ANAEROBIC DIGESTION BIOREFINERY TO PRODUCE BIOENERGY AND BIOBASED PRODUCTS USING HIGH YIELDING TROPICAL FEEDSTOCK

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAIʻI AT MĀNOA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

MOLECULAR BIOSCIENCES AND BIOENGINEERING

AUGUST 2017

By

Chayanon Sawatdeenarunat

Dissertation Committee:

Samir Kumar Khanal, Chairperson

PingSun Leung

Shihwu Sung

Rajesh Jha

Reza Ghorbani

To my wife, parents, and family

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my advisor Dr. Samir Kumar Khanal for the continuous support during my Ph.D. study, for his patience, motivation, and above all immense knowledge in the field of anaerobic digestion. His guidance helped me to successfully conduct my research and completion of this dissertation.

Besides my advisor, I would also like to thank the rest of my dissertation committee members: Dr. PingSun Leung, Dr. Shihwu Sung, Dr. Rajesh Jha, and Dr. Reza Ghorbani for their insightful comments and encouragements as well as critical comments which helped me broaden up my research skill.

My sincere thanks also go to Ryan Kurasaki for the bioreactor design and fabrication. Dr. Brian Turano and Dr. Richard Ogoshi for their help in supplying Napier grass samples, as well as Dr. Halina Zaleski for helping me with the experimental design. I am equally indebted to Dr. Sushil Adhikari and Dr. Hyungseok Nam of Auburn University for their help on the thermochemical processing of the digestate/raw Napier grass samples. Without their supports, it would not have been possible to conduct this research.

I would also like to thank my awesome friends as well as lab-mates in the Environment and Bioenergy Research Group: Duc, Sumeth (Fee), Chettaphong (Jom), Surendra, Ed, Fernanda, Kwon, and Misheel, and other former members for their friendships and supports throughout my study period at UHM.

I would like to thank my grandma, Kaneungsuk, my father, Chainun, and to my brothers and sisters in Thailand for supporting me spiritually throughout the Ph.D. study period and

my life in general. I would also like to deeply thank my mother, Wapara, the first person convincing me to pursue Ph.D. studies. This degree is particularly for you mom. Last but not the least; I would like to show my appreciation to my wife, Worapon (Poon), who is always the wind underneath my wings since the very first day she came to my life. It would not have been possible to complete this degree without her relentless supports. Thank you for your love, care and above all in believing and trusting me.

ABSTRACT

A series of batch experiments were conducted to investigate the effects of inoculum type, oxygen (O_2) dosage, and incubation time on volatile fatty acids (VFAs) production during anaerobic digestion (AD) of Napier grass (*Pennisetum purpureum*), a high yielding energy crop. The results showed that anaerobically digested cattle manure (ADCM) was the appropriate inoculum for VFAs production form Napier grass. Additionally, the incubation time of 3 days and O_2 dosage of 15 mL/g volatile solids $(VS)_{added}$ showed the highest VFAs production when ADCM was used as an inoculum. The semi-continuous bench-scale experiment was then performed using horizontal acid bioreactor to investigate the effect of micro-aeration on VFAs production from Napier grass. The VFAs produced during micro-aeration condition was significantly higher than that of anaerobic condition. The produced methane was significantly decreased during micro-oxygenation, thus, the methanogens were inhibited by the injected oxygen. The soluble chemical oxygen demand (SCOD) was also significantly enhanced by micro-aeration resulting in more available soluble organic substrates for acidogens. Hemicellulose was the main component of biomass degraded during AD, whereas cellulose and lignin were preserved in the digestate.

AD of Napier grass as a mono-substrate was also investigated for biomethane production. Two semi-continuous bench-scale horizontal bioreactors were operated in parallel for over 300 days, and the highest organic loading rate of 6 $kgVS/m^3$-d was achieved during long-term operation with average methane yield of 112.48±9.03 NmL/gVS_{added}. The methane yield accounted for more than 90% of the methane potential of the raw Napier grass.

Similar to the acid bioreactor, hemicellulose was the main component of lignocellulosic biomass contributed to methane production, while cellulose and lignin remained in the digestate. This cellulose-rich fiber and lignin was further examined for bioenergy potential via thermochemical conversion, e.g., torrefaction and hydrothermal carbonization (HTC), and showed the identical energy contents with that of bituminous coal. The techno-economic analysis indicated that torrefaction might be the most appropriate thermochemical process for digestate utilization. Thus, this study provided the first time successful integration of anaerobic digestion and thermochemical treatment for complete utilization of whole plant biomass representing a true biorefinery for lignocellulosic biomass.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AD:	Anaerobic digestion
ADCM:	Anaerobically digested cattle manure
ADF:	Acid detergent fiber
ADL:	Acid detergent lignin
ADWAS:	Anaerobically digested waste activated sludge
ANOVA:	Analysis of variance
AW:	Annual worth
BMP:	Biochemical methane potential
C/N:	Carbon to Nitrogen
CH_4:	Methane
CHP:	Combined heat and power
CO_2:	Carbon dioxide
COD:	Chemical oxygen demand
DM:	Dry matter
DTG:	Derivative thermogravimetric
FC:	Fixed carbon
H_2:	Hydrogen
HAc:	Acetic acid
HHV:	Higher heating value
HRT:	Hydraulic retention time
HTC:	Hydrothermal carbonization
MARR:	Minimum acceptable rate of return

MSL:	Mean sea level
NDF:	Neutral detergent fiber
OLR:	Organic loading rate
ORP:	Oxidation reduction potential
RCBD:	Randomized Complete Block Design
SCOD:	Soluble chemical oxygen demand
SEM:	Scanning electron microscopy
SRT:	Solids residence time
TGA:	Thermogravimetric analysis
TS:	Total solids
VCM:	Volatile combustible matter
VFAs:	Volatile fatty acids
VS:	Volatile solids

CHAPTER 1

INTRODUCTION

1.1 Background

Our heavy reliance on fossil-based fuels and products has serious implications on energy security and environment. Bioenergy and bio-based products derived from renewable bioresources are considered as potential alternatives to non-renewable fossil fuels and materials. There are two major pathways, namely thermochemical and biochemical, for converting the bioresources into bioenergy and bio-based products. Biochemical pathway is widely studied due to its potential for further cost reduction and environmentally benign process. Anaerobic digestion (AD) is one of the widely applied biochemical pathways to produce renewable bioenergy from diverse organic substrates ranging from high solid feedstocks (i.e., animal manures, food wastes, municipal solid waste, agri- and forest residues, and energy crops) to municipal and industrial wastewaters (Khanal and Li, 2017; Khanal, 2008). Although, AD technology was originally developed and applied for waste stabilization, especially human excreta and municipal sludge, AD process has now been widely applied for bioenergy production worldwide. There are over 14,000 commercial AD plants in operation in Europe, and Germany alone has more than 8,000 plants in operation (EBA, 2014). The produced biogas is used for producing electricity and heat using combined heat and power (CHP) unit, and/or upgraded to methane gas (> 97%) to be used as transportation fuels, or injected into natural gas grid (Khanal and Li, 2017). Recently, the focus on the bioenergy production via AD has shifted towards the use of lignocellulosic biomass due to year

round availability with consistent quality, and ubiquitous in distribution. Moreover, lignocellulosic biomass is the most abundant renewable bioresource on Earth, with year round availability of over 200 billion dry metric tons per year (Zhang et al., 2007). The United States alone has the potential to produce 1.1 billion dry metric tons of biomass annually which could replace 30% of transportation fuel demands (USDA and USDOE Joint Report, 2005). Lignocellulosic biomass primarily composes of cellulose, hemicellulose and lignin, and the interactions of these components make it highly recalcitrant to degradation. Several studies focused on enhancing the deconstruction of lignocellulosic biomass into simple sugars (such as C-5 and C-6 sugars) through physical, chemical, biological and hybrid pretreatments in the production of liquid biofuels (primarily ethanol) via biochemical pathways (FitzPatrick et al., 2010; Takara and Khanal, 2011). Pretreatment is not only costly; but it also generates large amount of liquid and solid wastes which require further treatment before disposal (Wan and Li, 2012) . The high pretreatment cost and biomass loss in the waste stream are the major challenges in achieving the economic viability as well as environmental sustainability of the lignocellulose-based liquid biofuels and bio-based products (Shrestha et al., 2008; Monlau et al., 2013; Hendriks and Zeeman, 2009; Alvira et al., 2010; Agbor et al., 2011; Kumar et al., 2009). AD has several inherent merits (e.g., no requirements for pretreatment and costly enzymes, no generation of toxic wastes, robustness of the process) with respect to digesting lignocellulosic biomass (Sawatdeenarunat et al., 2015). However, the challenge of AD of lignocellulosic biomass is scum formation (Thamsiriroj and Murphy, 2010), low digestibility, and nutrients deficiency during mono-digestion among others. Scum formation can result in floating of biomass on the top and

accumulation on the surface of reactor thereby causing poor contact between the microbes and the substrate. The poor mass transfer inside the bioreactor can result in low methane yield and increase the operation and maintenance costs (GÖMEÇ, 2006). To overcome the problem of scum formation, a horizontal bioreactor can be employed, which has several merits over a conventional vertical bioreactor, such as higher surface area per volume, lower propensity of scum formation and short circuiting, and better mixing (Karthikeyan and Visvanathan, 2012). Anaerobic co-digestion of lignocellulosic biomass with nutrient-rich substrates such as animal manure, food waste etc., would balance the nutrient deficiency and would enhance the biogas production with better process stability (Mussoline et al., 2012). However, the access of nutrient-rich substrates in close vicinity of lignocellulosic biomass is often limited (Lebuhn et al., 2008). Thus, mono-digestion becomes the only option in developing a decentralized AD system for bioenergy production. One of the avenues for achieving both the economic viability as well as environmental sustainability of bioenergy production is to adopt a biorefinery concept. According to National Renewable Energy Laboratory (NREL), "a biorefinery is a facility that integrates biomass conversion processes and equipment to produce fuels, and chemicals from biomass." Thus, the biorefinery approach aims at converting biomass into multiple products very similar petroleum refinery. Bioenergy, which is considered low-value and high volume product, will meet our energy demand whereas the high-value low value bio-based products will enhance profitability. In conventional biomass-to-biofuel conversion process, variation in biomass composition (which varies with biomass species, geographical locations, and crop growing conditions among others) has been treated as a challenge because such differences in the biomass composition result

variation in the consistency and yield of the end products. However, under biorefinery concept such variation is treated as an opportunity for producing diverse value added products. Anaerobic biorefinery is one of the biorefinery concepts, in which AD serves as a centerpiece to produce high-value, but low volume products (i.e., biochemicals to enhance economic viability of the system) and high-volume but low value products (i.e. heat, electricity, and conventional transportation biofuels to achieve energy security).

1.2 Objectives of the study

The overall goal of this research is to optimize AD process to maximize bio-based (i.e., VFAs, torrefied biochars, and hydrochars) and bioenergy (i.e., methane) production utilizing Napier grass by adopting biorefinery concept and conduct techno-economic analysis of the system. The specific objectives are to:

(1) optimize AD process to maximize VFAs production from Napier grass using micro-oxygenation via series of batch studies

(2) examine VFAs yield from Napier grass using an micro-aerated horizontal bioreactor

(3) examine anaerobic biorefinery potential of Napier grass as a feedstock by integrating anaerobic digestion with thermochemical conversion

(4) conduct a techno-economic analysis of AD biorefinery processes

1.3 Scope of the study

The study evaluates VFAs production during AD of Napier grass using micro-oxygenation in both batch studies and semi-continuous bench-scale horizontal reactors.

The horizontal reactors were also used to produce multiple products via AD (i.e. methane from raw Napier grass and biochar and hydrochar from digested fiber) were also studied. Techno-economic analysis was also performed to assess the economic feasibility of the biorefinery process.

CHAPTER 2

LITERATURE REVIEW

2.1. Anaerobic digestion

Anaerobic digestion (AD) is a microbial process mediated by diverse microbial communities to convert various organic substrates into biogas (primarily the mixture of methane (CH_4) and carbon dioxide (CO_2)) in the absence of oxygen. AD process involves four major metabolic stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Khanal, 2008). Hydrolysis is the first step of the conversion process in which complex organic matters such as protein, carbohydrate and lipid are broken down to simple soluble forms. The facultative hydrolytic or fermentative bacteria play an important role in this bioconversion by excreting extracellular enzymes. Hydrolysis can be a rate-limiting step for AD of complex substrates such as lignocellulosic feedstocks due to their recalcitrance to biodegradation, which is attributed to strong interactions among lignin, cellulose and hemicellulose. The simpler compounds from the first stage are fermented by acidogenic bacteria and produce hydrogen (H_2) CO_2, alcohols, volatile fatty acids (VFAs) with C > 2. In acetogenesis stage, alcohols and VFAs are then converted into acetate by acetogenic bacteria. Hydrogen-oxidizing acetogenic bacteria, known as homoacetogens can also utilize H_2 and CO_2 to generate acetate via homoacetogenesis. Finally, in methanogenesis stage, acetate is converted into CH_4 by aceticlastic methanogens. The hydrogenotrophic methanogens can also produce CH_4 by utilizing H_2 and CO_2. The schematic of AD process is shown in Figure 2.1.

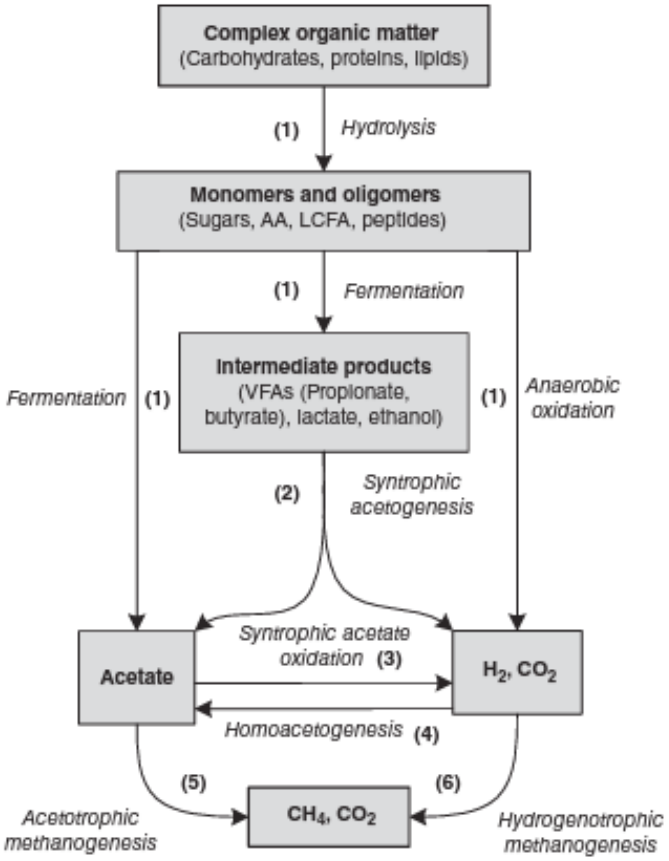


Figure 2.1. The schematic of anaerobic digestion process (AA is amino acid, VFAs is volatile fatty acids, and LCFA is long chain fatty acid) (adopted from Li and Khanal, (2017))

2.2. Lignocellulosic biomass as a feedstock

Lignocellulosic biomass is the most abundant feedstock on earth with availability of over 200 billion dry metric tons per year (Zhang et al., 2007), and has been widely reported as a potential feedstock for producing biofuels and bio-based products. Some of the examples of lignocellulosic biomass include agri and forest residues, and energy crops (Cherubini, 2010). Lignocellulosic biomass is primarily composed cellulose,

hemicellulose, and lignin along with small amount of other organic compounds such as proteins, lipids, and extractives (Frigon and Guiot, 2010). The proportion of these components significantly varies with the plant types, growth conditions as well as the maturity stages (Sawatdeenarunat et al., 2015). Cellulose is a homopolysaccharide, which is monomer of β-D-glucopyranose. The degree of polymerization of cellulose is around 10,000 where single units are linked to each other by β-(1-4)-glycosidic bond (Kumar et al., 2008). Typically, cellulose has two different structural forms, namely crystalline and amorphous. The crystalline cellulose has high packing density resulting from the high hydrogen bonding and makes it highly resistant to chemical and biological degradation. (Brown, 2003; Cherubini, 2010; Kumar et al., 2008). Hemicellulose, on the other hand is a heteropolysaccharide presenting in the plant cell wall with a degree of polymerization between 100 and 200. Hemicellulose consists of several monomeric sugars, namely xylose, glucose, galactose, arabinose, and mannose (Brown, 2003). The lower degree of polymerization and its amorphous structure makes hemicellulose more vulnerable to chemical, thermal or biological degradation than the cellulose (Cherubini, 2010). Lignin is a phenylpropane-based polymer consisting of aromatic alcohols, namely coniferyl, sinaply and coumaryl alcohols as the building blocks. Typically, lignin acts as glue and provides rigidity to plant cell wall. Since, lignin is a non-carbohydrate component of biomass, it cannot be hydrolyzed into monomeric sugars. Thus, it remains in the digestate. Lignocellulosic biomass can be categorized to 3 groups namely, agricultural residues, forest resources and residues, and energy crops. The details are presented in the following section.

2.2.1. Agricultural residues

Agricultural residues are biomass (i.e. leaves, stalk, stem and seed) left in the cultivation field after harvesting. This feedstock could be generated almost 0.3 billion dry metric tons annually (Perlack et al., 2005). Normally, agricultural residues could be used as soil amelioration, for nutrient recycle, and animal bedding (Bentsen et al., 2014). However, it also has been burnt during land preparation of the next cultivation (Monlau et al., 2015a). The emission of many air pollutants during this process could cause a serious health to a nearby community. However, it was estimated that the potential of global energy production from agricultural residues could reach between 10 and 69 EJ/year in 2050 (Bentsen et al., 2014). The agricultural residue could be used to produce bioenergy (i.e. hydrogen and methane via AD, and bioethanol via fermentation) and solid-fuels (i.e. biochar via torrefaction and hydrochar via hydrothermal carbonization) following biorefinery concept (Sawatdeenarunat et al., 2016). Thus, the conversion of agricultural residue to bioenergy and bio-based product could simultaneously mitigate energy and environmental issues.

2.2.2. Forest resources/residues

The forest resources include residues produced during many activities during forestry i.e. the harvesting of forest products, fuelwood from forestlands, and residues generated at processing mills (Perlack et al., 2005). However, forest residues such as logging residues and clean wood chips could be thermochemically processed to produce high-density solid fuels via torrefaction (Phanphanich and Mani, 2011). Moreover, the forest residues could also be used to produce biochar, syngas, and bio-oil via gasification (Anderson et al., 2013). It was presented that the potential of forest resources production

was approximately 1 billion dry metric tons/year (Perlack et al., 2005). Thus, it could be sustainable served as the feedstock to produce bioenergy and bio-based products.

2.2.3. Energy crops

Energy crops are many species of plants cultivated specifically for producing varieties of bioenergies and biobased-products (Brown, 2003). McKendry (2002) informed the idle properties of energy crops as high yield, low energy requirement for producing, low nutrient consumption and low overall cost. It was estimated that energy crops had potential to generate approximately 400 EJ/year by 2050 (Sims et al., 2006). Energy crops can also be divided into many groups based on the characteristics and expected product from the conversions. Oil crops such as oilseed rape, linseed, field mustard, hemp, sunflower, safflower, castor oil, olive, palm, coconut, and groundnut can be used to produce biodiesel via transesterification (Sims et al., 2006). Starch and sugar crops including but are not limited to sugar beet, sugar cane, and energy cane are able to use as a substrate to produce bioethanol via fermentation and distillation (Sims et al., 2006). Lignocellulosic energy crops are plant cultivated to produce mainly, lignocellulose for biologically as well as thermochemically converted to biofuels (i.e. syngas, bio-oil, methane, hydrogen, and ethanol) and bio-based products (VFAs, biochar, and hydro char) (Brown, 2003). The example of this biomass feedstock is herbaceous energy crops which consist of many species including sugarcane, energy cane, and Napier grass. The chemical characteristics of herbaceous crop are more similar to hardwood than softwood such as low lignin content. However, the high silica content might be an issue for thermochemical processes (Brown, 2003). The compositions of some typical lignocellulosic energy crops are presented in table 2.1.

