. Hagos, J. Zong, D. Li, C. Liu, and X. Lu, “Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives,” *Renew. Sustain. Energy Rev.*, vol. 76, no. November 2016, pp. 1485–1496, 2017.

[21] S. Czernik and A. V. Bridgwater, “Overview of applications of biomass fast pyrolysis oil,” *Energy and Fuels*, vol. 18, no. 2, pp. 590–598, 2004.

[22] J. L. Gaunt and J. Lehmann, “Energy balance and emissions associated with biochar sequestration and pyrolysis bioenergy production,” *Environ. Sci. Technol.*, vol. 42, no. 11, pp. 4152–4158, 2008.

[23] H. Yang, R. Yan, H. Chen, D. H. Lee, and C. Zheng, “Characteristics of hemicellulose, cellulose and lignin pyrolysis,” *Fuel*, vol. 86, no. 12–13, pp. 1781–1788, 2007.

[24] A. P. Pinheiro Pires *et al., Challenges and opportunities for bio-oil refining: A review*, vol. 33, no. 6. 2019.

[25] J. Akhtar and N. Saidina Amin, “A review on operating parameters for optimum liquid oil yield in biomass pyrolysis,” *Renew. Sustain. Energy Rev.*, vol. 16, no.

7, pp. 5101–5109, 2012.

[26] E. Butler, G. Devlin, D. Meier, and K. McDonnell, “A review of recent laboratory research and commercial developments in fast pyrolysis and upgrading,” *Renew. Sustain. Energy Rev.*, vol. 15, no. 8, pp. 4171–4186, 2011.

[27] A. V. Bridgwater, “Principles and practice of biomass fast pyrolysis processes for liquids,” *J. Anal. Appl. Pyrolysis*, vol. 51, no. 1, pp. 3–22, 1999.

[28] A. K. Varma and P. Mondal, “Pyrolysis of pine needles: effects of process parameters on products yield and analysis of products,” *J. Therm. Anal. Calorim.*, vol. 131, no. 3, pp. 2057–2072, 2018.

[29] A. V. Bridgwater, “Review of fast pyrolysis of biomass and product upgrading,” *Biomass and Bioenergy*, vol. 38, pp. 68–94, 2012.

[30] G. Duman, C. Okutucu, S. Ucar, R. Stahl, and J. Yanik, “The slow and fast pyrolysis of cherry seed,” *Bioresour. Technol.*, vol. 102, no. 2, pp. 1869–1878, 2011.

[31] A. K. Varma, L. S. Thakur, R. Shankar, and P. Mondal, “Pyrolysis of wood sawdust: Effects of process parameters on products yield and characterization of products,” *Waste Manag.*, vol. 89, pp. 224–235, 2019.

[32] A. K. Varma and P. Mondal, “Pyrolysis of sugarcane bagasse in semi batch reactor: Effects of process parameters on product yields and characterization of products,” *Ind. Crops Prod.*, vol. 95, pp. 704–717, 2017.

[33] F. H. Mahfud, F. P. Van Geel, R. H. Venderbosch, and H. J. Heeres, “Acetic acid recovery from fast pyrolysis oil. An exploratory study on liquid-liquid reactive extraction using aliphatic tertiary amines,” *Sep. Sci. Technol.*, vol. 43, no. 11–12,

pp. 3056–3074, 2008.

[34] M. R. Rover, P. A. Johnston, L. E. Whitmer, R. G. Smith, and R. C. Brown, “The effect of pyrolysis temperature on recovery of bio-oil as distinctive stage fractions,” *J. Anal. Appl. Pyrolysis*, vol. 105, pp. 262–268, 2014.

[35] A. Demirbas, “The influence of temperature on the yields of compounds existing in bio-oils obtained from biomass samples via pyrolysis,” *Fuel Process. Technol.*, vol. 88, no. 6, pp. 591–597, 2007.

[36] A. M. Azeez, D. Meier, and J. Odermatt, “Temperature dependence of fast pyrolysis volatile products from European and African biomasses,” *J. Anal. Appl. Pyrolysis*, vol. 90, no. 2, pp. 81–92, 2011.

[37] S.-S. Liaw, V. Haber Perez, S. Zhou, O. Rodriguez-Justo, and M. Garcia-Perez, “Py-GC/MS studies and principal component analysis to evaluate the impact of feedstock and temperature on the distribution of products during fast pyrolysis,” *J. Anal. Appl. Pyrolysis*, vol. 109, pp. 140–151, 2014.

[38] S. S. Liaw *et al.*, “Effect of pyrolysis temperature on the yield and properties of bio-oils obtained from the auger pyrolysis of Douglas Fir wood,” *J. Anal. Appl. Pyrolysis*, vol. 93, pp. 52–62, 2012.