Table 2.1.The composition of some typical lignocellulosic feedstocks (Karthikeyan and Visvanathan, 2012; Surendra and Khanal, 2014, and Monlau et al., 2012)

Lignocellulosic feedstocks	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Corn stover	37.5	22.4	17.6
Corn fiber	14.3	16.8	8.4
Wheat straw	38.2	21.2	23.4
Switch grass	31.0-45.0	20.0-31.0	12.0-18.0
Bagasse	38.2	27.1	20.2
Sugarcane	25.0	17.0	12.0
Rice straw	32.0	24.0	13.0
Giant reed stalk	33.1	18.5	24.5
Giant reed leaves	20.9	17.7	25.4
Sunflower stalk	31	15.6	29.2
Biomass sorghum	22.2	19.4	21.4
Napier grass	43.0	18.7	11.3

2.3. Napier grass (*Pennisetum purpureum*)

Napier grass (*Pennisetum purpureum*) is a perennial C-4 grass species that typically grows between 6.5 to 11.5 ft (2-3.5 m) tall. Although Napier grass is native to Africa, it has been naturalized in many of the tropic and sub-tropic regions of the world including Hawaii. Napier grass belongs to sugarcane family and has many morphological

similarities. Napier grass is often used as windbreaks due to its height and dense growth. Young Napier grass is extremely palatable and often is use forage for all ruminants, and can be fed as hay or pellets. As the plant starts to mature, the stalk becomes hard and coarse, and the plant has little use other than to prevent soil erosion or as a wind breaker. In recent years, there has been growing interest on Napier grass as a second generation feedstock for producing bioenergy and biobased products (Surendra and Khanal, 2014). Napier grass can be grown in tropical and sub-tropical regions of the world up to elevation of 2,000 m above mean sea level (MSL), at annual rainfall ranging from 750 to 2,500 mm (Bayer, 1990; Nyambati et al., 2010) and temperature of 30-35°C. Inputs of nutrient and irrigation have significant effect on the growth of Napier grass and fertilization is one of important factors affecting Napier grass yield. However, due to deep root system, Napier grass also is drought tolerant (Samson et al., 2005). The Napier grass yields as dry matter (DM) at different fertilization rate (e.g., nitrogen, phosphorus, potassium, and micronutrients) are summarized in Table 2.2. (Samson et al., 2005). Zewdu et al. (2002) reported the effect of nitrogen application on Napier grass yield in Ethiopia and recommended that a dosage of 92 kg nitrogen/ha could lead to the optimum dry matter and crude protein yields. In another study, Samson et al.(2005) recommended an application rate of 100 kgN/ha to achieve high yield.

Table 2.2. Napier grass yield at different fertilization rate (adopted from Samson et al. (2005))

Fertilization rate	Yield (dry metric ton/ha)	Reference
168-42-64 N-P_2O_5-K_2O	33.4	Prine and Woodard, 1994
200-22-83 N-P_2O_5-K_2O	45.7	Woodard and Prine, 1993
200-22-83 N-P_2O_5-K_2O	47	Woodard and Prine, 1993
168$(NH_4)_2SO_4$	56.5	Mislevy et al., 1989

Napier grass yield varies depending on several factors, including solar radiation, irrigation/rainfall, harvesting age, fertilizer input, and soil characteristics among others. In Florida, Napier grass yield was 2-fold when it was well fertilized and harvested at the height of 3 cm above the soil at the age of 6 months old compared to those harvested at the age of 1.5 months (Calhoun and Prine, 1985). The effect of ratooning on Napier grass yield was also studied by Osgood et al. (1996) as presented in table 2.3. Although, the wet weight yield of the ratoon crop was slight lower than the planted crop,the dry weight yield of the ratoon crop was higher due to it lower moisture content. The biomass yield, however decreased from 24.8 to 22.8 dry metric ton/ha when compared between year 1 and year 3 (Na et al., 2015). Woodard and Prine (1991) also showed the decreasing trend of the biomass yield with the ratooning of the plant.

Table 2.3. Napier grass yield of planted and ratoon crops (Adopted from Osgood et al., 1996).

Yield*	Planted crop	Ratoon crop
Fresh weight (metric ton/acre)	58.1	55.6
Total Solid (% fresh weight)	28.4	35.8
Dry weight (metric ton/acre)	16.7	20.0

(Note: * Harvest after 7.7 months of cultivation for the planted crop and 8 months for the ratoon crop.)

2.4. Methane Production Potential of Various Energy Crops

Energy crops are specifically cultivated to use as feedstocks for bioenergy/biofuel production. One of the key parameters to assess the potential of energy crop for bioenergy production CH_4 yield per unit area. The factors affecting the CH_4 yield include crop species, crop maturity, biomass preprocessing/pretreatment etc. (Amon et al., 2007a). Biochemical Methane Potential (BMP) is an effective and reliable method to evaluate the methane production as well as the anaerobic digestibility of organic substrates. The methane potentials of various energy crops are summarized in the Table 2.4.

Table 2.4. The biomethane potentials of some energy crops (Prochnow et al., 2009; Weiland, 2003)

Crop	Methane potential (m^3CH_4/ha/year)
Forage beet with leave	5,800
Maize	5,780
Wheat	2,960
Barley	2,030
Ryegrass	4,060
Alfalfa	3,965
Clover	2,530
Perennial ryegrass	2,041
Meadow fescue	2,621
Sunflower	3,300
Pressed sugar beet pulp silage	6,173

The economic feasibility of AD is strongly depended on the CH_4 potential, which in turn is governed by the feedstock composition. The composition of the feedstock are affected by many factors, i.e. the geographical location, the biomass maturity, and the management practices (Amon et al., 2007b). Amon et al. (2007) examined the effect of harvesting time on the biogas production from maize silage in Austria and reported that the appropriate harvesting time was at the end of wax ripeness (harvested after 122 days)

in which the plant DM was around 35-39%. At full ripeness (harvested after 151 days), only slight increase in methane production was obtained. This was because the carbon to nitrogen (C/N) ratio of the harvested maize at full ripeness was 42, which was higher than the recommended range for AD, i.e., 20-30 (Khanal, 2008). In contrary, the maize hybrids showed the maximum methane yield per unit area at full ripeness because of the higher volatile solids (VS) content. which could have compensated the lower methane yield (Schittenhelm, 2008). For cereals, the harvesting should be done during "grain in the milk stage" to "grain in the dough stage" and the first cut of grasses should be done after "ear emergence stage" to obtain high CH_4 yield (Amon et al., 2007a). Other energy crops also showed different appropriate harvesting periods. The overall goal of feedstock production is to maximize methane production per unit area. The lignin content of the substrate is also a factor affecting the CH_4 potential of the energy crops. The higher lignin content in the plant structure could lead to the lower CH_4 production due to its recalcitrance to anaerobic biodegradation (Triolo et al., 2011). The authors also reported that the lignin concentration greater than 100 g/kg VS was a critical point for anaerobic biodegradation, and the CH_4 potential was significantly low.

2.5. Anaerobic biorefinery

As indicated earlier, AD has been widely adopted to produce renewable bioenergy (i.e. methane and hydrogen) from agri-wastes (i.e., animal manure and crop residues) and dedicated energy crops (e.g., lignocellulosic biomass). However, the conversion of dedicated energy crops into CH_4 alone may not sufficiently justify the capital and operational costs associated with building a commercial biogas facility (Sawatdeenarunat et al., 2016). More likely, AD can be integrated into a biorefinery as an effective

technology for treating and recovering high-value products with simultaneous pretreatment (biological) of lignocellulosic biomass. The schematic diagram of an anaerobic biorefinery concept for biofuel and bio-based production is presented in the Figure 2.2. AD technology for treating and recovering resources from organic substrates not only generates bioenergy and bio-based products but also reduces the cost of waste disposal and ultimately the environmental footprint of such industries. When operating parameters (e.g., solids residence time (SRT), pH etc.) in AD system are controlled properly, the consortium of microorganisms present in the digester can selectively convert the plant extractives and hemicellulose into biogas, while effectively exposing lignin and cellulosic fibers in the digestate (Teater et al., 2011).In later downstream processes, the commercial enzymes (i.e., cellulases) can be added to saccharify cellulose into soluble glucose. The monomeric sugars can then be used as precursors in the production of diverse products ranging from bioenergy/biofuel (i.e., CH_4, H_2, ethanol and butanol) (Agler et al., 2011; Rabelo et al., 2011; Kaparaju et al., 2009) to organic acids (e.g., succinic acid) and biopolymers (e.g., bioplastic) (FitzPatrick et al., 2010; Cherubini and Strømman, 2011). The insoluble solid residue following the enzymatic hydrolysis, consisting of mainly lignin, can either be combusted for heat and electricity generation or be further processed into different bio-based products such as lignosulfonates. The effluent (the liquid stream after separating solid residue from the digestate) in general is rich in nutrients and remaining organic matters thus needs to be treated before being disposed into the environment. Though the effluent from most of the AD plants can be land applied as fertirrigation, the high concentration of certain metals, such as copper and zinc (in the case of digester fed with animal manure where such elements originates from

the micro- and macro-nutrients supplemented in animal feed) or ammonia in the effluent could cause phytotoxicity (Alburquerque et al., 2012). A significant opportunity exists in utilizing the generated effluent for macro- and micro-algae production. Such effluent-based algae cultivation offers the benefit of nutrient removal from the effluent (which can be recycled back as process water into AD plant) as well as algal biomass production which can be further processed into biofuels and bio-based products. The integration of AD in biorefinery concept as a technology for biofuels and bio-based products generation is discussed in the following section.

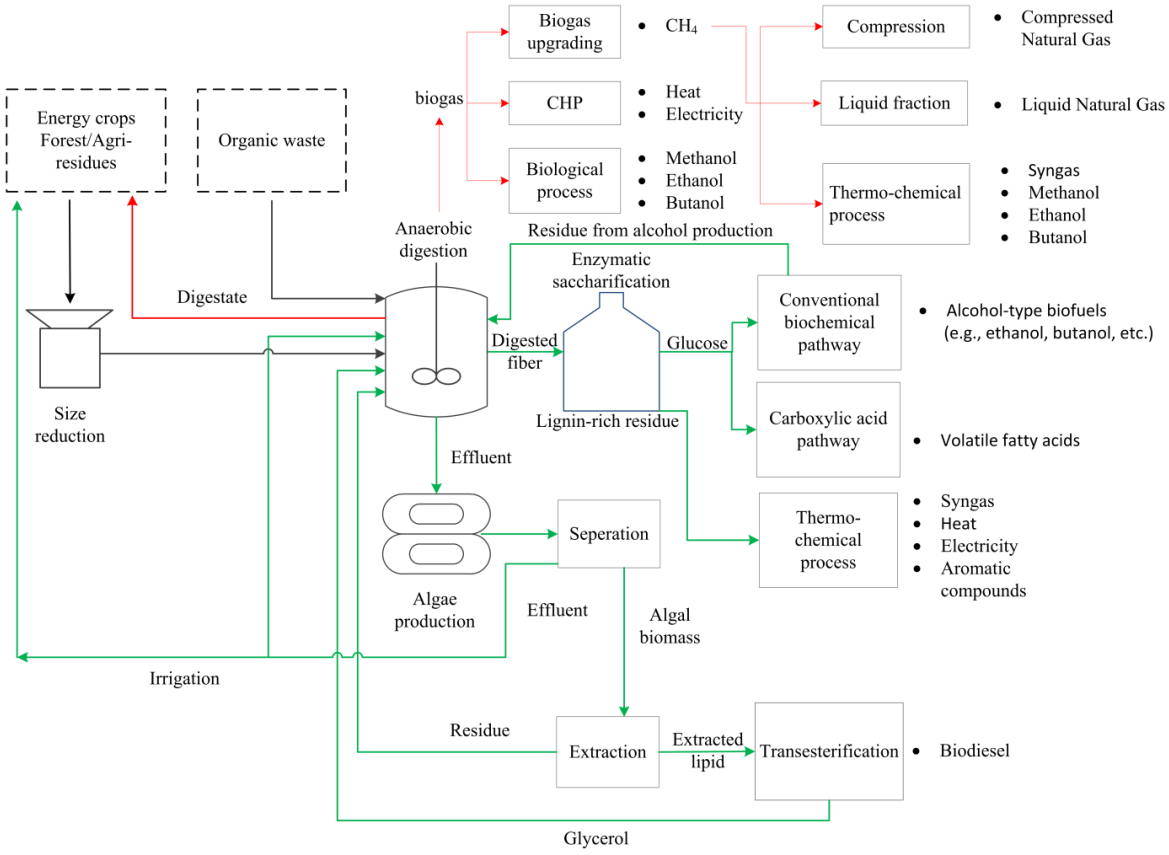


Figure 2.2. The schematic of an anaerobic digestion based biorefinery concept for producing biofuels and bio-based products (adopt from Surendra et al. (2015))

2.5.1. Anaerobic digestion for volatile fatty acids production

VFAs are low molecular weight organic acids (with carbon > 2) produced during acidogenesis stage of AD. VFAs could be biologically/chemically converted to biogas, alcohol-based fuels (e.g., ethanol and butanol), and other value-added products (e.g., polyhydroxyalkanoates) (Chen et al., 2013). Methanogenesis is considered as one of the rate limiting steps in biogas production for many substrates due to the long doubling time of methanogens (Khanal, 2008). Moreover, compared to biogas, certain higher-value products could be obtained through AD route. Hence, eliminating methanogenic step in AD process could favor the production of high-value products (i.e., VFAs) and cut down the capital and operational costs of the AD system by requiring short hydraulic retention time and subsequently smaller digester volume. AD has been reported to be a cost-effective and environmental friendly technology for producing VFAs (Alkaya and Demirer, 2011; Trevisan et al., 2014). Diverse substrates, such as crude glycerol from biodiesel production (Trevisan et al., 2014), food wastes (Yin et al., 2014), olive mill wastewater (Scoma et al., 2013), starch-rich potato processing wastewater (Elefsiniotis and Wareham, 2007), and waste activated sludge (Yuan et al., 2011), have been used for producing VFAs through AD process. Table 2.5. summarizes VFAs production from different substrates

Table 2.5. Volatile fatty acids production from various substrates (adopted from Surendra et al. (2015))

Organic substrates	Organic content (mg COD/L)	Reactor types and operating conditions	VFAs concentration (mg/L)
Kitchen waste	166,180	Batch reactor, pH 7.0, 35 ºC, 4 days	36,000
Organic fraction of municipal solid waste	196,700	Plug flow reactor, pH 5.7–6.1, 37 ºC, HRT=SRT 6 days, OLR 38.5 g VS/L-day	23,110
Palm oil mill effluent	88,000	Semi-continuous reactor, pH 6.5, 30 ºC, HRT 4 days	15,300
Dairy wastewater	4,420	Continuous flow-completely mixed reactor, pH 6.8–7.2, 35 ºC, HRT 0.5 day	3,100
Food waste and sludge	29,050	Continuous upflow reactor, pH 5.5–5.9, 18ºC, HRT 1 day, 25% food waste + 75% primary sludge (on weight basis)	3,610

VFAs could also be used as an external carbon source for biological nutrient removal in wastewater treatment process. Elefsiniotis and Wareham (2007) was successful in using acetic acid produced during anaerobic treatment of effluent from the potato processing and municipal primary sludge, as carbon source for denitrification. Moreover, VFAs can also be used as substrate to grow lipid accumulating oleaginous microorganisms and subsequently, biodiesel production. Heterotrophic microalgae (e.g., *Chlorella protothecoides* and *C. albidus)*, oleaginous yeasts, and molds could accumulate lipids 50 to 70% of their biomass under nutrient limiting condition (Fontanille et al., 2012). These lipids can serve as initial feedstock for biodiesel production via transesterification process. However, the high cost of carbon source (about 80% of the total medium cost when glucose was used) for cultivation of oleaginous microorganisms makes the process economically unfeasible (Fei et al., 2011). VFAs obtained from AD of variety of biodegradable organic substrates can serve alternative carbon source for lipid production (Fontanille et al., 2012). The yields and composition of VFAs strongly depends on the substrate characteristics and reactor operating conditions (i.e., pH, OLR, and HRT among others). Several studies examined the effects of pH on VFAs production. Alkali condition could enhance hydrolysis (and subsequently VFAs production) from waste activated sludge (Chen et al., 2007), in contrast, mild acidic condition (pH:5.25-6.0) was reported to enhance the VFAs production from food waste and industrial wastewater such as dairy whey effluent, and pulp and paper mill effluents (Bengtsson et al., 2008; Wang et al., 2014). The optimal pH for VFAs production varies with the substrate type. In case of dairy wastewater, production of propionate was higher at lower pH (pH:4.0-4.5) while the production of acetate and butyrate was favored at

higher pH (pH:6.0-6.5) (Yu and Fang, 2002). In case of cheese whey, the propionate concentration increased when pH was increased from 5.25 to 6.00 while acetate and butyrate concentrations decreased (Bengtsson et al., 2008). Since acidogens grow much faster compared to the methanogens, at longer HRT, methanogens could convert VFAs into methane, resulting in lower VFAs yield. Thus, HRT is one of critical operating parameters to maximize VFAs production. Alkaya and Demirer (2011) showed that there was higher total VFAs production (i.e., 2,159-3,635 mg/L as acetic acid) at HRT of 2 days compared to HRT at 4 days (i.e., 1,814-2,640 mg/L as acetic acid). Fang and Yu (2000) observed almost 2-fold increase in VFAs production from dairy wastewater in thermophilic condition when HRT was increased from 4 h to 12 h, but VFAs production improved only by 6% when HRT was further increased from 16 h to 24 h. Moreover, production of propionic acid was favored when HRT was increased during acidogenic fermentation of whey (HRT was increased from 20 h to 95 h) and paper mill effluent (HRT was increased from 11 h to 24 h); but the production of butyric acid decreased at longer HRT (Bengtsson et al., 2008).

2.5.2. Enhance volatile fatty acids production by micro-oxygenation

The VFAs composition and production strongly depend on both a type of substrate and operating conditions including temperature, pH, OLR, HRT, and SRT (Surendra et al., 2015). Thus, to optimize AD process for enhanced VFAs yield, these operating conditions should be controlled at optimum ranges. During acidogenesis stage, facultative bacteria was reported to be the dominant microorganism among the others to produce VFAs, carbon dioxide, alcohol, and hydrogen from organic feedstocks (Gerardi, 2003). The oxidation reduction potential (ORP) of -100 and -300 mV was found to be optimal to

facilitate VFAs production(Gerardi, 2003). Micro-aeration has been suggested as one of effective methods for stimulating the growth of facultative microorganisms for enhancing hydrolysis and acidogenesis during AD. Micro-aeration was reported to enhance the excretion of extracellular enzymes there by facilitating hydrolysis, which leads to higher VFAs yield (Botheju and Bakke, 2011). Several studies reported the positive effects of micro-aeration on hydrolysis of various substrates including lignocellulose biomass (Sawatdeenarunat et al., 2017), primary sewage sludge (Johansen and Bakke,2006). The authors observed that the micro-aeration could enhance the hydrolysis of protein and carbohydrate without consuming large amount of produced VFAs in a batch studies at a mesophilic condition. Nguyen et al. (2007) also reported that no significant effect found in the enhancing of hydrolysis and acidogenesis stages of municipal solid waste in high-solid pilot-scale process. Table 2.6. summarizes the effect of micro-aeration on VFAs yield.

Table 2.6. The effect of micro-aeration on volatile fatty acids yield from previous studies (modified from Lim and Wang, 2013)

Substrates	Reactor operation	Aeration intensity	Effect on VFAs production	Reference
Vegetable and flower waste	Mesophillic batch	74, 147, 442, 1768 Lair/kgTS-d	Negative	Zhu et al., 2009
Grass silage	Mesophillic leach bed reactor	1 Lair/min	4-fold increase	Jagadabhi et al., 2010
Brown water and food waste	Mesophilic batch	0.0375 LO_2/Lreactor-d	Positive	Lim and Wang, 2013
Potato peel	Thermophilic batch	0.10, 0.25, 0.50 and 1.00 volume of air/volume of waste slurry/minute (vvm)	Decrease with increasing aeration rate	Obeta Ugwuanyi et al., 2005

Zhu et al. (2009) reported that the efficiency of acidogenesis depends on the degree of aeration and operating period in the two-phase anaerobic digestion using fresh vegetable and flower wastes as the substrates. Excessive and inappropriate micro-aeration can cause

the negative effect on VFAs yield. The authors suggested that the micro-aeration should be applied at the early stage of the digestion to promote acidogenesis as well as to prevent the lactic acid (non-VFA) accumulation, which could otherwise lead to the failure of the system. The increase in VFAs yield during the micro-aeration of AD of energy crop was also reported by Jagadabhi et al. (2010). The grass-silage served as the substrate in this study in a leach-bed bioreactor. The result showed nearly 4-fold increase in VFA production when micro-aeration was applied. Lim and Wang (2013) demonstrated positive effect of micro-aeration on easily biodegradable substrates (i.e., brown water and food wastes) with increase in VFAs accumulation. The increase in conversion of short chain fatty acids to acetic acid was also investigated in this study. The authors concluded that the increase in VFAs yield could be due to improvement in the activities of facultative hydrolytic and acidogenic microorganisms. Obeta Ugwuanyi et al. (2005) studied the effect of aeration on potato peel waste using thermophilic anaerobic digestion and reported that acetate concentration decrease with increasing oxygen dosage. At the optimum oxygen dosage (i.e., 0.1 vvm), the produced acetate dropped after 84 and 60 hours of digestion time when the pH was controlled around 7.0 and uncontrolled, respectively.

2.5.3. Digestate fiber for biofuel and bio-based products generation

Digestate fiber, a solid residue following AD of biodegradable material, has been treated as low value product and is commonly used as soil additive or animal beddings (Johnson et al., 2006). However, recent studies have shown that digestate fiber has comparable properties with the feedstock (input) and can be used as a potential feedstock for biofuel and bio-based product generation (Teater et al., 2011). Studies have

demonstrated that hemicellulose is selectively consumed during AD, which facilitates the breakdown of complex interaction of cellulose, hemicellulose and lignin. In addition, AD results significant reduction in size of raw feedstock. For example, AD of animal manure with 75% (dry matter) of fiber with less than 1mm in size resulted in AD fiber with about 88% (dry matter) of fiber with less than 1mm in size. The combined effect of significant reduction in hemicellulose content and biomass size effectively destabilizes the biomass structure, thus facilitating better solubilization (i.e., saccharification) of cellulose by commercial enzymes in the downstream processing (Maclellan et al., 2013;Yue et al., 2011). Moreover, the lower hemicellulose content in the AD fiber eliminates the problem of pentose sugar utilization in cellulosic biorefinery (Yu et al., 2010). Thus, AD could serve as an effective biological pretreatment technology to pretreat the complex lignocellulosic biomass.

Glucose, derived from the hydrolysis of cellulose, has several potential applications such as a substrate for producing drop-in biofuels (via the carboxylate platform) (Agler et al., 2011) or as a precursor for producing high-value products such as bioplastics, succinic acid, fungal protein, etc. (FitzPatrick et al., 2010; Cherubini and Strømman, 2011). Several studies have shown the potential of using pretreated digestate fiber for bioethanol production. For example, about 120 million dry metric tons of cattle manure produced annually in the United States could generate about 63 million dry metric tons of fiber following AD, which has a potential to produce more than 1.67 billion gallons of ethanol (Yue et al., 2010).

Recently, there has been growing emphasis on biobutanol production due to several merits, e.g., higher energy density, low volatility and low corrosiveness (Bramono et al.,

2011). Thus, there significant potential in converting AD fiber- derived glucose into butanol by using several Clostridia species such as *Clostridium acetobutylicum*, *Clostridium beijerinckii*, and *Clostridium pasteurianum* (Bramono et al., 2011). The butanol yields by Clostridia species have been reported around 0.235 g/g of initial glucose and 0.247 g/g of initial xylan (Bramono et al., 2011). Moreover, the produced monomeric sugars can also be biologically converted into carboxylates using enriched microbial culture during AD, which later can be used as a precursor for producing solvents and/or fuel such as carbonyl and ester (via thermo- and electro-chemical processes), alcohols and alkane (via decarbonation and reduction processes) (Agler et al., 2011) and bioenergy such as CH_4, H_2, and bioethanol (Rabelo et al., 2011; Kaparaju et al., 2009). Additionally, glucose can be used as initial substrate to produce lactic acid which can be further converted into lactate esters (solvent for cleaning industry) and acrylic acid (used in polyester resin). Polymerized lactic acid has several industrial applications as a biodegradable plastic (Octave and Thomas, 2009).

Succinic acid can also be synthesized from glucose by microbial fermentation. Succinic acid can be converted into products such as surfactants, detergents, and food and pharmaceuticals (Zeikus et al., 1999; Du et al., 2007). Cellulose in the AD digestate after lignin removed can be used as filler in polymer composites such as Polypropylene–microcrystalline cellulose composites to enhance their properties such as strength and heat resistance. Cellulose reinforcement has several superior attributes (such as environment-friendly, renewable, and biodegradable) over conventional filler (i.e., aramid, carbon, and glass)(Spoljaric et al., 2009). Cellulose can also serves as initial substrate for synthesis of many cellulose derivatives such as cellulose esters (e.g.,

cellulose acetate and cellulose acetate propionate), and ethers (e.g., methylhydroxyethyl cellulose, and carboxymethyl cellulose). Cellulose ester films can be used to produce optical media due to their good mechanical and optical properties (Klemm et al., 2005). Cellulose nitrate and cellulose acetate have been widely used material for production of ,micro-, ultra-, and nano-filtration membranes for water purifications, food productions, and medical processes (Edgar et al., 2001; Klemm et al., 2005). Cellulose ethers have also been applied for producing methylhydroxyethyl and methylhydroxypropyl celluloses as building materials), and carboxymethyl cellulose (as a stabilizer to enhance milk properties) (Klemm et al., 2005).

2.5.4. Thermochemical process

Thermochemical decomposition of variety of substrates in the absence of oxygen has been widely used to produce biofuels (i.e., syngas, bio-oil), and bio-product (i.e., biochar) (Brown, 2003). Several studies demonstrated successful applications of thermochemical processes (i.e. pyrolysis, torrefaction, and hydrothermal carbonization among others) to produce high energy density fuels and efficient adsorbent from diverse lignocellulosic feedstocks, However, the thermochemical conversion of raw lignocellulosic materials still has some drawbacks, i.e., high moisture content, and high volatility, which leads to lower energy density compared to the fossil fuels (Poudel et al., 2015). Thus, thermochemical conversion of AD digestate is one of the innovative approach to overcome the above state limitations and provides new opportunity for true AD-based biorefinery as elucidated in the following section

2.5.4.1. Pyrolysis

Pyrolysis is a thermochemical process in which biomass is heated at temperature of 350 to 600°C under limited supply of air (Garcia-Perez, 2017). The products generated via pyrolysis depends on the composition of biomass and the operating conditions (i.e. operating temperature, residence time etc.) (Brown, 2003). Pyrolysis produces two main products, namely pyrolysis oil or bio-oil, and charcoal or biochar. Biochar is a solid combustible material with high heating value of 30 MJ/kg, which can be used to produce energy. It can also be used as a soil amendment to increase nutrients and water holding capacity as well as a cost effective adsorbent in wastewater treatment system (Garcia-Perez, 2017; Yao et al., 2011). Bio-oil is a low-viscosity dark-brown liquid produced during pyrolysis and contains 15-20% of water and other chemicals such as acids, aldehyde, and sugars from carbohydrate decomposition and phenolic compounds from lignin fractionation (Brown, 2003). The higher heating value (HHV) of the produced bio-oil ranges from 15 to 20 MJ/kg. Bio-oil has attributes similar to petroleum oil, which could be upgraded into different liquid fuels. Pyrolysis can be broadly classified into slow pyrolysis and fast pyrolysis depending on the desired end products. Slow pyrolysis occurs at the heating rate of 5 to 10°C/min and the main product is biochar with bio-oil as secondary product. Fast pyrolysis on the other hand, has the heating rate as high as 1000°C/min with the short reaction time (less than few minutes) with bio-oil yield of 60-70% by wt. (Garcia-Perez; Monlau et al., 2015b).

The pyrolysis utilizing AD digestate is one of innovative approaches of expanding the scope of AD biorefinery for lignocellulosic biomass (Sawatdeenarunat et al., 2016). Monlau et al. (2015a) presented a full-scale AD process fed with co-substrates, chicken

manure, groats, olive oil cake and triticale, integrated with a pyrolysis process operating at 500°C for 10 mins and the heating rate of 20°C/min. The results indicated that the excess heat produced from AD system could offset the cost of drying the digestate. The syngas, bio-oil, and biochar accounted for 9, 58, and 33%, respectively, of the products. Moreover, the pyrolysis products could enhanced 40% of the electricity production compared with stand-alone AD process (Monlau et al., 2015a). The phosphate removal from wastewater using biochar derived from the slow pyrolysis of digestate of AD of sugar beet tailings at 600°C as an adsorbent was investigated by (Yao et al., 2011). The authors reported that biochar produced from the digested sugar beet tailing showed higher phosphate removal efficiency compared with the biochar derived from raw sugar beet tailing and conventional activated carbon. This could be attributed to higher BET surface area and lower zeta potential of the biochar generated from the AD digestate. Similar results were also observed by Inyang et al. (2010) when the digestate from AD of sugarcane bagasse was used for producing biochar at the temperature of 600°C. The results showed that the biochar generated from the digestate had higher surface area, ion exchange capacity, hydrophobicity, and negative charge compared to that derived from raw sugarcane bagasse. As apparent from the characteristics of the digestate biochar, it could serve as a cost effective soil conditioner, and adsorbent for removal of pollutants from wastewater (Inyang et al., 2010).

2.5.4.2. Torrefaction

Torrefaction is a thermochemical process to produce high-density energy materials under inert condition and mild temperature (i.e., 200-300°C) with residence time between 30 to 180 min (Kambo and Dutta, 2015). In recent years, the process has been broadly

applied to increase energy density, HHV, and grindability to reduce the operation cost of grinding, and ignitability of biomass (Poudel et al., 2015). The high-energy product after palletization, torrefied biomass, has similar properties with coal (Batidzirai et al., 2013). Bridgeman et al. (2008) studied torrefaction of reed canary grass, wheat straw and willow at the temperature between 230 and 290 °C and residence time of 30 min. The authors reported that the volatile solids components of torrefied biomass decreases, which led to more thermally stable product (Bridgeman et al., 2008). Yan et al., (2009) reported that the increasing temperature of torrefaction of Loblolly pine (*Pinus taeda*) enhanced the energy density, and increased the carbon content with reduced volatility. However, it resulted in decrease in biomass recovery. Moreover, the obtained biochar had characteristics similar to a low-rank coal. The improvement of fuel characteristics and grindability of the torrefied pine chips and logging residue were reported by Phanphanich and Mani (2011). The enhanced properties of torrefied biochar could significantly cut down specific energy required for grinding which consequently lower the operating cost of the biomass processing. Moreover, the heating value of the torrefied biomass also significantly increased compared to the raw biomass. Similarly, Poudel et al. (2015) studied the torrefaction process of food waste, a non-lignocellulosic material, using a horizontal tubular reactor. The authors reported that the optimum temperature to maximize HHV of food waste was between 290-330°C and the operating temperature was more critical on torrefaction process than the residence time. Thus, torrefaction of biomass was found to enhance the fuel characteristics of the feedstock.

2.5.4.3. Hydrothermal carbonization

Unlike pyrolysis and torrefaction, hydrothermal carbonization (HTC) is a thermochemical process occurs at a high-pressure aqueous phase (i.e., between 2 and 6 MPa) (Libra et al., 2011) for producing three main products namely, hydrochar (solid phase), aqueous soluble (liquid phase), and CO_2 (gaseous phase) (Kambo and Dutta, 2015). The operating temperature of HTC is between 180 to 260°C (Kambo and Dutta, 2015) with optimum temperature being 250°C for HTC of biomass (Liu et al., 2013). The typical residence time between 5 and 240 min is recommend for HTC (Kambo and Dutta, 2015). The HTC of coconut fiber and dead eucalyptus leaves was studied for producing solid fuel by Liu et al. (2013). The authors concluded that the fuel quality, i.e., hydrophobicity, and carbon content of the biomass could be enhanced during HTC at temperature of 250°C and residence time of 30 min. The produced hydrochar had energy density similar with to a low-rank coal (i.e., lignite). Hitzl et al. (2014) presented the biorefinery concept in which HTC of wet biomass served as a centerpiece. Following a long-term observation, the authors concluded that the produced solid fuel had stable compositions. The carbon content was higher than 60% of dry ash free biomass. Moreover, the hydrochar after palletization could be used as solid biofuel as per the European standard (EN 14961-6). The ash after solid fuel combustion could be recycled as a phosphorus source for plant nutrient and the liquid from HTC could be used for irrigation. Thus, the generated waste was minimized with recovery of every component of feedstock representing a biorefinery concept. Since HTC is wet process, the feedstock drying could be eliminated especially wet feedstock. Thus, the process is ideal for a high moisture content biomass such as digestate from AD of lignocellulosic biomass (Hitzl et

al., 2014). Reza et al. (2014) presented a system integrating AD and HTC to produce renewable energy from wheat straw as a mono-substrate. The HTC was operated at 230°C with residence time of 6 hours. The energy obtained from the integrated system was over 20% and 60% higher than the stand-alone HTC and stand-alone AD processes, respectively. The similar concept was also studied by Mumme et al. (2011) by using the digestate of maize silage as feedstock for HTC. The thermophilic AD system consisted of two-stage solid-state digester. The HTC was operated at 190°C for 2 h. The produced hydrochar had BET surface area of 12 m^2/g, which is appropriate for using as an adsorbent in pollutants removal from wastewater. Moreover, the HHV of the produced hydrochar in this study was 25–36 MJ/kg which is in the range of bituminous coal and could be used as solid fuel for gasification, and co-firing with coal. Kim et al. (2014) investigated the solid fuel production form HTC of non-lignocellulosic biomass (i.e., anaerobically digested sludge). The HTC process could increase the HHV by decreasing the ratio between oxygen and carbon and of the sludge by dehydration and decarboxylation reactions. After HTC process, the hydrochar derived from anaerobically digested sludge could be used as a solid fuel.

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Batch experiments

3.1.1 Micro-oxygenation

3.1.1.1 Substrate and inoculum

Napier grass was cultivated for three months and harvested from the University of Hawai'i's Waimanalo Research Station (Waimanalo, HI, USA) The hand-harvested Napier grasses was shredded using a commercial cutting mill (Vincent Corporation, Tampa, FL, USA) to a size of about 2 cm. Later, it was air-dried to a total solids (TS) content of over 90%, to prevent the substrate degradation. The dried biomass was then passed through a second laboratory cutting mill (Retch SM2000, Haan, Germany) with a screen size of 6 mm. Finally, the milled biomass was stored in vacuum bags for further analysis and was used as a feedstock for the entire experiments. The TS, volatile solids (VS), and fiber composition including Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and Acid Detergent Lignin (ADL) of the prepared biomass were also analyzed. Two different inocula, namely anaerobically digested cattle manure (ADCM) and anaerobically digested waste activated sludge (ADWAS), were used to investigate the effect of inocula on VFAs yield. ADWAS was collected from an anaerobic digester treating waste activated sludge from Hawaii Kai wastewater treatment facility (Hawai'i Kai, Honolulu, USA) at mesophilic conditions. Similarly, ADCM was taken from a 20-L mother reactor fed with cattle manure and operated at mesophilic conditions in the laboratory. The reactor contents were withdrawn from the digester after 42 days of

digestion and sieved using a #8 sieve (ASTM 2.36 mm, Thermo Fisher Scientific Inc., USA) with opening of 2.38 mm to remove the fibers, which could interfere with the compositions of experimental samples. The filtrate after sieving was used as an inoculum for the experiments. The TS and VS contents of the ADWAS were 2.96±0.04% and 2.00±0.04%, respectively. Corresponding TS and VS contents of the ADCM were 3.63±0.35% and 2.71±0.26%, respectively. The prepared inocula were purged with nitrogen gas and stored at 4°C under anaerobic condition and were reactivated for 3 days at 37±1°C, before being used in the batch experiments.

3.1.1.2 Experimental design

The experiments were performed based on a Randomized Complete Block Design (RCBD). Three factors were considered in RCBD including inoculum, O_2 dosage, and incubation time. The inoculum had two levels, while the O_2 dosage and incubation time had three levels as shown in Table 3.1. In total, 18 different treatment combinations were tested in each replication.

Table 3.1. Experimental design pattern

Factor	Type	Levels	Types or Values	Unit
Inoculum	Fixed	2	ADCM, ADWAS	-
Oxygen dosage	Fixed	3	0, 15, 30	mL/gVS_{added}
Incubation time	Fixed	3	1, 3, 5	day(s)

3.1.1.3 Experimental setup

The preliminary batch experiments were performed using a series of 250 mL Erlenmeyer flasks with a working volume of 160 mL. The batch experiments were setup as per the experimental design discussed earlier. The processed Napier grass was used as a substrate and the substrate-to-inoculum ratio was maintained at 1:1 based on VS. TS content in the flask was adjusted to 4% using distilled water. Blanks were set up in the same way as the samples, except adding Napier grass to assess the VFAs and biogas production from inoculum alone during the experiments. The flasks were then injected with O_2 following the experimental design prior to the start of the incubation. All the experiments were conducted in an incubator shaker (New Brunswick Scientific Excella™ E25, New Brunswick Scientific Co., Inc., USA), in which the temperature and shaking speed were maintained at 37±1°C and 100 rpm, respectively. The experiments were terminated at different designated periods based on the experimental design. After each incubation period, the contents from the flasks were sieved through a 0.85 mm screen. The filtrate after sieving in the samples were centrifuged and subsequently analyzed for individual VFAs concentration. The produced biogas was quantified and the biogas composition was analyzed. Based on the results from the 250 mL flask experiments, the conditions that resulted the highest, medium, and the lowest VFAs yields from each inoculum were retested in 2 L serum bottles with a working volume of 1.5 L to collect enough fibers for fiber composition analysis. All the experiments were conducted in triplicates to confirm the repeatability and reproducibility of the results. The statistical correlation between VFAs production and the O_2 dosage was also tested. The physical appearance of digestate fiber was compared with raw grass using Scanning Electron

Microscopy (SEM) to examine the structural changes during AD. The overall experimental steps are illustrated in Figure 3.1.

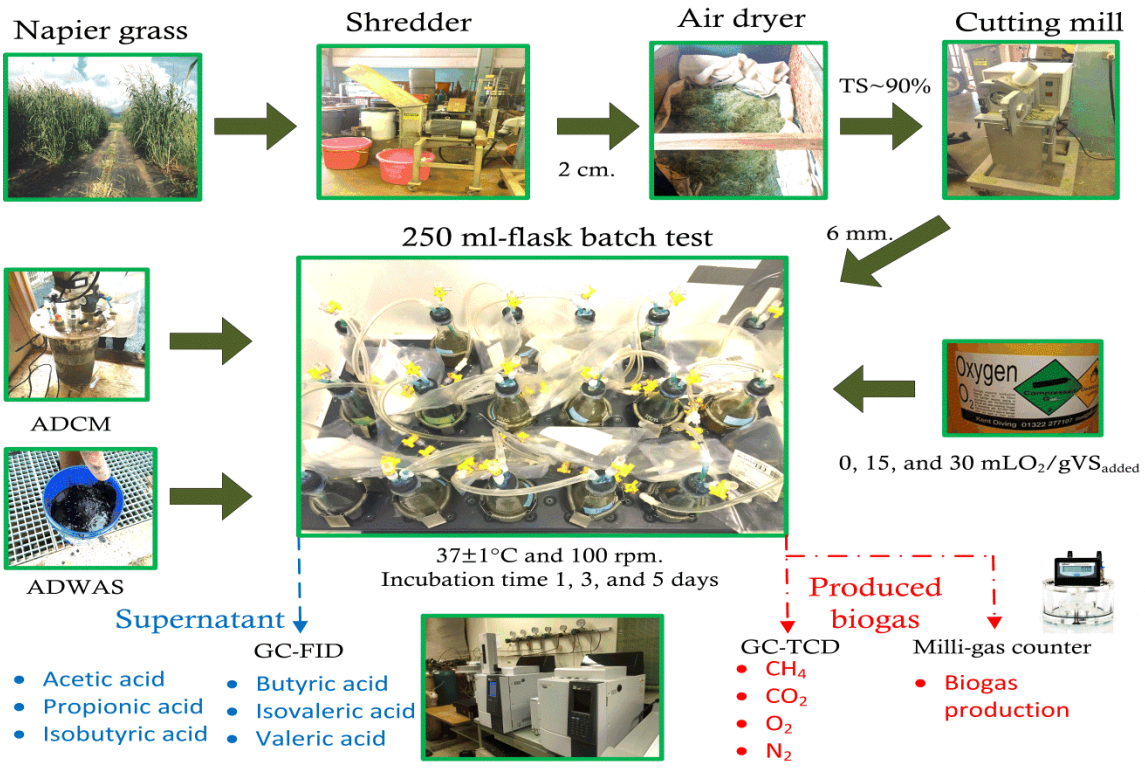


Figure 3.1. The experimental steps of the batch study for volatile fatty acids production from Napier grass using micro-oxygenation

3.1.1.4 Optimization studies and experimental verification

The results obtained from the batch experiments with different O_2 dosages, incubation times and inoculum types were modeled using a quadratic regression. The first-degree derivative of the equation from the quadratic regression was performed with respect to O_2

dosage and was set to zero to predict the O_2 dosage which gives the maximum VFAs production as illustrated in eq. (1):

$$Y_{VFAs} = AX^2{}_{ox} + BX_{ox} + C \quad (1)$$

Where, Y_{VFAs} is VFAs yield (mg/gVS_{added}), X_{ox} is O_2 dosage (mL/gVS_{added}), and A, B and C are regression coefficients. A series of batch tests were then reconducted in a 250-mL Erlenmeyer flask to test the prediction model using the predicted O_2 dosage obtained from the model that resulted in the maximum VFAs yield. The experiment was conducted in triplicate. Furthermore, the predicted and experimental values were statistically compared.