[39] Q. Lu, X. Yang, C. Dong, Z. Zhang, X. Zhang, and X. Zhu, “Influence of pyrolysis temperature and time on the cellulose fast pyrolysis products: Analytical Py-GC/MS study,” *J. Anal. Appl. Pyrolysis*, vol. 92, no. 2, pp. 430–438, 2011.

[40] Z. Wang, J. Cao, and J. Wang, “Pyrolytic characteristics of pine wood in a slowly heating and gas sweeping fixed-bed reactor,” *J. Anal. Appl. Pyrolysis*, vol. 84,

no. 2, pp. 179–184, 2009.

[41] J. Yanik, C. Kornmayer, M. Saglam, and M. Yüksel, “Fast pyrolysis of agricultural wastes: Characterization of pyrolysis products,” *Fuel Process. Technol.*, vol. 88, no. 10, pp. 942–947, 2007.

[42] W. N. R. W. Isahak, M. W. M. Hisham, M. A. Yarmo, and T. Y. Yun Hin, “A review on bio-oil production from biomass by using pyrolysis method,” *Renew. Sustain. Energy Rev.*, vol. 16, no. 8, pp. 5910–5923, 2012.

[43] T. Kan, V. Strezov, and T. J. Evans, “Lignocellulosic biomass pyrolysis: A review of product properties and effects of pyrolysis parameters,” *Renew. Sustain. Energy Rev.*, vol. 57, pp. 1126–1140, 2016.

[44] M. Bertero, H. A. Gorostegui, C. J. Orrabalis, C. A. Guzmán, E. L. Calandri, and U. Sedran, “Characterization of the liquid products in the pyrolysis of residual chañar and palm fruit biomasses,” *Fuel*, vol. 116, pp. 409–414, 2014.

[45] A. R. Mohamed[a], Z. Hamzah[a], M. Zulkali, and M. Daud, “The Effects of Holding Time and The Sweeping Nitrogen Gas Flowrates On The Pyrolysis Of EFB Using A Fixed Bed Reactor,” vol. 53, pp. 185–191, 2013.

[46] P. Kim, S. Weaver, and N. Labbé, “Effect of sweeping gas flow rates on temperature-controlled multistage condensation of pyrolysis vapors in an auger intermediate pyrolysis system,” *J. Anal. Appl. Pyrolysis*, vol. 118, pp. 325–334, 2016.

[47] D. Shen, W. Jin, J. Hu, R. Xiao, and K. Luo, “An overview on fast pyrolysis of the main constituents in lignocellulosic biomass to valued-added chemicals: Structures, pathways and interactions,” *Renew. Sustain. Energy Rev.*, vol. 51, pp.

761–774, 2015.

[48] D. K. Shen, S. Gu, and A. V Bridgwater, “Study on the pyrolytic behaviour of xylan-based hemicellulose using TG–FTIR and Py–GC–FTIR,” *J. Anal. Appl. Pyrolysis*, vol. 87, no. 2, pp. 199–206, 2010.

[49] B. A. Black *et al.*, “Aqueous Stream Characterization from Biomass Fast Pyrolysis and Catalytic Fast Pyrolysis,” 2016.

[50] C. Torri and D. Fabbri, “Biochar enables anaerobic digestion of aqueous phase from intermediate pyrolysis of biomass,” *Bioresour. Technol.*, vol. 172, pp. 335–341, 2014.

[51] H. Zhou, R. C. Brown, and Z. Wen, “Anaerobic digestion of aqueous phase from pyrolysis of biomass: Reducing toxicity and improving microbial tolerance,” *Bioresour. Technol.*, vol. 292, no. June, p. 121976, 2019.

[52] C. Wen, C. M. Moreira, L. Rehmann, and F. Berruti, “Feasibility of anaerobic digestion as a treatment for the aqueous pyrolysis condensate (APC) of birch bark,” *Bioresour. Technol.*, vol. 307, no. December 2019, p. 123199, 2020.

[53] T. Hübner and J. Mumme, “Integration of pyrolysis and anaerobic digestion - Use of aqueous liquor from digestate pyrolysis for biogas production,” *Bioresour. Technol.*, vol. 183, pp. 86–92, 2015.

[54] K. Spokas, “Review of the stability of biochar in soils: Predictability of O:C molar ratios,” *Carbon Manag.*, vol. 1, Dec. 2010.

[55] N. Hagemann, K. Spokas, H. P. Schmidt, R. Kägi, M. A. Böhler, and T. D. Bucheli, “Activated carbon, biochar and charcoal: Linkages and synergies across pyrogenic carbon’s ABCs,” *Water (Switzerland)*, vol. 10, no. 2, pp. 1–19, 2018.