3.1.2 Biochemical methane potential (BMP) of raw Napier grass

Napier grass was grown in research plots of Hawaiian Commercial and Sugar (HC&S) on Maui, HI, and was harvested at the age of 6 months. The biomass feedstock and inoculum were also processed following the same methods discussed in section 3.1.1. The experiments were conducted following the same procedures as discussed in section 3.1.1.3 except that the aeration was not applied. The processed Napier grass and ADCM were used as the substrate and inoculum, respectively. The TS and VS of raw Napier grass were 90.5±0.4% and 92.3±0.1% TS, respectively. ADCM was used as an inoculum at the beginning of the experiment. The TS and VS contents of the inoculum were 3.7±0.1% and 74.9±0.9% TS, respectively. The experiments were terminated when the accumulated methane production reach a plateau (i.e., after 45 days). The biogas production was measured daily and biogas composition was analyzed three times a week. The serum bottle containing only the inoculum was used as a control to observe the

volume of methane produced from the inoculum alone. At the end of the experiment, the content from each flask was withdrawn and analyzed for TS, VS, and VFAs. The experiments were duplicated to confirm the repeatability. The results from batch experiments could reflect the methane potential of the feedstock.

3.2 Horizontal bioreactor

3.2.1 Substrate and inoculum

The processed Napier grass and inoculum as mentioned in section 3.1.2 was used as a substrate for mono-digestion in this study. Reactor configuration and experimental set up

3.2.2 Experimental setup

Two 10-L acrylic horizontal bioreactors with working volume of 4 L were used in this study. The mechanical mixers were designed to horizontally mix the reactor contents from inlet to outlet ports. Moreover, the mixtures could break down an accumulated scum as well as degas the high-solid reactor contents to prevent the formation of thick scum layer. Reactors were maintained at mesophilic condition (33±2˚C) using a silicone heater (BriskHeat OH, USA). Type T-thermocouple (Omega Engineering, Inc., Connecticut, USA) coupled with data locker (Dataq Instruments, Inc., Ohio, USA) were used to monitor the reactor temperature. AD of Napier grass was conducted in semi-continuous mode to accurately reflect the commercial practices. The feedstock was manually fed through an inlet port located at one end of the reactor. Digestate was withdrawn from the bottom of the reactor during mixing at another end. Mixing was set at 100 rpm and intermittently turned on for 5 minutes in every 30 minutes. The reactor setup is shown in Figure 3.2.

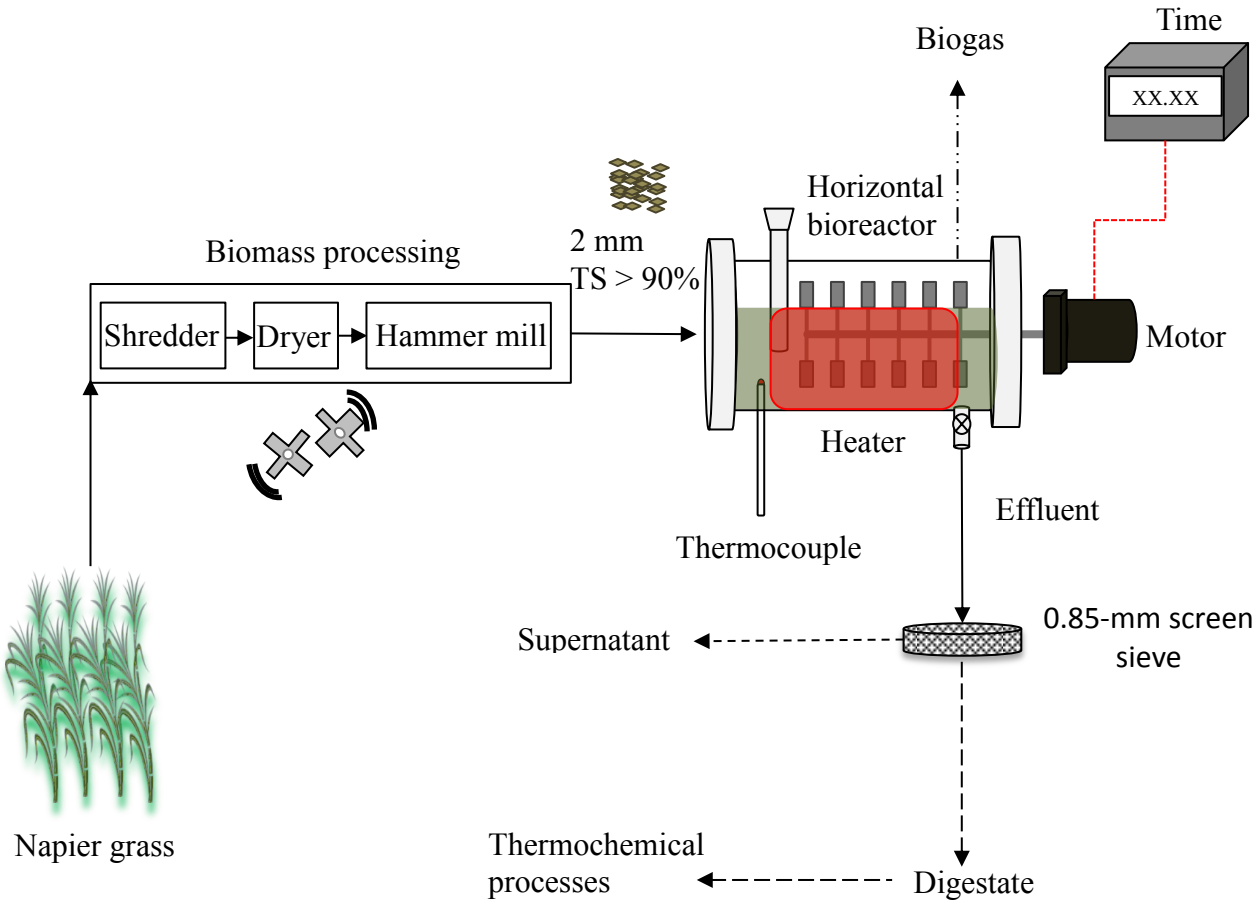


Figure 3.2. The horizontal bioreactor setup

3.2.3 Reactor start up and operation

The horizontal bioreactors were seeded with 2L of the prepared inoculum and reactivated at 33±2°C for a week. TS of feedstock was adjusted to 12% by distilled water and C/N ratio was adjusted to 25 by adding NH_4Cl daily prior to feeding. Trace elements were added weekly to maintain active methanogenic activity as recommended by Wilkie et al. (1986). $NaHCO_3$ was also added once a week to maintain the buffering capacity of the system. The reactors were started up at an initial OLR of 2 kgVS/m^3-d. After startup period, the OLR was increased step-wise at an interval of 1 kgVS/m^3-d and operated for

at least 3 HRTs to allow complete wash-out of reactor contents from the previous loading condition and to reach steady/quasi-steady state condition before collecting all the experimental data (Usack et al., 2012). The OLR was increased until the reactor failure occurred due to significant accumulation of VFAs. The reactor contents were withdrawn daily and sieved through a 0.85-mm screen for a solid-liquid separation. The filtrate of the samples following sieving was centrifuged and subsequently analyzed for individual VFAs. The produced biogas was measure and its composition was analyzed. The frequency of analysis of the operating parameters is summarized in Table 3.2.

Table 3.2. The frequency of analysis of operating parameters

Operating parameters	Frequency of analysis
pH	3 times a week
Total VFAs concentration	3 times a week
Individual VFA concentration	3 times a week
Alkalinity	3 times a week
Biogas production	Daily
Biogas composition	Daily
TS and VS	2 times a week

3.2.4 Volatile fatty acids production using aerated horizontal bioreactor

3.2.4.1 Reactor configuration and experimental setup

Two 5-L acrylic horizontal bioreactors with working volume of 2.7L were used in this study. The reactor configuration and set-up were similar to that discussed in section

3.2.2. The aeration was carried out using an air pump with an adjustable flowmeter to maintain a designed airflow rate.

3.2.4.2 Reactor start up and operation

The horizontal bioreactors were seeded with 1.5 L of the prepared ADCM and reactivated at 33±2°C for a week. The reactors were started up at an initial OLR of 4 kgVS/m^3-d for a month. The feedstock preparation and nutrients supplementation were also applied as discussed in section 3.1.1.2 to maintain the favorable conditions for the microorganisms. After startup period, the OLR were increased at an interval of 1 kgVS/m^3-d until reactor failure occurred. The reactor contents were withdrawn and analyzed for important parameter as discussed in section with respect to section 3.2.3. The reactor contents were aerated for 1 min in every 6 hours using an air pump (Super pond, WA, USA) at constant flowrate of 450 mL/min.

3.3 Thermochemical conversion of digestate

3.3.1 Digestate preparation

The digested fiber from the horizontal bioreactors was air dried at 40°C using the incubator (Isotemp Incubator, Thermo Fisher Scientific Inc., Hudson, NH, USA) for 4 days. The final moisture content was kept below 10% to prevent the biodegradation and facilitate its transportation. The digested fiber was used as the substrate for thermochemical conversions, namely torrefaction to produce a torrefied biochar and hydrothermal carbonization to produce a hydrochar, which are the high energy content solid fuels. The raw Napier grass was also processed following the same procedures to

compare the characteristics of the products with that of the thermochemically processed AD digested fiber.

3.3.2 Experimental setup

Two thermochemical conversion processes, namely hydrothermal carbonization (HTC) and torrefaction, were applied to investigate the energy value of anaerobically digested Napier grass as a solid fuel. The major difference between the two methods was the presence of water during the thermal reactions. The HTC was performed using a 450-mL Parr pressure reactor (Parr Instrument Company, Moline, IL, USA) equipped with an electric heating mantle. A J-type thermocouple was located between a cylindrical reactor and an electric furnace and another was placed inside the reactor. Approximately 7 g of raw Napier grass/digestate samples was mixed with 42 g of deionized water and stored at room temperature overnight (1:6 weight basis). Then, the prepared sample was placed inside the reactor and nitrogen, as an inert gas, was introduced for 10 min to remove the air. The reactor was initially filled with nitrogen gas at 50 psi (0.3 MPa), heated to 240°C at 7°C/min, and treated for an hour. The maximum vapor pressure itself increased to about 540 psi (3.7 MPa) during the HTC process. After the reaction, the reactor was quenched and cooled with water to room temperature and the samples were collected for further analysis after filtering and drying. For the torrefaction, a one inch tube reactor of AISI 316 stainless steel was used to treat the raw Napier grass/digestate samples. Initially, about 2 g of a sample was placed in the reactor and N_2 gas was used to remove air for 10 min. Then, the reactor was placed in an electric muffle furnace, which was heated to 240°C at about 8°C/min and held for an hour.

3.4 Statistical analysis

Analysis of Variance (ANOVA) with a threshold value (α) of 0.05 followed by a post-hoc Tukey's test of experimental data was conducted. Biogas and VFA yields were used as response variables for different treatment conditions. All the statistical analyses were performed using JMP statistical software (JMP Pro 12.0.1, SAS Institute Inc., Cary, NC, USA).

3.5 Analytical methods

pH was measured using a bench top pH meter (Accumet AB15, Fisher, Fairlawn, USA). TS and VS were analyzed following the Standard Methods (APHA, 2005). Fiber composition was analyzed before and after AD using a cell wall fractionation method according to Van Soest (Faithfull, 2002). Biogas production was quantified using a milligas counter (Ritter US LLC, NY, USA), which works based on the principle of buoyancy. The biogas compositions were analyzed using a gas chromatography (Shimadzu, GC-2014, Japan) equipped with a thermal conductivity detector (GC-TCD) and a packed column (80/100 Hayesep D column, 2 m length x 3.2 mm outer diameter x 2.1 mm inner diameter, Supelco, USA). The individual VFAs (i.e., acetic, propionic, isobutyric, butyric, isovaleric and valeric acids) were quantified using a gas chromatography (Shimadzu, GC-2014, Japan) equipped with a flame ionization detector (GC-FID) and a capillary column (ZB-Wax Plus column 30 m length x 0.25 mm inner diameter x 0.25 µm film thickness, Phenomenex, USA). For SEM analysis, the fiber specimens were mounted on a conductive carbon tape with aluminum stubs and the sputter was coated with gold/palladium in a Hummer 6.2 sputter coater. The specimens were then viewed with a field emission SEM (Hitachi S-4800, japan) at an accelerating

voltage of 5.0 kV. Hydrothermally carbonized and torrefied solid products were analyzed for volatile combustible matter (VCM), ash, fixed carbon (FC) and heating value. VCM and ash were measured according to ISO 562; whereas FC was determined from the difference. HHV was measured using a bomb calorimeter (IKA, model C2000, IKA Works, Inc., NC, USA) in accordance with ASTM E870. In order to compare the efficacy of two thermal processes, mass and energy yields were determined according to the Equations (2) and (3).

$$\text{Mass yield (\%)} = \text{mass}_{final} / \text{mass}_{initial} \quad (2)$$

$$\text{Energy yield (\%)} = (\text{mass}_{final} \times \text{HHV}_{final})/(\text{mass}_{initial} \times \text{HHV}_{initial}) \quad (3)$$

The elemental analysis was performed in both raw Napier grass and digestate samples. The samples (1.0000 g ± 0.0010 g) were ashed at 550°C using muffle furnace for six hours followed by acid digestion of the ash following the method No. 968.08 of AOAC International (AOAC , 1996). The residue was then transferred to a digestion tube which is washed 2 times with 5 mL of 25% HCl. The digestion tubes were then placed onto a pre-heated (125°C) digestion block (Martin Machine, Ivesdale, IL, USA) and samples were digested for 30 min. The digests were removed from the digestion block, cooled down to room temperature, and diluted to a volume of 100 mL using deionized water. Finally, the solutions were analyzed for various elements following EPA Method 200.7 (USEPA , 1994) using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Spectro Arcos FHS16, Germany)

3.6 Techno-economic analysis

The main purpose of techno-economic is to determine the appropriate technology to maximize the benefit of the low-value digestate following the anaerobic biorefinery concept. The thermochemical processes (e.g. torrefaction, and HTC) were assumed to be installed in Hawaii and the analysis was independent from the entire anaerobic biorefinery process. Since, the thermochemical processes are in the development phase, the capital as well as operating cost are based on the previous studies (i.e., Xu et al. (2014) and Suwelack et al. (2016) for torrefaction and hydrothermal carbonization, respectively). The scale-up capital costs are based on equation (4) presented by Batidzirai et al. (2013 with the exponential factor (α) of 0.7.

$$\text{New cost} = \text{Base cost}\left(\frac{\text{New size}}{\text{Base size}}\right)^{\propto} \quad (4)$$

The lifetime of the equipment is assumed to be 15 years of both thermochemical technologies as recommended by Batidzirai et al. (2013). Assume that the produced chars after palletization, in which the HHV are identical to low–rank coal (i.e. subbituminous C). The char pellets is used for onsite co-firing process and the coal's selling price in Hawaii of $ 56.70/metric ton (U.S. Energy Information Administration (EIA), 2016).The pretax minimum acceptable rate of return (MARR) was 10% recommended by García-Gusano et al., 2016. The break-even point of the plant capacity, selling price, and the MARR which result the balance annual work were presented.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Optimize anaerobic digestion process to maximize volatile fatty acids production from Napier grass using micro-oxygenation via series of batch studies

AD of Napier grass for VFAs production was evaluated in batch mode under micro-oxygenation condition. A series of experiments were conducted based on a RCBD to investigate the VFAs yield using two different inocula, and at three different O_2 dosages and three different incubation periods. The major findings of this study are discussed in the following sections.

4.1.1. Effect of inoculum on volatile fatty acids yield

ADCM as an inoculum produced significantly (α=0.05) higher total VFAs under different O_2 dosages and incubation times as shown in Figure 4.1. and Table A1 in appendix A.

The lowest VFAs yield from ADCM batch at O_2 dosage of 30 mL/gVS_{added} and incubation time of 1 day was two-folds higher than the highest VFAs yield when ADWAS used as an inoculum under similar conditions. Overall, the batch tests using ADCM as an inoculum resulted in nearly 13-folds higher VFAs yield compared with the tests using ADWAS an inoculum during the incubation time of 3 days. In general, rumens found in cattle manure have phylogenetically diverse microbial communities including many cellulolytic bacteria that release hydrolytic enzymes capable of enhancing hydrolysis of lignocellulosic biomass (Hu and Yu, 2005; Tsavkelova and

Netrusov, 2012). Thus, ADCM inoculum plays key role in the enhanced VFAs production from lignocellulosic feedstocks. Similar results were also reported by Gu et al. (2014) when digested dairy manure and digested municipal sludge were compared as inocula for AD of lignocellulosic biomass (i.e., rice straw). The study showed an 8-fold higher specific methane yield with a shorter lag phase when the digested cattle manure was used as an inoculum compared to digested municipal sludge as an inoculum. The higher enzyme activities (i.e., cellulases and xylanases) and the availability of suitable nutrients in anaerobically digested dairy manure were a likely contributor to the higher VFAs yield seen when using ADCM over ADWAS in this study (Gu et al., 2014). Surendra and Khanal (2014) also used ADCM as an inoculum for AD of Napier grass in a series of batch studies to examine biomethane production potential. In the United States alone, over 120 million dry tons of cattle manure is produced annually (Yue et al., 2010), which could be used in co-fermentation with lignocellulosic feedstocks for VFAs production in full-scale AD plants. These apparently suggest that cattle manure-derived inoculum is ideal for VFAs production from lignocellulosic feedstocks

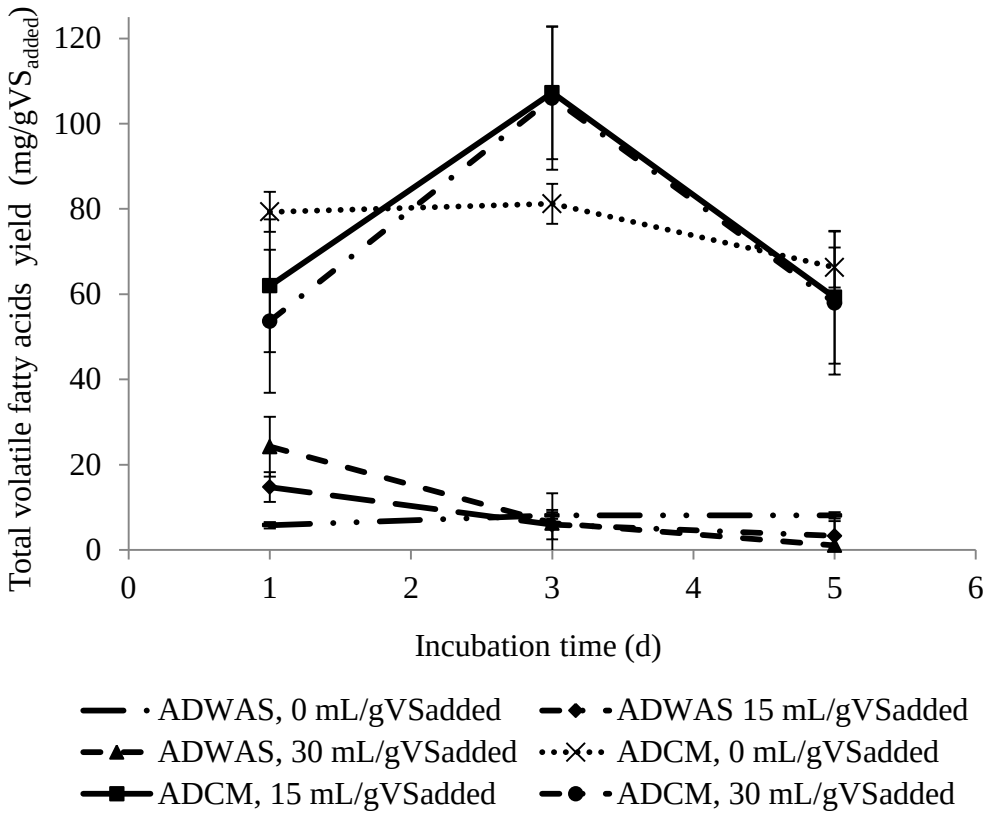


Figure 4.1. Total volatile fatty acids yield from Napier grass

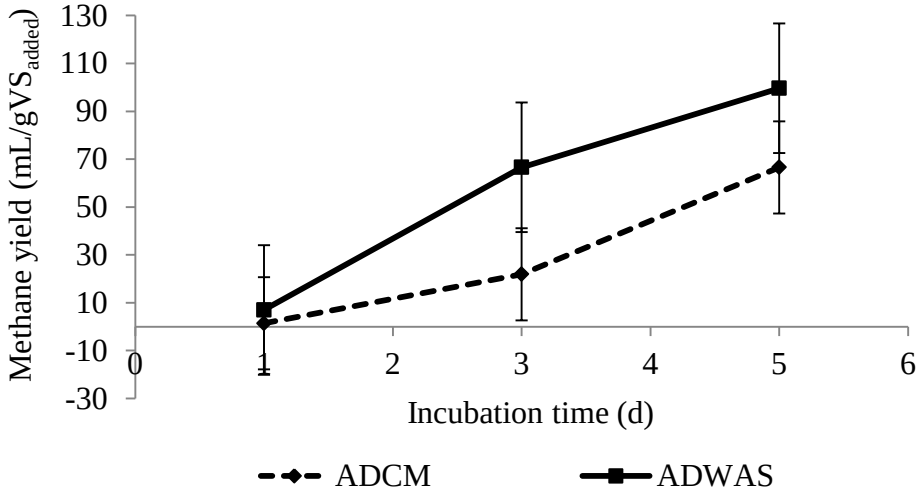


Figure 4.2. Methane yield at various incubation times

4.1.2. Effect of oxygen dosage and incubation time on volatile fatty acids yield

O_2 dosage by itself did not show a significant difference in VFAs yield between the two inocula. However, incubation time as well as the interaction between O_2 dosage and incubation time showed a significant difference. This could be attributed to an increase in the population of facultative microorganisms (Botheju and Bakke, 2011) and the enhancement of hydrolytic extracellular enzymes produced (Johansen and Bakke, 2006) during micro-oxygenation. The incubation time is also an important factor, which could affect VFAs yield and composition (Bengtsson et al., 2008). The importance of incubation time for microorganisms to acclimate and adapt to the AD's operating conditions under micro-oxygenation was investigated in the leach-bed reactor using fresh vegetable and flower wastes as the substrates without inoculation (Zhu et al., 2009). The VFAs production in the micro-oxygenated reactor increased from day 2 to day 5 and then started to decrease. However, in this study, the VFAs yield of batch tests with ADCM as inoculum reached a plateau on day 3, even though Napier grass has a higher lignin (ADL) content compared to the mixture of flower and vegetable wastes (i.e.,10.2% and 2.3% TS, respectively). The enhanced and sustained VFAs yield at shorter incubation time in this study could be attributed to a well-acclimated inoculum used in this study. Moreover, Zhu et al. (2009) also reported that VFAs produced during oxygenation depended on the amount of O_2 input. The authors further reported that insufficient micro-aeration could negatively affect the development of facultative microorganisms thereby resulting in poor system performance. Contrary to this, an appropriate micro-aeration rate could promote the hydrolysis process and result in higher VFAs yield. Jagadabhi et al. (2010) reported over a 4-fold increase in VFAs production during AD of grass silage using leach-bed

reactors, when aeration was supplied at a flow rate of 1 L/min compared with non-oxygenated conditions. As presented in Figure 4.2., with batch tests using ADCM as inoculum, the average daily methane yield between day 1 and day 3 of incubation period was 10.3±3.0 mL/gVS_{added}-d, which was less than half of the yield between day 3 and day 5. Thus, it is apparent that methanogens started to grow during longer incubation time. On the other hand, the batch tests using ADWAS as an inoculum had the average daily methane yield of 29.8±2.6 mL/gVS_{added}-d between day 1 and day 3 of incubation period which was higher than the methane yield of 16.5±5.9 mL/gVS_{added}-d for the incubation period between day 3 and day 5. Thus, the use of ADWAS resulted in higher conversion of VFAs into methane in the first 3 days of incubation period. The highest VFAs yield observed in this study was 107.3±2.6 mg/gVS_{added} at O_2 dosage of 15 mL/gVS_{added}, incubation time of 3 days and ADCM as an inoculum. The VFAs yield was not significantly different from that at O_2 dosage of 30 mL/gVS_{added} and incubation time of 3 days. The highest VFAs yield obtained in this study (i.e., 107.25±2.19 mg/gVS_{added}) was lower than that reported by Jagadabhi et al., (2010) (i.e., 139.50 mgVFAs/$gVS_{added,}$) when grass silage was used as the substrate. The higher yield of VFAs from the grass silage compared to raw Napier grass used in this study was attributed to ensiling, which often serve as a biological pretreatment at acidic conditions (i.e., pH 4 to 4.5), and further enhanced the hydrolysis during AD.

4.1.3. Volatile fatty acids composition

As shown in Figure 4.3., the use of ADCM as an inoculum generated a more diverse variety of individual VFAs compared to ADWAS as an inoculum. For both inocula, acetic and propionic acids dominated the VFAs profile. However, with ADWAS as an

inoculum, acetic and propionic acids were the major individual VFAs accounting for 50 to 100% of the total VFAs. Zhu et al. (2009) also reported a similar pattern of dominance of acetic, propionic, and butyric acids during AD of fresh vegetable and flower wastes under sufficient micro-aerated conditions. Similarly, acetic and butyric acids were the major VFAs produced during AD of grass silage (Jagadabhi et al.,2010). With ADWAS as an inoculum, acetic acid concentration increased with increasing incubation time and contributed over 80% of VFAs at an incubation time of 5 days during micro-aeration. This was mainly due to conversion of higher carbon VFAs into acetic acid by the acclimated acetogenic microorganisms in ADWAS. Under non-oxygenated conditions, the produced acetic acid was not significantly different. Conversely, in the batch test with ADWAS inoculum, the amount of acetic acid produced with ADCM as an inoculum decreased with longer incubation time. Acetic acid, which is the only VFAs that methanogens can consume, was converted into methane during long incubation times, which facilitated the growth of methanogens as evident from the increasing methane yield as shown in Figure 4.2. Thus, the distribution of individual VFAs strongly depended on the type of inoculum used.

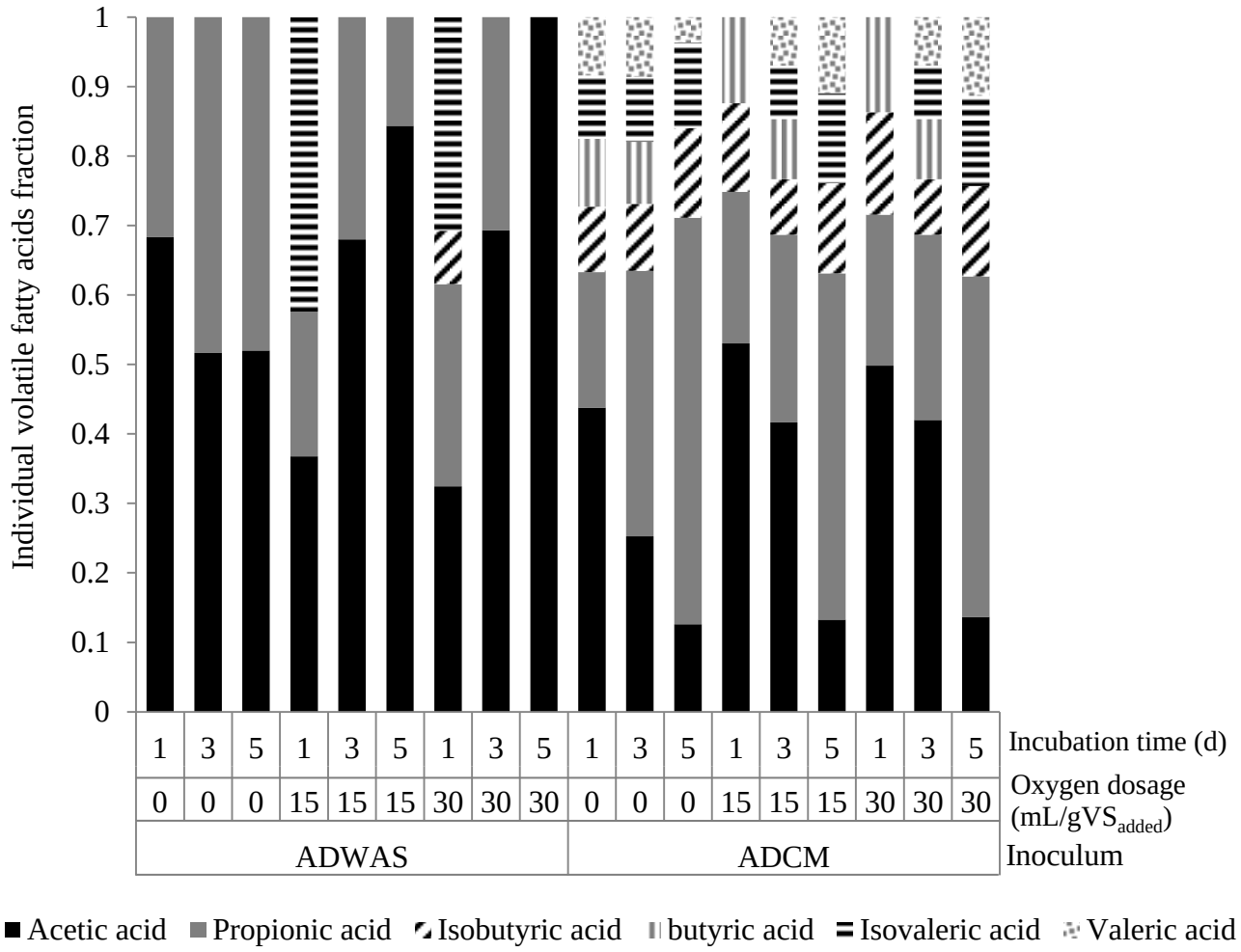


Figure 4.3. The distribution of individual volatile fatty acids

4.1.4. Statistical prediction model of volatile fatty acids yield

As seen from Figure 4.1., the optimum condition for high VFAs yield was when ADCM was used as an inoculum for the incubation time of 3 days. The correlation between VFAs yield and O_2 dosage under the selected condition was further studied by applying quadratic regression model that is shown in Figure 4.4.

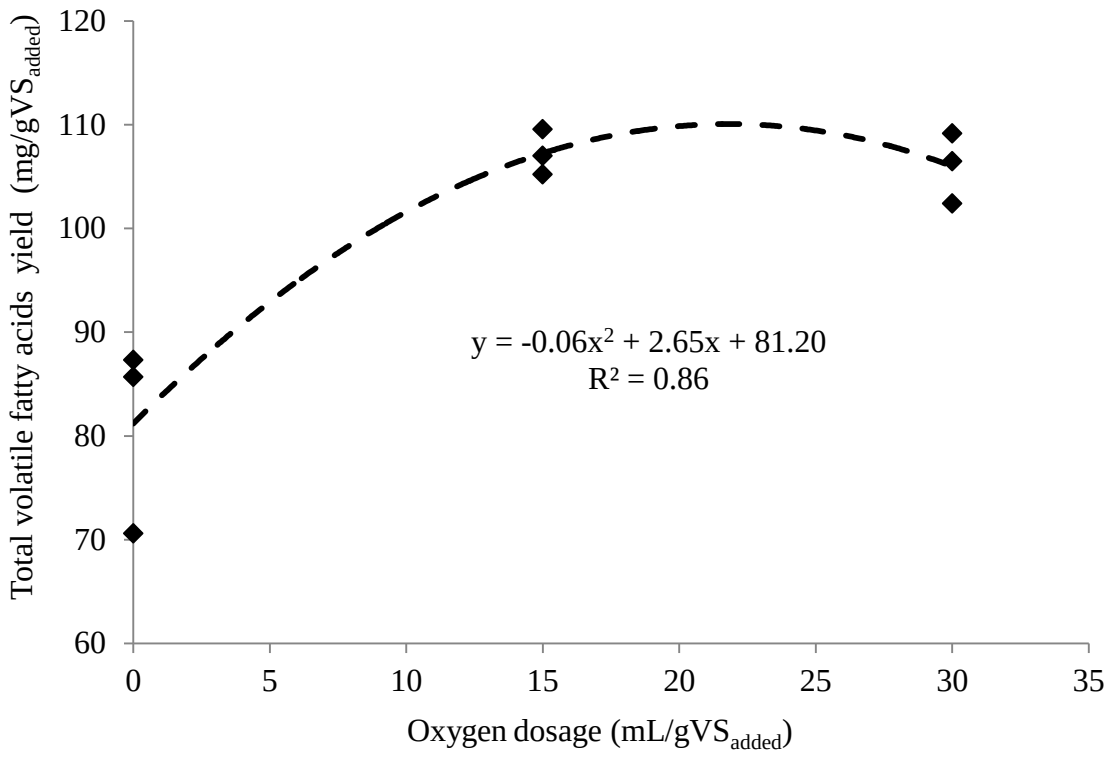


Figure 4.4. Quadratic regression of the volatile fatty acids yield with different O_2 dosages at incubation time of 3 days using ADCM as inoculum

The VFAs yield showed a close fit to the quadratic model with the coefficient of determination (R^2) of 0.86. The predicted model was found to be Y_{VFAs}= -$0.06X^2_{ox}$+$2.65X_{ox}$+81.20, where Y_{VFAs} and X_{ox} represent VFAs yield and O_2 dosage, respectively. The optimum O_2 dosage was then calculated from the first-order differentiation of the equation. The predicted optimum O_2 dosage in this study was found to be 22 mL/g VS_{added}, which resulted in the maximum VFAs yield of 110.05±5.46 mg/g VS_{added}. To further prove that the predicted value truly reflects the actual value, a series of batch experiments was conducted in triplicate using the O_2 dosage of 22 mL/g VS_{added}, incubation time of 3 days, and ADCM as inoculum. The experimental results showed that

the VFAs yield of 112.70±5.15 mg/$gVS_{added,}$ was not significantly different from the predicted value (i.e., 110.05±5.46 mg/gVS_{added}). Thus, the proposed model was precise enough to effectively predict the VFAs yield during AD of Napier grass using ADCM as inoculum during incubation time of 3 days. Since using ADCM as inoculum and incubation time of 3 days was the optimum operating condition in this study, the constructed model could effectively predict the required O_2 dosage to obtain the expected VFAs production without performing any additional experiments.

4.1.5. Change in structural composition of biomass

The best, the median, and the worst operating conditions for VFAs productions of each inoculum were selected as presented in table 4.1. to investigate the changes in structural composition of biomass during AD.

Table 4.1. The selected conditions for studying the change in structural composition of biomass

Operating condition	Inoculum			
	ADCM		ADWAS	
	Oxygen dosage (mL/gVS_{added})	Incubation time (day)	Oxygen dosage (mL/gVS_{added})	Incubation time (day)
Best	15	3	30	1
Median	0	1	30	3
Worse	30	1	30	5

The structural carbohydrates (i.e., cellulose and hemicellulose) and lignin (ADL) content of Napier grass before and after AD are summarized in Table 4.2. Compared with raw Napier grass, the fibers obtained from the batch tests with ADCM as inoculum showed significantly lower amount of hemicellulose (i.e., 25-34%) and cellulose (i.e., 2-18%). Conversely, for the fibers obtained from batch tests using ADWAS as inoculum, there were no significant difference in both hemicellulose and cellulose contents in the biomass before and after AD as presented in Table 4.2. and Table A5 in appendix A. The higher cellulose and hemicellulose degradation of fibers obtained from the batch tests using ADCM as inoculum thus resulted significantly higher VFAs yields compared with the batch tests using ADWAS as an inoculum. Gu et al. (2014) also presented a similar discussion when digested dairy manure and digested municipal sludge, and rice straw were used as the inoculum and substrate, respectively for methane production. The authors reported an increase in cellulose and hemicellulose degradation resulting in higher specific methane yield. For the batch tests using ADWAS as an inoculum, there were no significant differences in biomass composition after AD among all operating conditions. This further showed that ADWAS was the least effective inoculum in the AD of Napier grass. Surendra and Khanal (2014) also observed high methane yield from Napier grass, when ADCM was used as an inoculum. Typically, the macromolecules, such as lignocellulose are degraded during hydrolysis step of AD to produce monomeric sugars, which are subsequently converted into VFAs and then to methane. Thus, the higher the rate of degradation of these compounds, the higher the product yield (i.e., VFAs and/or methane) should be. Based on the compositional analysis, it was apparent that microorganisms preferably consumed hemicellulose while leaving cellulose and

lignin in the digestate during AD. Approximately 25-34% and 21-23% of hemicellulose was degraded from the raw Napier grass using ADCM and ADWAS as inoculum, respectively. However, the respective cellulose degradations were only 2-18% and 9-18% of total cellulose using ADCM and ADWAS as inoculum. Yue et al. (2010) studied the composition change of cow manure fiber during AD and reported that hemicellulose was the favorable component of the fiber to be converted to methane. The AD digested fiber contained 11% less hemicellulose compared with raw manure (Yue et al., 2010). The cellulose-rich fiber has undergone partial biological pretreatment during AD and could directly be subjected to enzymatic hydrolysis to release monomeric sugars as a-potential feedstock for producing other bio-based chemicals via anaerobic biorefinery approach (Sawatdeenarunat et al., 2016). Ethanol is one of the products bio-converted from cellulose-rich fiber. The ethanol production from low-hemicellulose feedstock could avoid the problem of fermenting five carbon sugar into ethanol, which has low ethanol yield and requires specific microorganisms (Lee, 1997).

The lignin content measured as ADL increased for all experimental conditions (i.e., 75-125% for biomass samples when ADCM was used as inoculum and only 8-40% for biomass samples when ADWAS was used as inoculum) following AD compared to the raw Napier grass due to decrease in cellulose and hemicellulose contents of the fiber. Lignin, a phenylpropane-based polymer, is highly recalcitrant to microbial attack including AD. Since the Ankom method was used for fiber analysis, which is a gravimetric mass balance approach, residual fibers showed higher lignin content. Lignin can also be used as a substrate to produce many bio-based products i.e. vanillin (a

flavoring reagent), a binding agent in an animal feed, carbon fiber as well as heat and electricity via thermochemical processes (Surendra et al., 2015).

Table 4.2. The characteristics and structural carbohydrates of the selected conditions

Sample	NDF (% TS)	ADF (% TS)	Lignin (ADL) (% TS)	Hemicellulose (% TS)	Cellulose (% TS)
Raw Napier grass	75.0±1.0	49.7±0.6	10.2±0.3	25.3±1.6	39.5±0.9
ADCM,15,3	73.4±0.6	55.4±1.8	23.0±1.0	19.0±1.3	32.3±1.0
ADCM,0,1	73.0±0.6	55.3±2.9	18.9±0.6	17.7±2.2	36.3±0.6
ADCM,30,1	73.3±1.1	56.5±0.9	17.8±2.4	16.8±1.0	38.6±2.4
ADWAS,30,1	66.6±2.4	46.7±0.2	11.0±1.3	19.9±1.4	35.7±1.3
ADWAS,30,3	69.2±4.0	49.6±0.4	13.7±4.7	19.5±1.4	36.0±4.7
ADWAS,30,5	66.7±1.5	46.8±0.2	14.3±3.1	19.9±2.8	32.5±3.1

4.1.6. Microscopy examination of biomass

Digested fiber samples were collected from the serum bottles that yielded the highest VFAs, and were subjected to SEM examination. The SEM micrographs of the digested fiber showed rough and crumbled surface structures (Figure 4.5(b) and 4.5(c)) as opposed to the undigested fiber, which had smooth intact surface structures (Figure 4.5(a)).