[56] J. Pan, J. Ma, X. Liu, L. Zhai, X. Ouyang, and H. Liu, “Effects of different types of biochar on the anaerobic digestion of chicken manure,” *Bioresour. Technol.*, vol. 275, pp. 258–265, 2019.

[57] M. O. Fagbohungbe, B. M. J. Herbert, L. Hurst, H. Li, S. Q. Usmani, and K. T. Semple, “Impact of biochar on the anaerobic digestion of citrus peel waste,” *Bioresour. Technol.*, vol. 216, pp. 142–149, 2016.

[58] M. Zhang, J. Li, Y. Wang, and C. Yang, “Impacts of different biochar types on the anaerobic digestion of sewage sludge,” *RSC Adv.*, vol. 9, no. 72, pp. 42375–42386, 2019.

[59] F. Lü, C. Luo, L. Shao, and P. He, “Biochar alleviates combined stress of ammonium and acids by firstly enriching Methanosaeta and then Methanosarcina,” *Water Res.*, vol. 90, pp. 34–43, 2016.

[60] Y. Chen, J. J. Cheng, and K. S. Creamer, “Inhibition of anaerobic digestion process: A review,” *Bioresour. Technol.*, vol. 99, no. 10, pp. 4044–4064, 2008.

[61] C. Deng, R. Lin, X. Kang, B. Wu, R. O. Shea, and D. Murphy, “Improving gaseous biofuel yield from seaweed through a cascading circular bioenergy system integrating anaerobic digestion and pyrolysis,” vol. 128, no. April, 2020.

[62] H. Zhou, R. C. Brown, and Z. Wen, “Biochar as an Additive in Anaerobic Digestion of Municipal Sludge: Biochar Properties and Their Effects on the Digestion Performance,” *ACS Sustain. Chem. Eng.*, vol. 8, no. 16, pp. 6391–6401, Apr. 2020.

[63] Q. Li, M. Xu, G. Wang, R. Chen, W. Qiao, and X. Wang, “Biochar assisted thermophilic co-digestion of food waste and waste activated sludge under high

feedstock to seed sludge ratio in batch experiment," *Bioresour. Technol.*, vol. 249, pp. 1009–1016, 2018.

[64] C. Luo, F. Lü, L. Shao, and P. He, "Application of eco-compatible biochar in anaerobic digestion to relieve acid stress and promote the selective colonization of functional microbes," *Water Res.*, vol. 68, pp. 710–718, 2015.

[65] S. Chen, J. Zhang, and X. Wang, "Effects of alkalinity sources on the stability of anaerobic digestion from food waste," *Waste Manag. Res.*, vol. 33, no. 11, pp. 1033–1040, Sep. 2015.

[66] M. Mun and H. Cho, "Mineral Carbonation for Carbon Sequestration with Industrial Waste," *Energy Procedia*, vol. 37, pp. 6999–7005, 2013.

[67] J. Mumme, F. Srocke, K. Heeg, and M. Werner, "Use of biochars in anaerobic digestion," *Bioresour. Technol.*, vol. 164, pp. 189–197, 2014.

[68] C. Torri, G. Pambieri, C. Gualandi, M. Piraccini, A. G. Rombolà, and D. Fabbri, "Evaluation of the potential performance of hyphenated pyrolysis-anaerobic digestion (Py-AD) process for carbon negative fuels from woody biomass," *Renew. Energy*, vol. 148, pp. 1190–1199, 2020.

[69] V. Patel, S. Pandit, and K. Chandrasekhar, "Basics of Methanogenesis in Anaerobic Digester BT - Microbial Applications Vol.2: Biomedicine, Agriculture and Industry," V. C. Kalia, Ed. Cham: Springer International Publishing, 2017, pp. 291–314.

[70] T. Abbasi, S. M. Tauseef, and S. A. Abbasi, "A Brief History of Anaerobic Digestion and 'Biogas' BT - Biogas Energy," T. Abbasi, S. M. Tauseef, and S. A. Abbasi, Eds. New York, NY: Springer New York, 2012, pp. 11–23.

[71] P. Bajpai, “Anaerobic Technology in Pulp and Paper Industry,” pp. 7–13, 2017.

[72] M. Madsen, J. B. Holm-Nielsen, and K. H. Esbensen, “Monitoring of anaerobic digestion processes: A review perspective,” *Renew. Sustain. Energy Rev.*, vol. 15, no. 6, pp. 3141–3155, 2011.

[73] X. Yu, C. Zhang, L. Qiu, Y. Yao, G. Sun, and X. Guo, “Anaerobic digestion of swine manure using aqueous pyrolysis liquid as an additive,” *Renew. Energy*, vol. 147, pp. 2484–2493, 2020.

[74] N. R. Buan, “Methanogens : pushing the boundaries of biology,” vol. 0, no. October, 2018.

[75] S. Paul and A. Dutta, “Challenges and opportunities of lignocellulosic biomass for anaerobic digestion,” *Resour. Conserv. Recycl.*, vol. 130, no. March 2017, pp. 164–174, 2018.

[76] Q. Feng and Y. Lin, “Integrated processes of anaerobic digestion and pyrolysis for higher bioenergy recovery from lignocellulosic biomass: A brief review,” *Renew. Sustain. Energy Rev.*, vol. 77, no. March, pp. 1272–1287, 2017.

[77] I. H. Franke-Whittle, A. Walter, C. Ebner, and H. Insam, “Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities,” *Waste Manag.*, vol. 34, no. 11, pp. 2080–2089, 2014.

[78] P. P. Karhadkar, J.-M. Audic, G. M. Faup, and P. Khanna, “Sulfide and sulfate inhibition of methanogenesis,” *Water Res.*, vol. 21, no. 9, pp. 1061–1066, 1987.

[79] Zeshan, O. P. Karthikeyan, and C. Visvanathan, “Effect of C/N ratio and ammonia-N accumulation in a pilot-scale thermophilic dry anaerobic digester,”

Bioresour. Technol., vol. 113, pp. 294–302, 2012.

[80] M. Rasapoor, B. Young, R. Brar, A. Sarmah, W. Zhuang, and S. Baroutian, "Recognizing the challenges of anaerobic digestion : Critical steps toward improving biogas generation," *Fuel*, vol. 261, no. October 2019, p. 116497, 2020.

[81] O. Yenigün and B. Demirel, "Ammonia inhibition in anaerobic digestion: A review," *Process Biochem.*, vol. 48, no. 5, pp. 901–911, 2013.

[82] P. Jha and S. Schmidt, "Reappraisal of chemical interference in anaerobic digestion processes," *Renew. Sustain. Energy Rev.*, vol. 75, pp. 954–971, 2017.

[83] P. M. Fedorak and S. E. Hrudey, "The effects of phenol and some alkyl phenolics on batch anaerobic methanogenesis," *Water Res.*, vol. 18, no. 3, pp. 361–367, 1984.

[84] W. Yi-Tin, D. G. H., and P. Pin-Chieh, "Inhibition of Acetate Methanogenesis by Phenols," *J. Environ. Eng.*, vol. 117, no. 4, pp. 487–500, Jul. 1991.

[85] S. R. Shanmugam, S. R. Chaganti, J. A. Lalman, and D. D. Heath, "Effect of inhibitors on hydrogen consumption and microbial population dynamics in mixed anaerobic cultures," *Int. J. Hydrogen Energy*, vol. 39, no. 1, pp. 249–257, 2014.

[86] I. Itabaiana Junior, M. Avelar do Nascimento, R. O. M. A. de Souza, A. Dufour, and R. Wojcieszak, "Levoglucosan: a promising platform molecule?," *Green Chem.*, vol. 22, no. 18, pp. 5859–5880, 2020.

[87] M. Rezaee, S. Gitipour, and M.-H. Sarrafzadeh, "Different Pathways to Integrate Anaerobic Digestion and Thermochemical Processes: Moving Toward the

Circular Economy Concept," *Environ. Energy Econ. Res.*, vol. 4, no. 1, pp. 57–67, 2020.

[88] X. Yue, U. Arena, D. Chen, K. Lei, and X. Dai, "Anaerobic digestion disposal of sewage sludge pyrolysis liquid in cow dung matrix and the enhancing effect of sewage sludge char," *J. Clean. Prod.*, vol. 235, pp. 801–811, 2019.

[89] E. Taskin, C. de Castro Bueno, I. Allegretta, R. Terzano, A. H. Rosa, and E. Loffredo, "Multianalytical characterization of biochar and hydrochar produced from waste biomasses for environmental and agricultural applications," *Chemosphere*, vol. 233, pp. 422–430, 2019.

[90] M. H. McCrady, "STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTE-WATER (12th ed.)," *Am. J. Public Heal. Nations Heal.*, vol. 56, no. 4, p. 684, Apr. 1966.

[91] E. K. Orhorhoro, P. O. Ebunilo, and G. E. Sadjere, "Experimental Determination of Effect of Total Solid (TS) and Volatile Solid (VS) on Biogas Yield," vol. 3, no. 6, pp. 131–135, 2017.

[92] A. Waterhouse, "Waterhouse Lab," pp. 5–7, 2013.