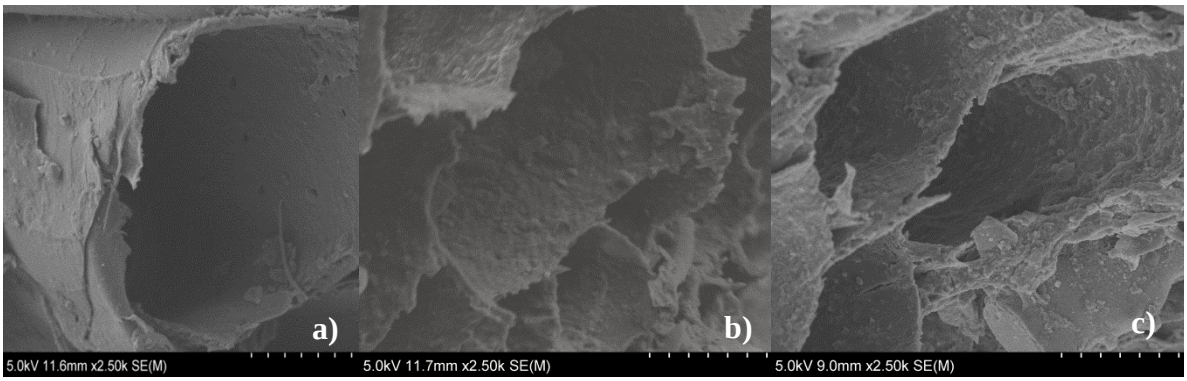


Figure 4.5. Scanning electron micrographs of the cross section of Napier grass fibers, (a) raw, (b) using ADCM as inoculum, O_2 dosage of 15 mL/g VS_{added}, incubation time of 3 days and (c) using ADCM as inoculum, O_2 dosage of 30 mL/g VS_{added}, incubation time of 1 day

The rough surface of the fiber was mainly due to degradation of hemicellulose from the biomass as evident from the fiber analysis presented in Table 4.2. The surface structure showed some correlation between the fiber destruction and VFAs yield. It is important to note that the rough surface structure does not necessarily correlate to VFAs yield as the fibers collected from the serum bottles at O_2 dosage of 30 mL/gVS_{added} and incubation time of 1 day did not yield significantly higher VFAs yield. Thus, additional parameter such as the composition of the raw and digested fiber needed to be examined as discussed in Section 4.1.5.

4.2 Examine volatile fatty acids yield from Napier grass using micro-aerated horizontal bioreactor

The horizontal bioreactors were inoculated with ADCM and started up at OLR 4 kgVS/m^3-d. The OLR was gradually increased at an interval of 1 kgVS/m^3-d. The operating OLRs was 6 kgVS/m^3-d and the bioreactors was operated for more than 30

days after reaching steady state. Micro-aeration was initiated on day 57 at the flow rate of 450 mL/min for 1 minute every 6 hours. The VFAs concentration are shown in Figure 4.6.

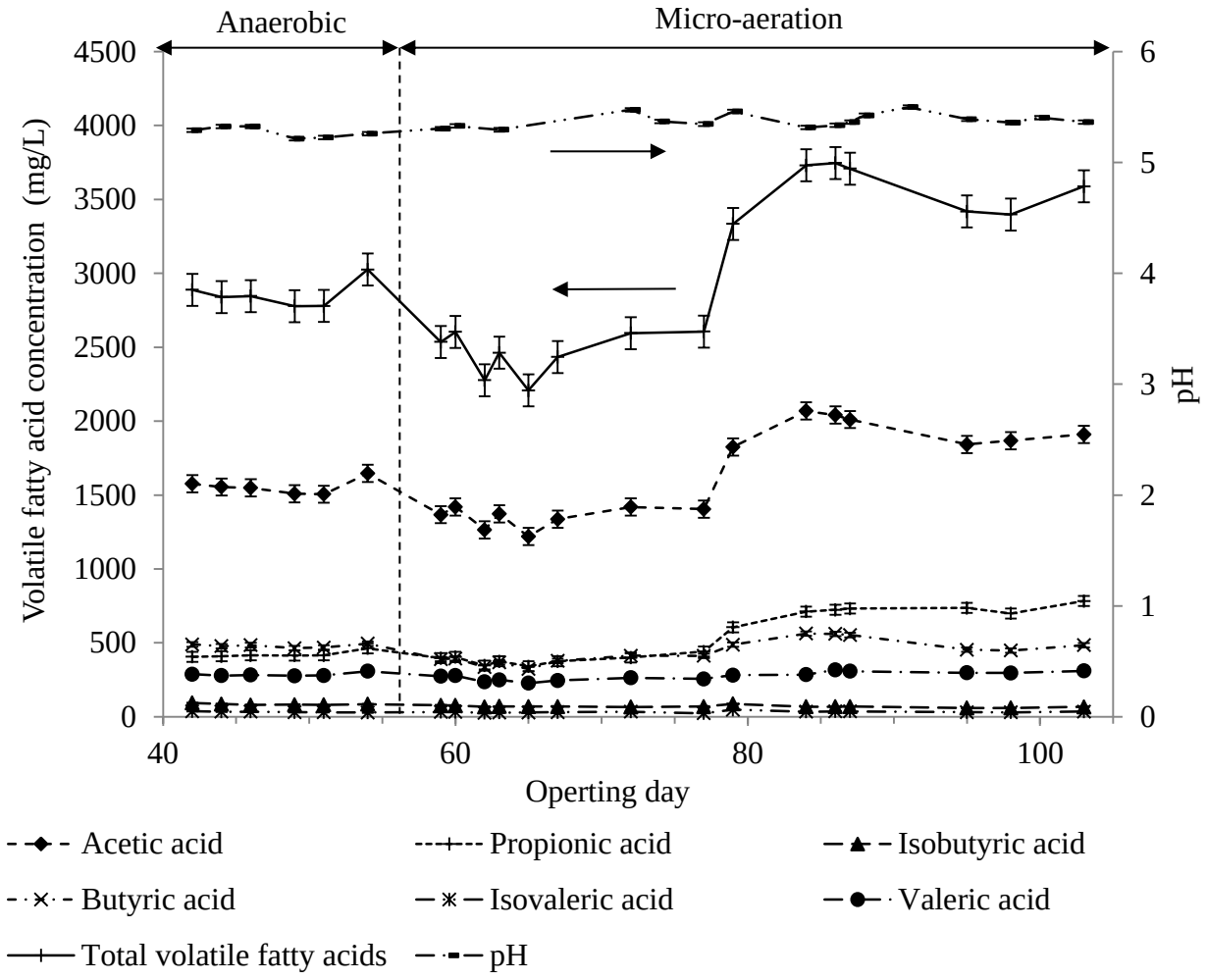


Figure 4.6. Total and individual volatile fatty acids concentration under anaerobic and micro-aeration condition in horizontal bioreactor

4.2.1 Volatile fatty acids concentration

The effluent pH from horizontal reactor was 5.27±0.04 and 5.37±0.06 for aerated and anaerobic conditions, respectively. These values were slightly lower than the

recommended range for acidogenesis (i.e., 5.50-6.50) (Li and Khanal, 2017) to prevent the growth of methanogens. $NaHCO_3$ was added on a weekly basis to maintain the desired pH of the systems. The total VFAs concentration was in between 2800 and 3030 mg of acetic acid (HAc)/L during anaerobic condition (i.e., day 42-57). However, following micro-aeration on day 57, the VFAs concentration significantly dropped and the concentration varied from 2500 to 2600 mg HAc/L between day 59 and 77. This phenomenon could be due to acclimatization of the microorganism during micro-aeration (Lim et al., 2014). After day 77, VFAs concentration sharply increased by almost 30%. At the stable operating condition, the VFAs concentration under micro-aeration condition was 3524±191 mg HAc/L, which was significantly higher than that under anaerobic condition (i.e., 2831±89 mg HAc/L). Acetic acid accounted for more than 50% of the produced VFAs in both anaerobic and micro-aeration conditions. Propionic, butyric, and valeric acids were also observed during the operating period. Similar result was also reported when leach-bed reactors were used during micro-aeration using grass silage as a mono-substrate (Jagadabhi et al., 2010). The results showed nearly 4-fold increase in VFAs production following micro-aeration, and acetic acid was the predominant VFA (i.e. over 40% of total VFAs). Similar results were also reported by Zhu et al. (2009) using vegetable and flower wastes as the substrates during two-phase anaerobic digestion. The authors reported that the appropriate amount of air dosage could enhance the hydrolysis of carbohydrates and proteins which could subsequently produce higher VFAs compared to conventional acid production. The acetic and propionic acids accounted for 50, and 40% of the produced VFAs, respectively. Obeta Ugwuanyi et al. (2005) studied the effect of aeration on potato peel waste using thermophilic anaerobic digestion and

reported that acetate concentration decrease with increasing oxygen dosage. At the optimum oxygen dosage (i.e., 0.1 vvm), the produced acetate dropped after 84 and 60 hours of digestion time when the pH was controlled at 7.0 and without control, respectively.

4.2.2 Soluble chemical oxygen demand (SCOD) production

Typically, SCOD represents the product formed during hydrolysis stage of AD process. During micro-aeration condition, the produced SCOD and VFAs was 10 and 24% higher than those of anaerobic condition, respectively. The SCOD and VFAs concentrations are summarized in table 4.3.

Table 4.3. The comparisons of soluble chemical oxygen demand and volatile fatty acids productions from this study

Condition	SCOD (mg/L)	Volatile fatty acids (VFAs) (mg/L)	VFAs/SCOD
Anaerobic	8017±1039	2831±89	0.36±0.04
Micro-aeration	8839±482*	3524±191*	0.40±0.03*

(Note: the values are presented in average±standard deviation (n=5), * significantly higher)

Several studies reported the enhancement of hydrolysis during AD of various substrates and reactor configurations (Jagadabhi et al., 2010; Zhu et al., 2009) during micro-aeration. The positive effect micro-aeration on hydrolysis of protein and carbohydrate of primary sludge in a batch mode was reported by Johansen and Bakke

(2006). The authors reported over than 50% increase (based on COD) in hydrolysis compared to anaerobic batch. However, no effect of micro-aeration on lipid hydrolysis was observed. Charles et al. (2009) concluded that the increasing in the hydrolytic enzymes (i.e., cellulose and protease) during the pre-aeration period using organic fraction of municipal solid waste as the substrate under thermophilic condition. However, enhancement of hydrolysis by micro-aeration depends on the applied air dosage. Insufficient aeration rate could decrease the hydrolysis efficiency compared with anaerobic condition when fresh vegetable and flower wastes were used as substrates (Zhu et al., 2009). VFAs/SCOD ratio can be used as an indicator of efficiency of acidogenesis during AD. The higher ratio indicates the higher efficiency of acidogenesis to convert the intermediate products from hydrolysis (i.e., alcohol, sugars, amino acid, and fatty acids among others) to VFAs. Xu et al. (2014) presented that after 10 days of operation of leach bed reactors coupled with methanogenic upflow anaerobic sludge blanket reactor using synthetic food waste as the substrate, the VFAs/COD ratio during aerated condition was 2-fold higher than that of anaerobic condition. Botheju and Bakke (2011) reported that the higher VFAs production during micro-oxygenation might be from the high yield of facultative microorganisms resulting in higher biomass and more extracellular hydrolytic enzymes production. It should be noticed that during aeration, acetic, propionic, and butyric acids significantly increased; however, iso-butyric, iso-valeric, and valeric acids concentrations were not significantly different compared to that under anaerobic condition. Zhu et al. (2009) concluded that during micro-areared AD of food waste and brown water, microorganism belonging to the *Firmicutes* phylum increased, which resulted in a higher substrate hydrolysis rate.

4.2.3 Biogas composition

Aeration was found to inhibit the strict anaerobic microorganisms (i.e., methanogens) (Botheju and Bakke, 2011) and produced alternative products (e.g., hydrogen and VFAs) during AD (Chae et al., 2010). The authors reported significantly lower CH_4 yield compared to anaerobic condition (at α=0.05). However, CO_2 yield was not significantly different in both operating conditions. Methanogens usually lack enzyme, *superoxide dismutase* which mitigates toxic oxygen ions and radicals. Kiener and Leisinger (1983) reported that the methanogenic species, *Methanococcus voltae a*nd *Methanococcus vannielii*, were extremely sensitive to oxygen. The average CH_4 and CO_2 yields from this study are presented in table 4.4.

Table 4.4. The biogas yield of the horizontal bioreactor form this study

Condition	CH_4 yield (NmL/gVS_{added})	CO_2 yield (NmL/gVS_{added})
Anaerobic	13.7±1.8*	21.7±3.0
Micro-aeration	8.5±1.8	20.8±2.9

(Note: the values are presented in average ± standard deviation (n = 5), * significantly higher)

Similar to this study, Botheju and Bakke (2011) also reported that the initial aeration or anaerobic inoculum could lead to longer lag phase (nearly 3-fold) of methanogen compared to anaerobic inoculum. Botheju et al. (2010) conducted a long-term study to examine the effect of oxygenation on the performance of AD using synthetic substrate under mesophilic condition. The authors indicated that methane production decreased

with increasing oxygen dosing. It could be concluded that the higher VFAs concentration of micro-aeration condition was associated with the inhibition of methanogens. With the introduction of appropriate oxygen dosage, the facultative microorganisms maintain the metabolic function via fermentation to produces VFAs rather than aerobic respiration to produce CO_2 (Botheju and Bakke, 2011). When the excess oxygen was added, CO_2 increased which indicated that the metabolic pathway switched from fermentation to aerobic respiration (Botheju and Bakke, 2011; Johansen and Bakke, 2006). The more the CO_2 produced, the less the available carbon source for VFAs production. Thus, appropriate reactor operation in this study contributed to VFAs production during AD of Napier grass.

4.2.4 Fiber composition

The change in structural carbohydrate (i.e., cellulose and hemicellulose) and ADL were investigated. Hemicellulose contents of the anaerobic and micro-aeration conditions were 26.2±0.6 and 26.9±0.7%TS, respectively. These values were significantly lower than that of raw Napier grass (i.e., 29.59±0.1%TS). In addition, the hemicellulose removals were 12% and 8% for the micro-aeration and anaerobic conditions, respectively. Conversely, cellulose and lignin were not degraded and exposed in the digestate which could be further utilized to produce plethora of biobased products as discussed in section 2.5. (Sawatdeenarunat et al., 2016). Similar removal of hemicellulose during acidogenesis of AD of lignocellulosic biomass was also reported by Sawatdeenarunat et al. (2017) using a series of batch study. The structural carbohydrates

(i.e., cellulose and hemicellulose) and ADL of raw Napier grass and digestate from different operating conditions are presented in Figure 4.7.

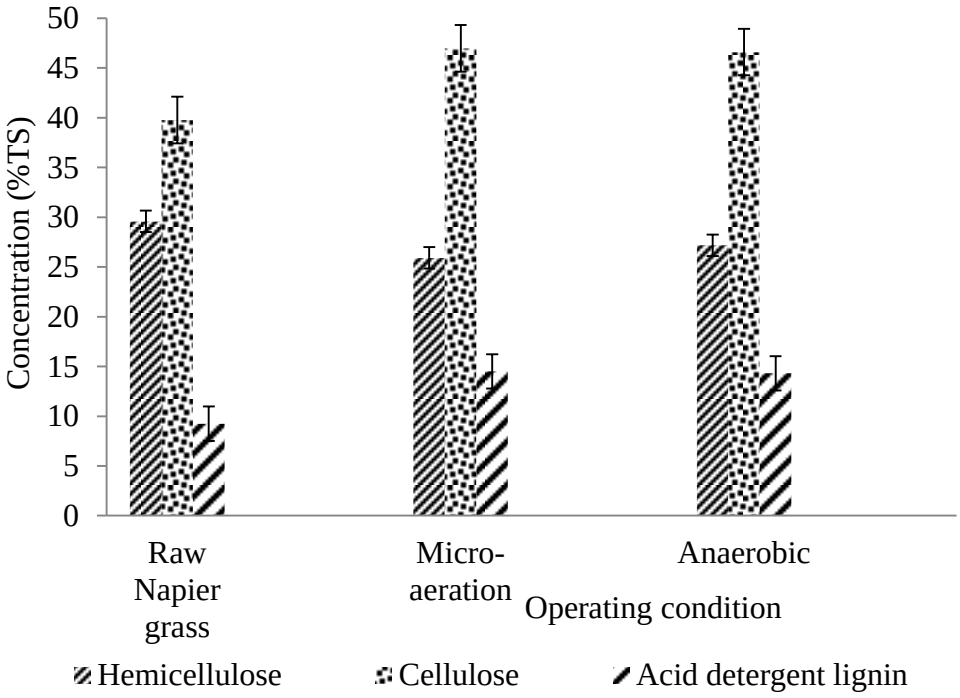


Figure 4.7. The fiber composition of raw Napier grass, and digested fibers from anaerobic and micro-aeration operating conditions

It should be pointed out that the hemicellulose removals from the acid tank were higher than that from methane reactor (i.e., 7%) presented in section 4.3.2.3 when operated at the same OLR (i.e., 6 kgVS/m^3-d). Operation under acidic condition was likely played a role as a mild pretreatment to mitigate the recalcitrant structure of lignocellulosic biomass and subsequently, enhanced the accessibility of the hydrolytic enzymes.

4.3 Long-term anaerobic mono-digestion of Napier grass using horizontal bioreactors

4.3.1 Methane potential of Napier grass

The batch experiments were performed to evaluate the methane potential of raw Napier grass. The results indicated that the cumulative methane production curve plateaued after 30 days of incubation as presented in Figure 4.8. The net methane yield on day 45 was 124.0±11.9 NmL/gVS$_{added}$ which was around 17% lower than the yield reported by Surendra and Khanal (2014) when 6-month Napier grass with 6 mm in size was used as the substrate. This phenomenon might be due to the different in feedstock composition as well as the activity of the inoculum. The obtained methane yield could be used as a baseline to compare with those from bench-scale bioreactor in the following section to determine an efficiency of the bioreactor.

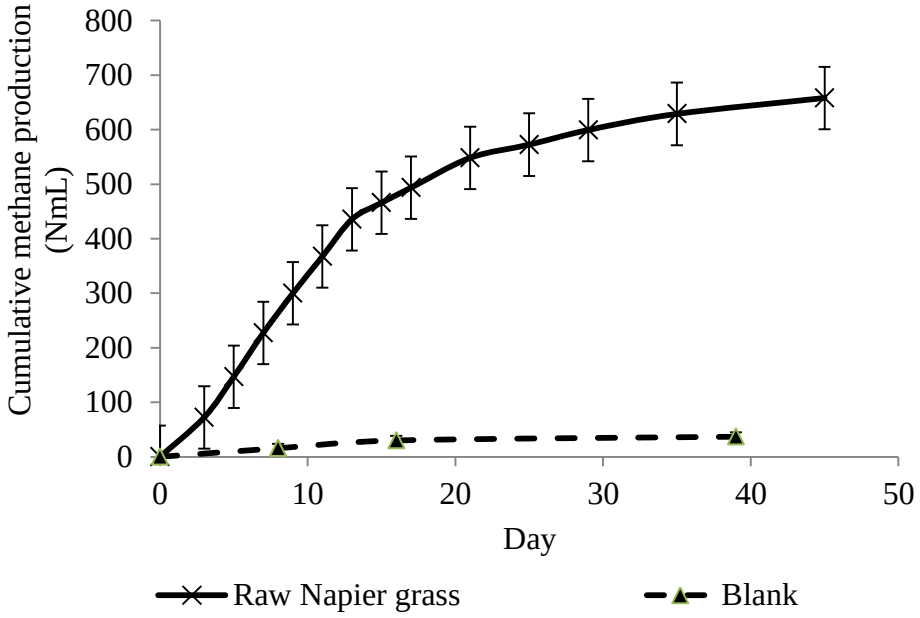


Figure 4.8. The cumulative methane production from raw Napier grass (presented in average values±standard errors)

4.3.2 Long-term mono-digestion of Napier grass

The long-term mono-digestion of Napier grass, a model lignocellulosic feedstock, was examined using two semi-continuous horizontal bioreactors operating in parallel. The reactors performance is in Figure 4.9. The bioreactors were initially started up at OLRs of 2 and 3 kgVS/m^3-d for more than 30 days and then the OLR was increased stepwise at an interval of 1 kgVS/m^3-d. The bioreactors were operated for nearly 300 days before the reactor failure occurred when the OLR was increased to 7 kgVS/m^3-d. This is the highest OLRs ever reported for mono-digestion of lignocellulosic biomass.

4.3.2.1 Reactor performances

The methane yield showed an increasing trend during the startup period (i.e., at OLRs of 2 and 3 kgVS/m^3-d) and reached 80.80±12.24 Nml/gVS_{added} at OLR of 3 kgVS/m^3-d. The average methane yields at higher OLRs of 4 and 5 kgVS/m^3-d were 103.70±6.15 and 106.82±6.16 NmL/gVS_{added}, respectively, which were significantly higher (at α =0.05) than that at OLRs of 2 and 3 kgVS/m^3-d. At OLR of 6 kgVS/m^3-d, the maximum average methane yield obtained was 112.48±9.03 NmL/gVS_{added}. The ratio of total VFAs and alkalinity (VFAs/Alk) was maintained within the recommended range of 0.10-0.25 by adding $NaHCO_3$ (Khanal, 2008). Average pHs of the systems were 7.00±0.03, 7.00±0.04, and 6.95±0.09, respectively, operated at OLR 4, 5, and 6 kgVS/m^3-d, which were the optimal range for anaerobic digestion (Khanal, 2008).The VS removals, however, showed a decreasing trend when OLR was increased. The reactor performance data are summarized in Table 4.5.