[93] ASTM D 1762-84, "Standard Test Method for Chemical Analysis of Wood Charcoal.," *ASTM Int.*, vol. 84, no. Reapproved 2007, pp. 1–2, 2011.

[94] C. E. Brewer, K. Schmidt-Rohr, J. A. Satrio, and R. C. Brown, "Characterization of biochar from fast pyrolysis and gasification systems," *Environ. Prog. Sustain. Energy*, vol. 28, no. 3, pp. 386–396, Oct. 2009.

[95] X. Wang, X. Lu, F. Li, and G. Yang, "Effects of Temperature and Carbon-Nitrogen (C/N) Ratio on the Performance of Anaerobic Co-Digestion of Dairy

Manure, Chicken Manure and Rice Straw: Focusing on Ammonia Inhibition," *PLoS One*, vol. 9, no. 5, p. e97265, May 2014.

[96] M. Usman *et al.*, "Characterization and utilization of aqueous products from hydrothermal conversion of biomass for bio-oil and hydro-char production: A review," *Green Chem.*, vol. 21, no. 7, pp. 1553–1572, 2019.

[97] R. Boopathy, "Methanogenesis from furfural by defined mixed cultures," *Curr. Microbiol.*, vol. 44, no. 6, pp. 406–410, 2002.

[98] C. j. Rivard and karel Grohmann, "Degradation of furfural (2- furaldehyde) to methane and carbon dioxide by an anaerobic consortium," *Appl. Biochem. Biotechnol.*, vol. 28–29, no. 1, pp. 285–295, 1991.

[99] R. Kaur, R. D. Tyagi, and X. Zhang, "Review on pulp and paper activated sludge pretreatment, inhibitory effects and detoxification strategies for biovalorization," *Environ. Res.*, vol. 182, p. 109094, 2020.

[100] M. Stefaniuk and P. Oleszczuk, "Characterization of biochars produced from residues from biogas production," *J. Anal. Appl. Pyrolysis*, vol. 115, pp. 157–165, 2015.

[101] S. Alghashm, S. Qian, Y. Hua, J. Wu, H. Zhang, and W. Chen, "Properties of Biochar from Anaerobically Digested Food Waste and Its Potential Use in Phosphorus Recovery and Soil Amendment," 2018.

[102] Y. Shen, J. L. Linville, M. Urgun-Demirtas, R. P. Schoene, and S. W. Snyder, "Producing pipeline-quality biomethane via anaerobic digestion of sludge amended with corn stover biochar with in-situ CO2 removal," *Appl. Energy*, vol. 158, pp. 300–309, 2015.

[103] D. J. Brockway and R. S. Higgins, “Chapter 5 - BROWN COAL SAMPLING, ANALYSIS AND COMPOSITION,” R. A. B. T.-T. S. of V. B. C. Durie, Ed. Butterworth-Heinemann, 1991, pp. 247–278.

[104] S. A. Opatokun, T. Kan, A. Al Shoaibi, C. Srinivasakannan, and V. Strezov, “Characterization of Food Waste and Its Digestate as Feedstock for Thermochemical Processing,” 2016.

[105] J. H. Windeatt, A. B. Ross, P. T. Williams, P. M. Forster, M. A. Nahil, and S. Singh, “Characteristics of biochars from crop residues: Potential for carbon sequestration and soil amendment,” *J. Environ. Manage.*, vol. 146, pp. 189–197, 2014.

[106] Y. Shen, J. L. Linville, P. A. A. I. Leon, R. P. Schoene, and M. Urgun-demirtas, “Towards a sustainable paradigm of waste-to-energy process : Enhanced anaerobic digestion of sludge with woody biochar,” vol. 135, pp. 1054–1064, 2016.

[107] S. Bakshi, C. Banik, and D. A. Laird, “Estimating the organic oxygen content of biochar,” *Sci. Rep.*, vol. 10, no. 1, p. 13082, 2020.

[108] E. Lorenc-Grabowska, “Effect of micropore size distribution on phenol adsorption on steam activated carbons,” *Adsorption*, vol. 22, no. 4, pp. 599–607, 2016.

[109] T. R. K. C. Doddapaneni, R. Praveenkumar, H. Tolvanen, M. R. T. Palmroth, J. Konttinen, and J. Rintala, “Anaerobic batch conversion of pine wood torrefaction condensate,” *Bioresour. Technol.*, vol. 225, pp. 299–307, 2017.

[110] R. Subramanyam and I. M. Mishra, “Treatment of catechol bearing wastewater

in an upflow anaerobic sludge blanket (UASB) reactor: Sludge characteristics," *Bioresour. Technol.*, vol. 99, no. 18, pp. 8917–8925, 2008.

7. Appendix

A1. Total Phenolics Estimation – Gallic Acid Calibration

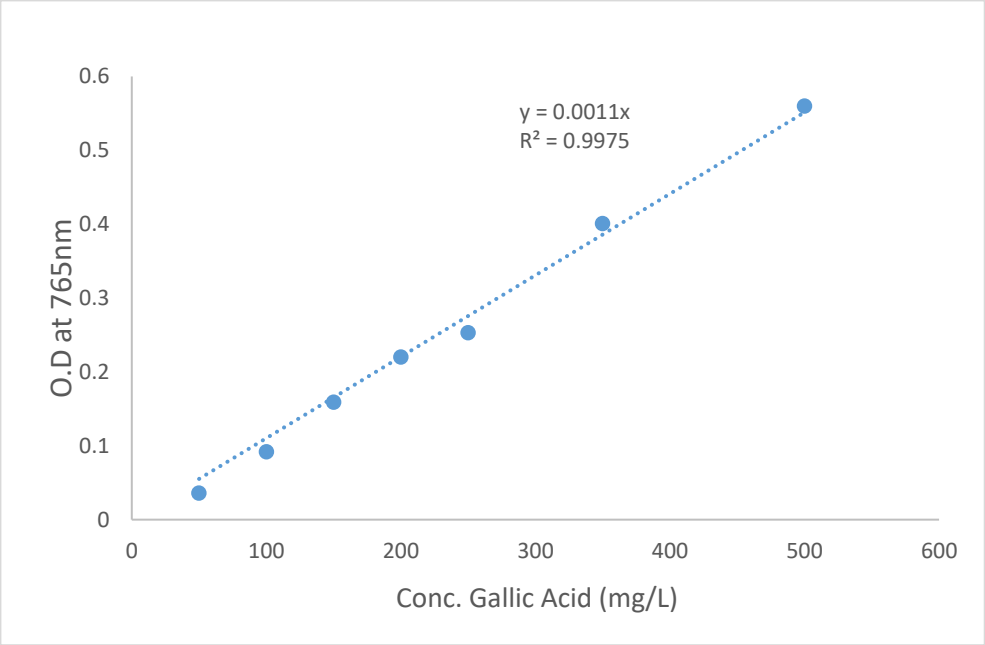

Figure A1: Standard curve for total phenolic estimation

A2. GC-MS Analysis

Table A1: List of all components identified in the APC, using GC-MS

% Area	Name
36.31	**Acetic acid**
7.73	Cholan-24-oic acid, 3-(acetyloxy)-7-oxo-, methyl ester, (3.alpha.,5.beta.)-
0.17	2-Propanol, 1-(1-methylethoxy)-
0.72	2-Butanone, 3-hydroxy-
0.7	**2-Propanone, 1-hydroxy-**
0.48	Silane, trimethylpropyl-
0.42	3-Penten-2-one, (E)-
6.69	**Propionic acid**
0.78	1-Hydroxy-2-butanone

0.13	Acetic acid, methyl ester
0.14	Butanedial
0.76	2-Furanol, tetrahydro-
0.21	Acetic acid, [(aminocarbonyl)amino]oxo-
0.09	meso-3,4-Hexanediol
0.22	Ethanol, 2-(diethylamino)-, N-oxide
0.2	Propanoic acid, 2-hydroxy-, 2-methylpropyl ester
0.33	**Furfural**
0.09	Butanal, 3-methyl-
0.33	2-Pentanone, 4-hydroxy-
1.15	3,5-Dimethylpyrazole-1-methanol
0.3	2-Cyclopenten-1-one
0.16	Ethanol, 2-(1-methylethoxy)-
0.23	Methanol, (methyl-onn-azoxy)-, acetate (ester)
0.23	1,6-Heptadien-4-ol
0.3	2-Butanone
0.36	2-Ethoxytetrahydrofuran
0.28	2-Propanone, 1-(acetyloxy)-
0.15	1,3-Butanediol, diacetate
0.33	2-Cyclopenten-1-one, 2-methyl-
0.29	Di(3-Methylbutyl)amine
0.17	Ethanone, 1-(2-furanyl)-
0.69	Butanoic acid, 4-hydroxy-
0.21	2,5-Hexanedione
0.43	2-Furancarboxaldehyde, 5-methyl-
0.22	1-Penten-3-ol, 4-methyl-
0.18	But-1-ene-3-yne, 1-ethoxy-