Table 4.5. Summary of horizontal bioreactor performance data

OLR (kgVS /m^3-d)	Hydraulic retention time (d)	Average methane yield* (NmL/gVS_{added})	% of methane potential	%VS removal*	Remarks
2	49	75.62±16.45	60	-	Startup
3	32	80.80±12.24	67	-	Startup
4	24	103.70±6.15	83	55.87±2.83	Stable operation
5	19	106.82±6.16	86	54.72±2.65	Stable operation
6	16	112.48±9.03	93	49.54±1.79	Stable operation
7	14	24.93±12.19	20	-	Reactor failure

*Based on minimum of 5 data obtained after reactor operation for 3 hydraulic retention times. The data presented are average values ± standard errors (n=5).

The horizontal bioreactor started to show a sign of instability when the OLR was increased to 7 kgVS/m^3-d on day 259 and the bioreactors was on the verge of failure on day 287 with a perpetual decline in pH to as low as 5.65±0.23 on day 299. The total VFAs increased sharply to over 3,000 mg HAc/L, which apparently suggested that methanogens were inhibited (Khanal, 2008). Moreover, the final propionic acid concentration also reached as high as 1006.9±9.9 mg/L. Propionic acid over 900 mg/L

was reported to inhibit methanogenic activity (Wang et al., 2009). The methane contents during stable operation at OLR of 4, 5, and 6 kgVS/m^3-d were 49.5±0.1, 48.7±1.4, and 47.9±0.6% (v/v), respectively and were not significantly different. However, it dropped to as low as 15.2±5.2% when the bioreactor was on the verge of failure at OLR of 7 kgVS/m^3-d. The solid accumulation (the average TS content in the reactor was 15.7±1.0%) in the reactor at OLR of 7 kgVS/m^3-d led to insufficient mixing of the reactor contents that resulted in high VFAs concentration. Thamsiriroj and Murphy (2010) reported difficulties during AD of grass silage at feed TS content less than 15% and biomass could only submerge after a long period of operation. The scum formation, which is one of important operating issues in AD of lignocellulosic biomass, did not hinder the performance of the horizontal bioreactor during the long-term operation. This could be attributed to unique design of horizontal bioreactor employed in this study, which was able to handle significantly higher OLR that the other studies reported in the literature (See Table 4.6.).

Table 4.6. Anaerobic digestion of lignocellulosic feedstocks

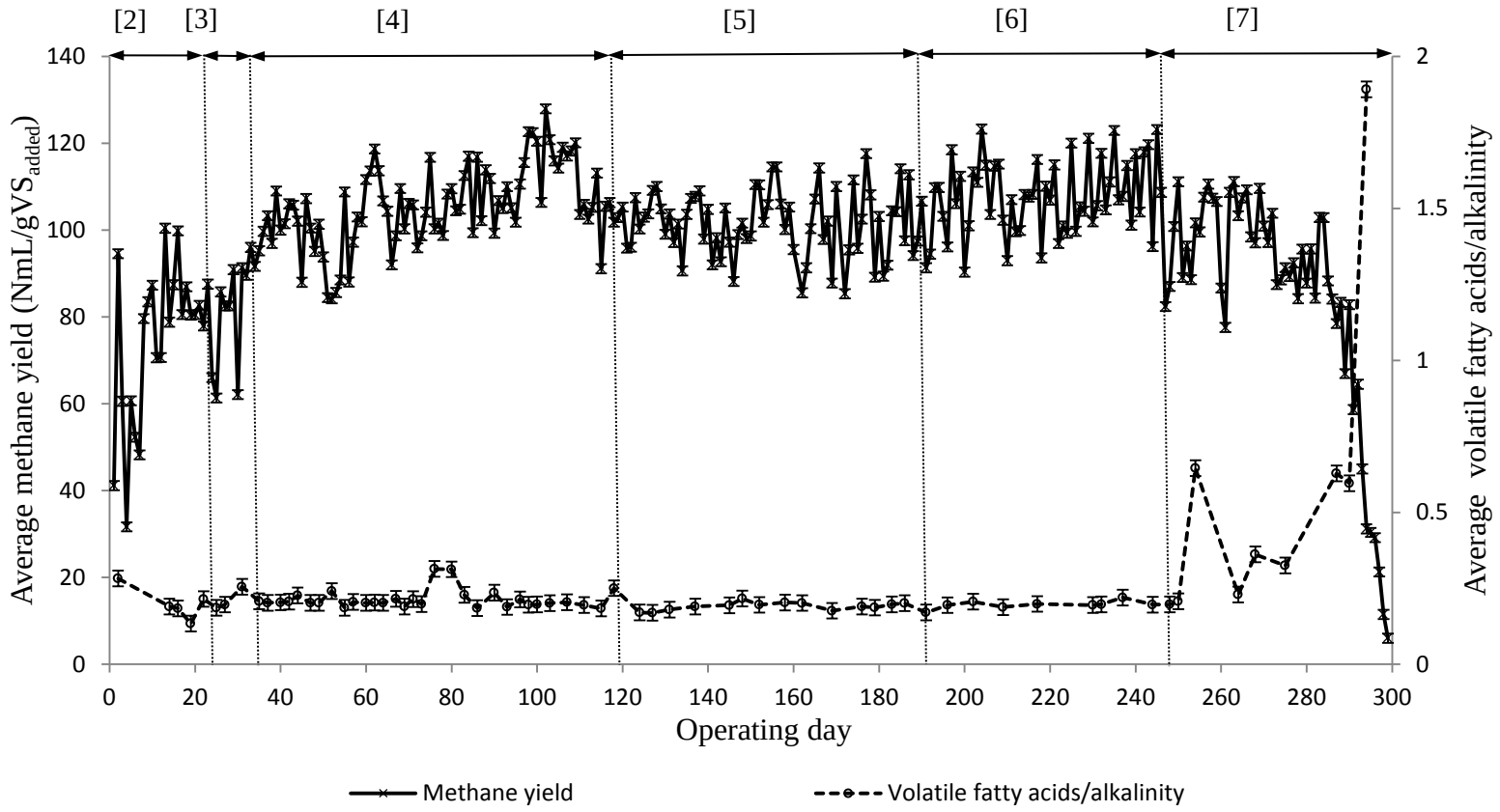
Feedstock	Operating condition	TS of feedstock (%)	Methane yield (NmL/gVS_{added})	OLR ($kgVS/m^3$-d)	References
7-month-old Napier grass	CSTR reactor, Semi-continuous, 170 days, 35°C	Not presented	139	1.23	Wilkie et al., 1986
1.5-month-old Napier grass (Pak Chong 1)	CSTR, 35 days, room temperature	3.6	242	0.57	Janejadkarn and Chavalparit, 2013
Chicken manure and corn stover (42%:58% VS basis)	CSTR reactor, Semi-continuous, 140 days, 37°C	12%	196	4	Li et al., 2014
Cow manure and sugar beet tops (60%:40% VS basis)	CSTR reactor, Semi-continuous, 55 days, mesophilic	5%	194	2	Lehtomäki et al., 2007

Table 4.6. (continued) Anaerobic digestion of lignocellulosic feedstocks

Feedstock	Operating condition	TS of feedstock (%)	Methane yield (NmL/gVS_{added})	OLR ($kgVS/m^3$-d)	References
Cow manure and oat straw (60%:40% VS basis)	CSTR reactor, Semi-continuous, 55 days, mesophilic	5%	139	2	Lehtomäki et al., 2007
6-month-old Napier grass	Horizontal CSTR reactor, Semi-continuous, 299 days, 33±2°C	12	113±9	6	This study

Although the maximum methane yield obtained in this study was lower than those reported in other studies, the methane yield of the bench-scale study was nearly 93% of the biomethane potential of the Napier grass. The difference in the yield could be attributed to feedstocks characteristics, age of the feedstocks and prior pretreatment such as ensiling (Hendriks and Zeeman, 2009). To the best of our knowledge, this is the first

long-term successful mono-digestion of lignocellulosic biomass, especially using high yielding energy crop at OLR as high as 6 kgVS/m^3-d. It is important to note that the focus of this study was not on maximizing methane yield; but to degrade hemicellulose and to obtain energy-rich fiber.

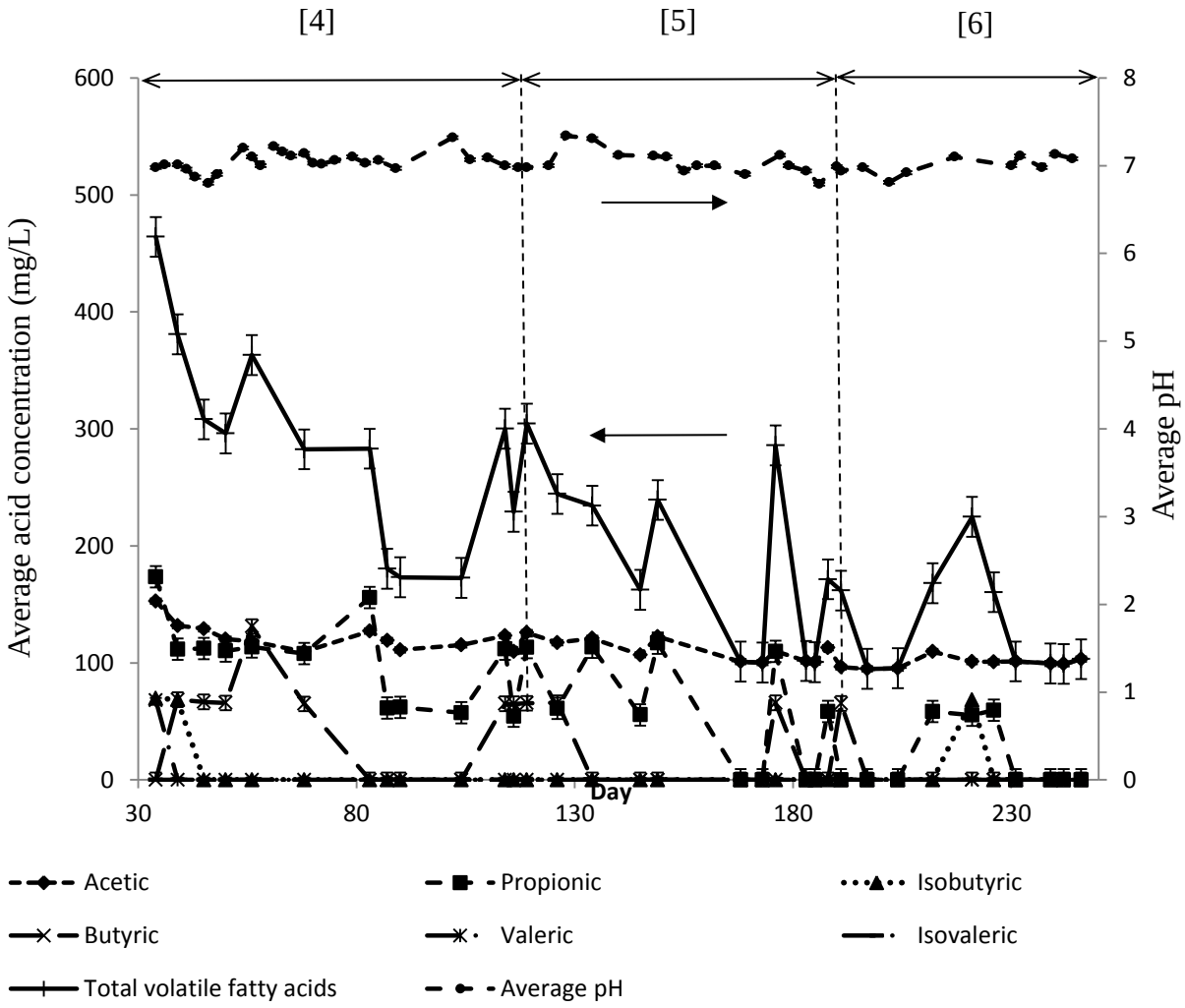


(Note: The number in the bracket refers to organic loading rate (OLR) (gVS/m^3-d))

Figure 4.9. Horizontal bioreactor performance at different organic loading rates

4.3.2.2 Volatile fatty acids profile

The individual VFAs were analyzed at different OLRs to evaluate the stability of the bioreactor and the results are shown in Figure 4.10.



(Note: The numbers in the bracket represent organic loading rate (OLR) ($kgVS/m^3$-d))

Figure 4.10. Total and individual volatile fatty acids concentration at different organic loading rates

The average total VFAs concentration at OLR 4, 5, and 6 kgVS/m^3-d were 239±29, 125±33, and 100±2 mg HAc/L, respectively as presented in Figure S2 in SI. Typically, a normal operating AD system has VFAs in between 50 to 250 mg HAc/L (Khanal, 2008). However, higher VFA concentration was reported when lignocellulosic feedstock was used as a substrate. Nizami et al. (2012) reported total VFAs concentration between 100 and 590 mg HAc/L when completely stirred tank reactor was used for grass silage digestion. Acetic and propionic acids were the predominant VFAs followed by butyric acid at all OLRs. Small concentrations of valeric and isovaleric acids were also detected. Orozco et al. (2013) also reported that acetic acid was the dominant VFA followed by butyric and propionic acids when grass silage was used as the substrate. In another study with AD of Napier grass, Janejadkarn and Chavalparit (2013) reported predominance of acetic acid. In this study, when the OLR was increased to 7 kgVS/m^3-d, total VFAs concentration sharply increased from 133±15 mg HAc/L on day 279 to over 3,300±318 mg HAc/L on day 299. The corresponding individual VFAs: acetic, propionic, and butyric acids increased from 116±13, 7±1 and 17±2 mg/L to 1,970±280, 975±23 and 626±65 mg/L, respectively. The results clearly showed that methanogenesis was inhibited, and the reactor was on the verge of failure at higher OLR of 7 kgVS/m^3-d as described in section 3.2.1.

4.3.2.3 Composition analysis of the fiber from bench-scale study

In order to identify which component of biomass was degraded during AD, the changes in fiber compositions before and after AD were also examined at all OLRs. The structural carbohydrates (i.e., cellulose and hemicellulose) and acid detergent lignin

(ADL) of raw Napier grass and digestate, and the removal of cellulosic material are presented in Figure 4.11.

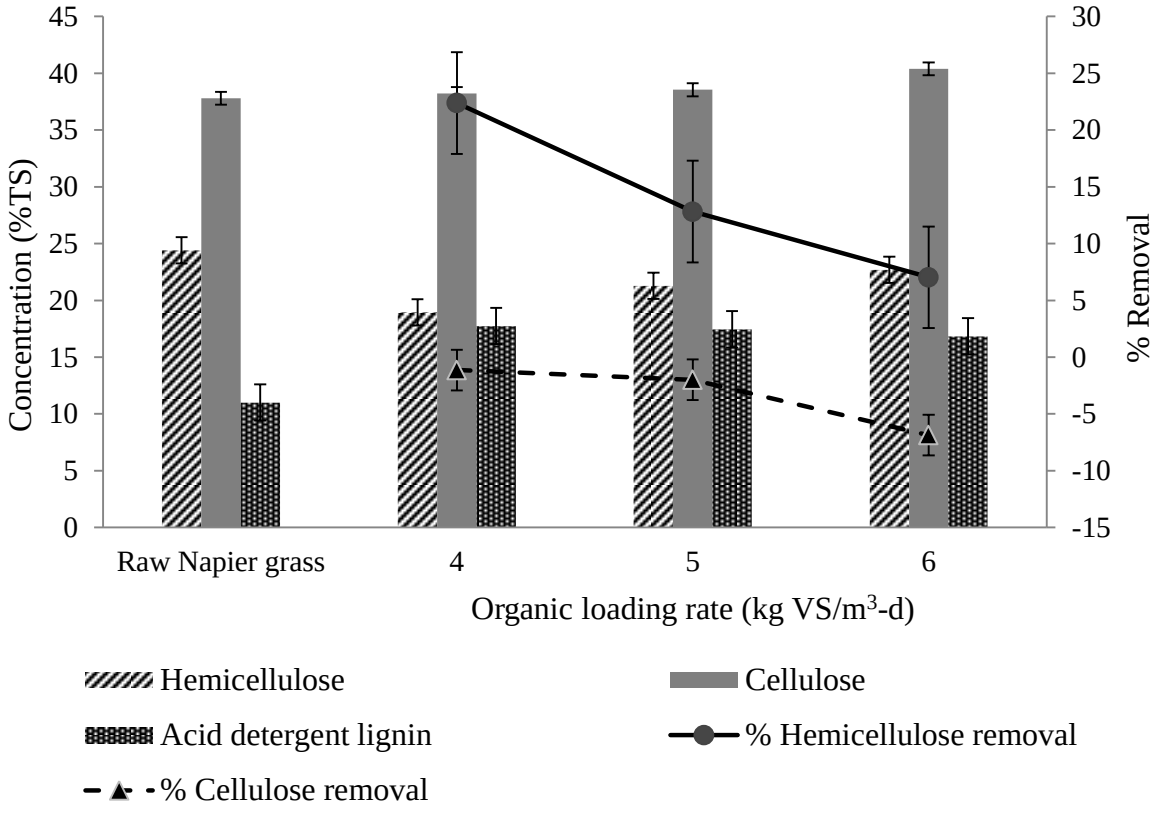


Figure 4.11. The fiber composition of raw Napier grass and digested fiber and their removals at different organic loading rates

Hemicellulose contents of the digested fibers obtained at OLRs of 4, 5, and 6 kgVS/m^3-d, were 18.9±1.5, 21.3±1.0, and 22.7±0.6% of TS, respectively. These contents were significantly lower (statistically) than that of raw Napier grass (i.e., 24.4±0.4% of TS). However, cellulose contents of the digestate at OLRs of 4, 5, and 6 kg VS/m^3-d were 38.2±0.7, 38.5±0.7, and 40.4±0.2% of TS, respectively; whereas the respective ADL

contents of the digestate at OLRs of 4, 5, and 6 kg VS/m^3-d were 17.7±1.2, 17.4±0.9, and 16.8±0.3% of TS. The cellulose and lignin contents were higher than those of raw Napier grass, i.e., 37.8±0.2% and 11.0±0.5%, respectively. Since the fiber analysis via ANKOM method uses a gravimetric mass balance approach, the residual fibers would show higher cellulose and lignin contents to account for loss of hemicellulose content in the fiber. Similar findings were also reported in several studies when ANKOM method was used for fiber composition analysis (Teater et al., 2011; Yue et al., 2010). Teater et al. (2011) found that cellulose and lignin contents in the digested fiber of dairy manure increased by 56, and 46%, respectively compared to raw dairy manure when 8% of hemicellulose was removed during AD. Hemicellulose, a hetero-polysaccharides, is easier to be anaerobically degraded compared to cellulose (Karimi, 2015) and thus, it is the preferable component of lignocellulosic biomass during AD. The better hemicellulose degradation is attributed to several factors, namely, it doesn't form aggregate even when bonded with cellulose (Pérez et al., 2002); the amorphous short chain structure of hemicellulose leads to better enzymatic hydrolysis compared with cellulose (Karimi, 2015); and location of hemicellulose in plant cell wall (i.e., on a surface of cellulose) facilitates the accessibility of enzymes.

4.4 Thermochemical processes of digestate following biorefinery concept

The compositional analysis of AD digestate showed significant decrease in hemicellulose and ash contents, and increase in cellulose and lignin contents. This, therefore, provided a new opportunity for effective utilization of digestate via thermochemical conversion. The digestate obtained at all OLRs and raw Napier grass

samples were dried at 50°C to achieve a moisture content of less than 10% and shipped to Auburn University, Auburn, AL, USA for the proximate analysis as well as HHV. The results are presented in Table 4.7.

Table 4.7. Proximate analysis and higher heating value of raw Napier grass and digestate on a dry weight basis

Sample	%VCM	%Ash	%FC	HHV (MJ/kg)
Raw Napier grass	77.4 ±1.2	7.4 ±0.2	15.2 ±1.4	17.9 ±0.1
OLR 4 kgVS/m^3-d	77.4 ±1.7	6.2 ±0.9	16.4 ±0.9	18.8 ±0.2
OLR 5 kgVS/m^3-d	79.3 ±3.6	6.2 ±1.1	14.5 ±2.6	18.6 ±0.4
OLR 6 kgVS/m^3-d	78.0 ±0.1	6.7 ±0.5	15.3 ±0.6	18.9 ±0.1

(Note: The data are presented as average value ± standard error)

The digestate showed a higher HHV compared to raw biomass due to an increase in lignin and cellulose contents, and a decrease in the hemicellulose content following AD. Demirbaş (2005) reported the HHVs of 17–18 MJ/kg for cellulose and hemicellulose, and 26 MJ/kg for lignin. Accordingly, a slight increase in VCM and FC, and a decrease in ash content was observed in the digestate samples. However, these values were not statistically different (α=0.05) compared to raw Napier grass samples. The inorganic elements in the raw Napier grass and the digestate samples at various OLRs are presented in Table 4.8.