0.15	2(5H)-Furanone, 3-methyl-
2.06	Phenol
0.14	Pentanoic acid, 4-oxo-, methyl ester
0.23	2-Furanone, 2,5-dihydro-3,5-dimethyl
0.09	Tetrahydrofuran, 2-propyl-
0.09	1H-Pyrazole-5-carboxamide, N-(2-hydroxyethyl)-
0.11	Propanoic acid, 2-methyl-, propyl ester
1.07	**Lactic acid, monoanhydride with 1-butaneboronic acid, cyclic ester**
0.09	2H-Pyran-2-one, 5,6-dihydro-
0.18	2-Cyclopenten-1-one, 2,3-dimethyl-
0.17	Benzaldehyde, 2-hydroxy-
0.11	Oxirane, butyl-
1.48	Phenol, 2-methyl-
0.43	Benzoic acid, 3,17-diacetoxy-4,4,10,13-tetramethylhexadecahydrocyclopenta[a]phenanthren-7-yl ester
0.61	3-Ethyl-4-methyl-3-heptanol
1.81	Phenol, 3-methyl-
0.4	Phenol, 2-methoxy-
0.73	**Butyric acid**
0.39	1,3-Dioxolane, 2-methyl-2-(2-methylpropyl)-
0.95	Pentanal
1.18	Heptanoic acid, 6-oxo-
0.29	5-Ethyl-2-furaldehyde
0.29	Phenol, 2-ethyl-
0.42	Phenol, 2,4-dimethyl-
0.13	Benzaldehyde, 2-hydroxy-4-methyl-
0.34	Phenol, 3-ethyl-

0.16	Phenol, 2,3-dimethyl-
0.11	1-(3-Isopropenyl-2,2-dimethylcyclopropyl)-2-methylpropan-1-one
0.11	Phenol, 2-methoxy-4-methyl-
4.55	**1,2-Benzenediol ; Pyrocatechol ; Catechol**
1.93	**Isovaleric acid, 3-methylbutyl-2 ester**
0.93	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose
1.47	**2-Furancarboxaldehyde, 5-(hydroxymethyl)- ; 5-Hydrxoymethylfurfural**
0.22	2,3-Anhydro-d-mannosan
1	1,2-Benzenediol, 4-methyl-
0.3	**Isovaleric acid**
0.23	Resorcinol, 2-acetyl-
0.57	Hydroquinone
2.2	1,2-Benzenediol, 4-methyl-
0.2	Benzaldehyde, 3-hydroxy-
0.2	4(1H)-Isobenzofuranone, hexahydro-3a,7a-dimethyl-, cis-(.+/-.)-
0.35	Cyclohexanecarboxylic acid, phenyl ester
0.58	3,3,5,5-Tetramethylcyclohexanol
0.52	1,4-Benzenediol, 2-methyl-
0.28	p-Dodecyloxybenzaldehyde
0.71	4-Ethylcatechol
1.13	1,6-Anhydro-.beta.-d-talopyranose
0.24	1,3-Benzenediol, 4,5-dimethyl-
0.08	2(1H)-Pyridinone, 5-hydroxy-
0.19	**Formic acid, 2-propylphenyl ester**
0.13	2-Cyclopenten-1-one, 2,3,4,5-tetramethyl-
0.09	Phenol, 2-(1,1-dimethylethyl)-

0.08	4-Hydroxy-2,4,5-trimethyl-2,5-cyclohexadien-1-one
0.18	6-Nonenoic acid, methyl ester
0.13	2(3H)-Furanone, 4,5-dihydro-4-(2-methyl-3-methylenebut-4-yl)-
0.35	1,4-Diisopropyl cyclohexane
0.12	Sulfurous acid, nonyl 2-pentyl ester
10.3	**1,6-Anhydro-.beta.-D-glucopyranose (levoglucosan)**
0.09	Idosan triacetate

8. Curriculum Vitae

NEHA BATTA

SUMMARY

Biochemical Engineer with a background in Microbial Biotechnology and 5 years of research experience in bioremediation, sustainable and green technology. Strong communication skills. Technical expertise in

- Microbiological techniques – aerobic & anaerobic culture methods; sterilization; fermentation; strain development
- Analytical procedures – HPLC; GC; DNA isolation; PCR; protein estimation and purification; enzyme kinetics; UV-Vis; bacterial cloning
- Data Analysis; MS Word, MS Excel, MS Powerpoint
- Strong scientific communication skills

EDUCATION

Western University London, ON (Canada).
M.E.Sc Chemical and Biochemical Engineering Dec. 2020

National Law School of India University Bangalore, India
PG Diploma IPR Law Dec. 2011

Panjab University Chandigarh, India
M.Sc Microbial Biotechnology Aug. 2010

Panjab University Chandigarh, India
B.Sc (Hons.) Biotechnology Aug. 2008

PROFESSIONAL EXPERIENCE

University of Western Ontario London, Ontario, Canada

Graduate Research Assistant Sep. 2018 – Dec. 2020

- Worked on the research project entitled "Anaerobic digestion of Aqueous Pyrolysis Condensate for the production of Biogas".
- Part of the W2R (Waste to Resource) initiative at ICFAR (Institute for Chemicals and Fuels from Alternative Resources), a part of the University of Western Ontario.

University of Western Ontario London, Ontario, Canada

Graduate Teaching Assistant
Fundamentals of Bioprocess and Bio-reaction Engineering Sept 2020-Dec 2020

- Designed and conducted labs for enzyme kinetics and microbial growth kinetics
- Responsible for marking reports; office hours for students

Genetics Jan 2020-April 2020

- Held tutorials; marking reports and quizzes; proctoring exams; office hours

Fundamentals of Bioprocess and Bio-reaction Engineering Sept 2019-Dec 2019

- Designed and conducted labs for enzyme kinetics and microbial growth kinetics
- Responsible for marking reports; office hours for students

DST- Centre for Policy Research, Panjab University **C**handigarh, India

Scientific Officer Mar 2014 – Mar 2015

- Knowledge partner to the Government of India for development of Science and Technology indicators to enhance Industry Academia interactions and PPP in R&D at national level
- Perform case studies to observe the working of industry-institute facilitating bodies
- To conduct conferences, seminars, round table meetings and so on to bring industry-institute compliance and promote PPP in R&D.

The Energy and Resources Institute (TERI) New Delhi, India

Project Associate (Junior Research Fellow) Apr 2012 – Oct 2013

- Entrusted with the responsibility of DBt-NER funded, collaborative project entitled *'Treatment of Oil Field Formation Water with In-situ Generated Bioflocculant'*

Sanjeevni Institute of Biosciences and Applied Research (SIBAR) India

Faculty Oct 2011 – Mar 2012

- Giving Industrial Training to students in Microbiology and Molecular Biology.
- Formulation and reviewing of respective Industrial training Modules.

Orbit Biotech Pvt. Ltd. Mohali, Punjab, India.

Scientific Officer Mar 2011 – Sep 2011

- Imparting industrial training to students in the following: Microbiology, Molecular Biology, Proteomics and Anti-microbial chemotherapy.
- Formulation and reviewing of all the Industrial training Modules.
- Conducting customized workshops in colleges.
- Planning, organizing and executing contract research projects.

OTHER ACADEMIC RESEARCH

- **Project Title: '***Cloning, expression, partial purification and quantification of deletion mutant of human protein – Gelsolin':*

The project involved cloning of the gene of interest into *E.coli* strain DH5α and its expression in *E.coli* strain BL21 (DE3) using vector pET 303 CT-His tag. The protein was purified using sonication and affinity chromatography; quantification was done using bioinformatics tools and SDS-PAGE.

- **Project Title:** *'Isolation and partial Characterization of Probiotic Bacteria from food samples'*
  A total of 20 samples were screened to get pure cultures which were tested for bacteriocin production against 4 test organisms.

PUBLICATIONS:

- Sarchami, T., Batta N., Rehmann, L., Berruti, F**.** (2020) '**Removal of Phenolics from Aqueous Pyrolysis Condensate by Activated Biochar**'. Canadian Journal of Chemical Engineering (Submission number: CJCE-20-0654)
- Subudhi S, Bisht V, Batta N, Pathak M, Devi A, Lal B. **Purification and characterization of exopolysaccharide bioflocculant produced by heavy metal resistant *Achromobacter xylosoxidans***. Carbohydr Polym. 2016 Feb 10; 137:441-451.
- Subudhi S, Batta N, Pathak M, Bisht V, Devi A, Lal B, Al khulifah B. **Bioflocculant production and biosorption of zinc and lead by a novel bacterial species, *Achromobacter sp*. TERI-IASST N, isolated from oil refinery waste**. Chemosphere. 2014 Oct; 113:116-24.
- N. Batta, S. Subudhi, B. Lal, A. Devi. **Isolation of a lead tolerant novel bacterial species, *Achromobacte*r sp. TL-3: assessment of bioflocculant activity**. Indian J Exp Biol, 51 (2013), pp. 1004-1011

VOLUNTEER EXPERIENCE

- Partner, On-COVID-19 Project (August 2020 – Present)
- V.P Finance, Graduate Engineering Society (September 2019 – September 2020)
- Committee member. Chemical & Biochemical Engineering Grad Society (September 2019 –December 2020)
- Councilor, Graduate Engineering Society (September 2018 - August 2019)
- Volunteer at CAGIS (Canadian Association for Girls in Science)– September 2018 until April 2019

SCHOLARSHIPS / AWARDS

- The Ross and Jean Clark Scholarship in Environmental Engineering at the University of Western Ontario.
- DST-Travel Grant for international conference (India).