Table 4.8. Elemental analysis of raw Napier grass and digestate based on dry weight basis.

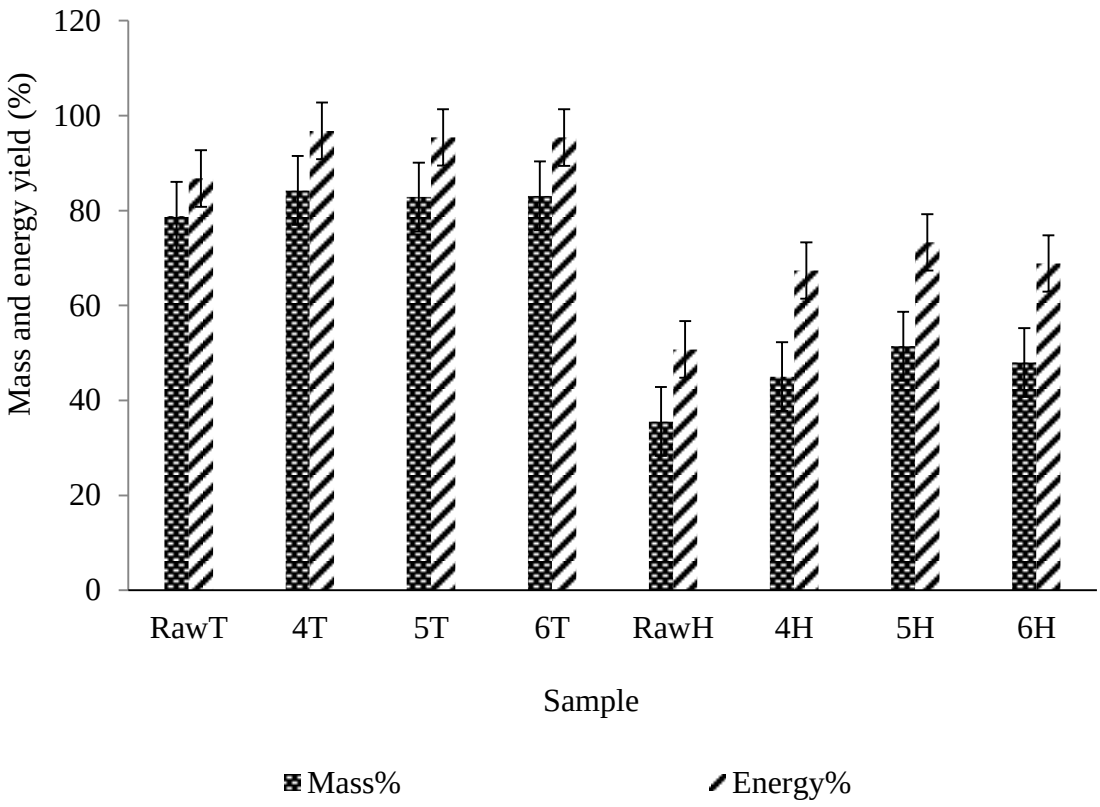
Sample	Element										
	P (%TS)	K (%TS)	Ca (%TS)	Mg (%TS)	Mn (ppm)	Fe (ppm)	Al (ppm)	B (ppm)	Cu (ppm)	Zn (ppm)	Na (ppm)
Raw Napier grass	0.32	4.26	0.6	0.39	138.79	1121.68	1054.47	39.38	7.25	36.08	515.25
Digestate	0.26	1.89	0.41	0.23	105.43	1331.34	1373.97	34.08	8.30	22.08	4994.07
% Change	-18	-56	-32	-42	-24	19	30	-13	14	-39	869

(Note: The elements of the digestate are presented in the average values of OLR 4, 5, and 6 kgVS/m^3-d and the negative and positive values mean decrease and increase, respectively)

Majority of the elements decreased during AD which thus contributed to reduced ash content in the digestate. During AD, phosphorus and potassium serve as macro-nutrients and many other inorganic elements as micro-nutrients for the growth of anaerobic microorganisms (Demirel and Scherer, 2011). The HHV of the digestate enhanced in which the ash content showed a significant correlation with biomass HHV (Sheng and Azevedo, 2005).

The digestate samples obtained at different OLRs were subjected to thermochemical conversion via torrefaction and hydrothermal carbonization (HTC, also known as wet torrefaction), to investigate the effect of AD as a pretreatment on the thermochemical conversion products. Figure 4.12. shows the mass and energy yields of thermally-treated solid products. The mass recovery of the digestate through torrefaction ranged from 83±4 – 84±1% at 240 $^{\circ}$C, which was higher than that of raw torrefied Napier grass (79±2%). As significant part of hemicellulose was already consumed during AD, only small amount of biomass structure was degraded during torrefaction compared to raw Napier biomass. Lower mass yields of 74% for rice straw and 78% for grape pomace at 250 $^{\circ}$C were reported without AD process (Nam and Capareda, 2015; Pala et al., 2014). On the other hand, the mass yield from the HTC process was significantly low (36±3%) for raw Napier grass. Again, a significantly higher mass yield (45±1-51±3%) was obtained for the digestate samples through the HTC process. However, the mass yield in this study was much lower compared to the yields from other studies (~50 – 66%) at 250 $^{\circ}$C (Liu et al., 2013; Pala et al., 2014). This could be due to the long residence time (an hour) in this study as compared to 5 to 30 mins in other studies. Thus, residence time plays an important role on product yield in the HTC operation. When the residence time of over 2

hours was applied, the mass yield of raw biomass, such as eucalyptus and barely straw was reduced to 36–40%, which is close to mass yield of raw Napier grass in our study (Sevilla et al., 2011). As compared to torrefaction, the presence of subcritical water in HTC facilitates the hydrolysis reactions at the early stage, which reduces the activation energy of hemicellulose and cellulose thereby favoring better depolymerization and degradation (Libra et al., 2011; Sevilla et al., 2011). This therefore resulted in much lower mass yield in HTC process than from the torrefaction process. To better understand the degree of upgraded solid sample, an energy densification value (the ratio of energy yield divided by the final mass recovered) was calculated. The energy densification value of torrefied raw Napier grass was 1.10, and it increased to 1.15 with torrefied digestate. The densification value was close to that of torrefied rice straw (~1.1–1.2) at temperature of 250°C for 20 and 40 mins. On the other hand, the energy densification values for raw Napier grass and digestate samples after HTC process were 1.42 and 1.43–1.49, respectively. Thus, HTC process (wet torrefaction) yielded much higher energy density products compared to torrefaction process. It is, however, important to point out that overall higher amount of energy was recovered from torrefaction process than the HTC process due to significantly higher mass recovery of the former process.

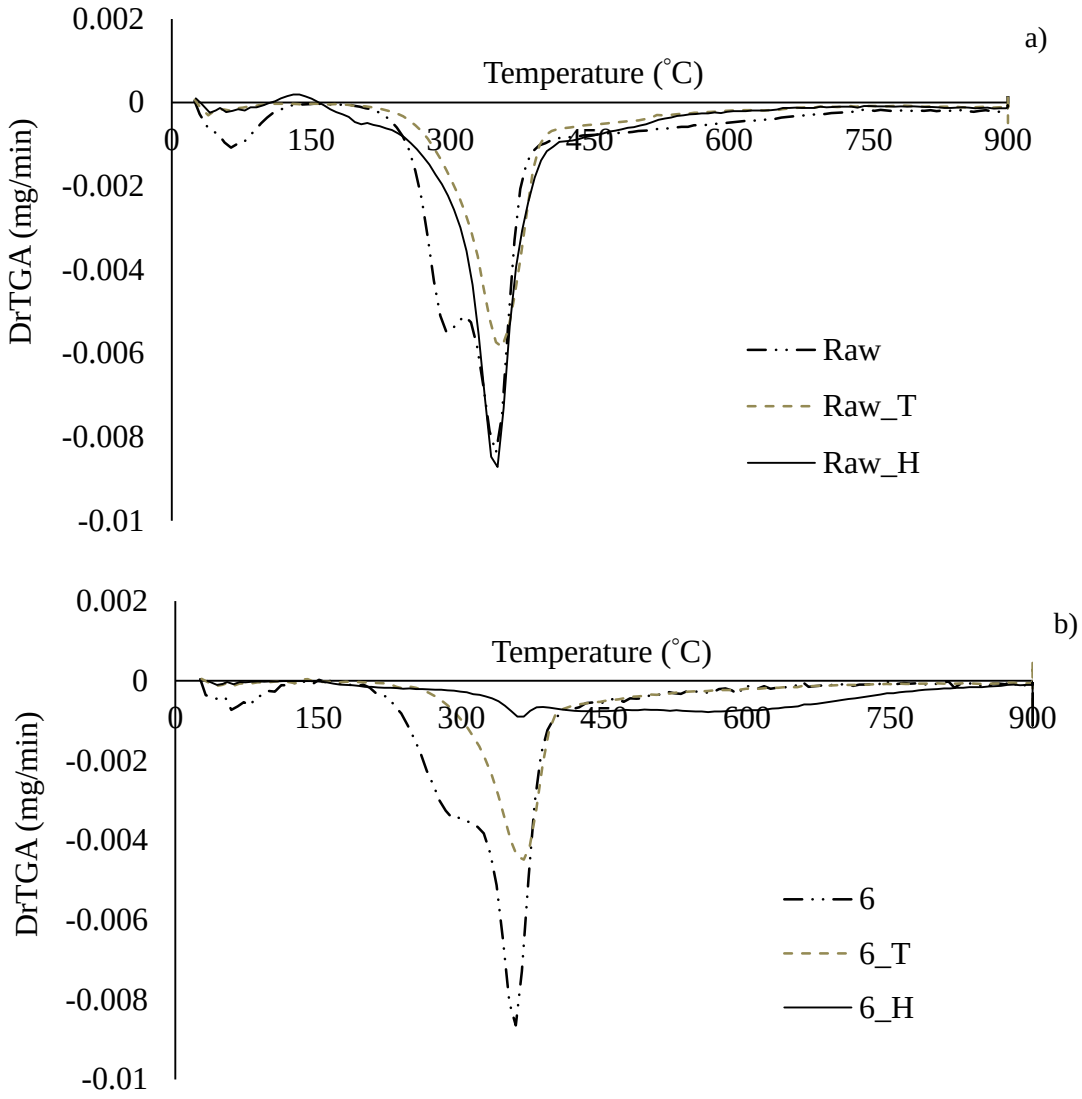


(Note: Raw = Raw Napier grass, the numbers in x-axis indicate operating OLR ($kgVS/m^3$-d), T = torrefaction, and H = hydrothermal carbonization, and the error bars represent standard errors)

Figure 4.12. Mass and energy distribution after torrefaction and HTC with raw Napier grass and digestate samples at 240°C

Derivative thermogravimetric (DTG) analysis from thermogravimetric analysis (TGA) was conducted to examine the degradation of biomass polymer compositions (e.g., hemicellulose, cellulose and lignin). The raw biomass had a shoulder peak at around 295°C corresponding to the hemicellulose as shown in Figure 5 (a) (Ren et al., 2013), whereas the digestate had the reduced shoulder peak at 295 °C (Figure 5 (b)). For

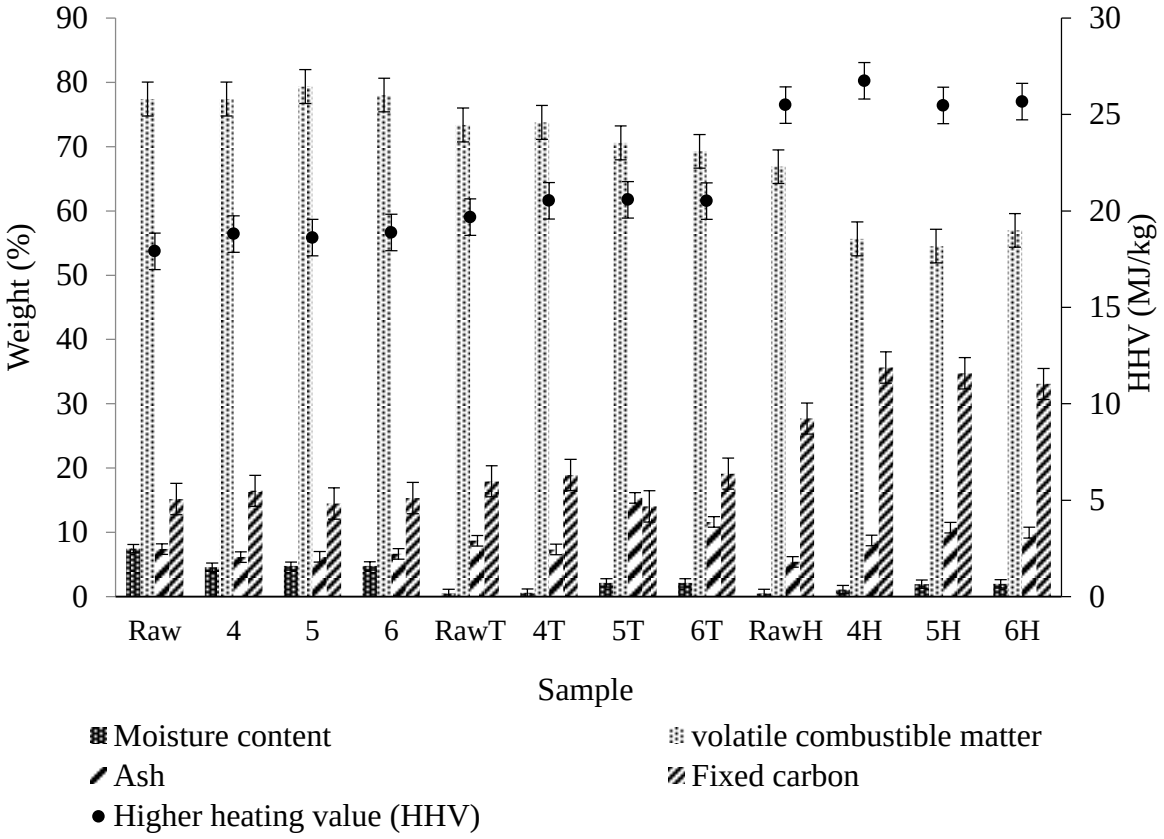
cellulose degradation, the maximum peak of raw Napier grass at 350°C shifted to 358°C in the digestate (OLR 6 kgVS/m^3-d). The smaller peak height at the same temperature represents the severity of the thermal conversion conditions. After the torrefaction process, the hemicellulose peaks from both raw Napier grass and digestate samples were completely removed (Figures 4.13 (a) and (b)). The smaller height of the cellulose peak of torrefied digestate was obtained compared to the torrefied raw Napier grass. With respect to HTC of digestate, almost complete removal of major peaks (e.g., cellulose and hemicellulose) was observed, which was quite different from that of HTC of raw Napier grass. This indicates that AD process played an important role as a pretreatment for facilitating better depolymerization during the HTC process.



Remark: Raw = Raw Napier grass, the number in the graph indicate operating OLR (kgVS/m^3-d), T = torrefaction, and H = hydrothermal carbonization

Figure 4.13. Thermogravimetric analysis of torrefied and hydrothermally carbonized samples

Figure 4.14. shows the proximate analysis for VCM, FC, Ash, MC and HHV. Higher VCMs were observed in both the raw Napier grass and digestate samples (77–79%). However, it was reduced significantly in the torrefied samples (69–73%) and HTC samples (55–67%). In contrast, the FC contents of digestate subjected to HTC increased. Importantly, HHV of raw Napier grass (17.9 MJ/kg) also increased to 19.7–19.6 MJ/kg and 25.5–26.7 MJ/kg for torrefied and HTC biomass, respectively. The upgraded heating value of solid char was close to the heating value of different types of coal (21 MJ/kg for sub-bituminous coal, and 27 MJ/kg for bituminous coal) (Patzek and Croft, 2010). Both torrefaction and HTC processes increased the heating value; but importantly HTC produced more energy dense product as compared to torrefaction. With respect to ash content, the HTC process helped in reducing the ash content because the initial hydrolysis stage facilitated solubilization of ash in water. Kambo and Dutta (2015) reported that the concentration of seven inorganic elements decreased in HTC biochar compared to torrefied biochar. Since high ash concentration can cause problems such as slagging, fouling, and corrosion in combustion plants, it is highly desirable to reduce the ash content of the feed.



(Remark: Raw = Raw Napier grass, the numbers in x-axis indicate operating OLR ($kgVS/m^3$-d), T = torrefaction, and H = hydrothermal carbonization and the error bars represent standard errors)

Figure 4.14. Higher heating value and proximate analysis of torrefied and hydrothermal carbonized samples

CHAPTER 5

TECHNO-ECONOMIC ANALYSIS OF DIGESTATE UTILIZATION

The solid-biofuels produced via thermochemical processes (e.g., torrefaction and hydrothermal carbonization (HTC)) have higher HHV and FC as well as lower VCM compared with those of the raw Napier grass and the AD digestate as presented in section 4.4. The HHV of the solid-biofuels is nearly same as sub-bituminous and bituminous coals. There are varieties applications of the biofuel pellets ranging from solid fuel for producing electricity via co-firing to as a feedstock for producing plethora of bio-based products as showed in Figure 6.1. In this chapter, however, the produced pellets are assumed to be used onsite for co-firing process to produce electricity and mitigate the local/regional air pollution issues. The main objective of conducting the techno-economic analysis was to determine the economic feasibility of the two selected thermochemical conversion processes, namely torrefaction and hydrothermal carbonization (HTC). In addition, the break-even points of product selling prices and the maximum pretax minimum acceptable rate of return (MARR) of each process are also examined. The cost break down of torrefaction and HTC including pelletization process are presented in Table 5.1.

Table 5.1 Total cost comparison of torrefaction and hydrothermal carbonization processes at the capacity of 25 dry ton Napier grass/day (assumed the plant is located in Hawaii)

Items	Torrefaction[a]	Hydrothermal carbonization[b]
Capital costs ($)		
Capital investment[c]	1,395,423	4,095,233
Operation cost ($/year)		
Fuel gas[d]	40,227	191,140
Process water	-	9,026
Utility[e]	3,600	39,106
Labor[f]	57,600	57,600
Maintenance	2,880	192,107
Plant overhead	16,200	40,952
Total	120,507	529,931
Revenue		
Pelletized biomass[g]	254,144	146,975

(Note: [a] estimated costs based on Xu et al. (2014), [b] estimated costs based on Suwelack et al. (2016), [c] calculated cost based on the equation recommended by Batidzirai et al. (2013) with the scaling factor of 0.7, [d] Natural gas price in Hawaii of $15.63/GJ (U.S. Energy Information Administration (EIA), 2017), [e] Electricity cost in Hawaii of $0.33/kW-h (https://hawaiienergy.com), [f] assume working period of 8 hours/day and 360

days/year, and minimum wage of $20/hour, and [g] Coal price of $51.60/short ton (U.S. Energy Information Administration (EIA), 2016).

The net cash flows of both technologies were performed with the following assumptions. The useful life times of the torrefaction and HTC were assumed to be 15 years as suggested by Batidzirai et al. (2013) and salvage values were negligible. There were no machine replacements during the useful life period (Batidzirai et al., 2013). Taxation and depreciation were not considered. In addition, the digestate had no market value since it contains low nutrients as mentioned in chapter 4 and cannot be used as a fertilizer. The pre-tax MARR was assumed to be 10% as recommended by García-Gusano et al. (2016). The results of the cashflow analysis indicated the negative present worth of -$378,966 and -$9,839,584 for torrection and HTC, respectively. Thus, both torrefaction and HTC are not enonomically viable options at the assumed conditions. It should be pointed that the devoped model might be more appropriate to developing countries where the operating costs are lower than that of Hawaii. For example, The electricity and labor cost in Hawaii are almost 3-folds and 8-folds higher than that in Thailand, respectively (Hawaii Clean Energy Initiative; Ministry of Labor and Ministry of Energy, Thailand). The selling price is one of the critical parameters affecing the economic feasibility of the system (Kam et al., 2003). To evaluate the break-even point of the plant capacity and selling price, which results in the zero annual worth (AW) at the designed MARR of 10%, sensitivity analysis was conducted by varying plant capacity starting from 25 dry ton Napier grass/day with respect to the annual yield of Napier grass per acre (Osgood et al., 1996) . The plant capacity was then stepwise increased at an interval of 25 dry ton Napier grass/day up to the final plant capacity of

100 dry ton Napier grass/day. The selling prices of the produced solid-biofuels pellets were also varied from $50 to $85 and $320 to $420/metric ton of pellet from torrefied biochar and hydrothermal carbonized hydrochar, respectively, as presented in Figure 5.1.

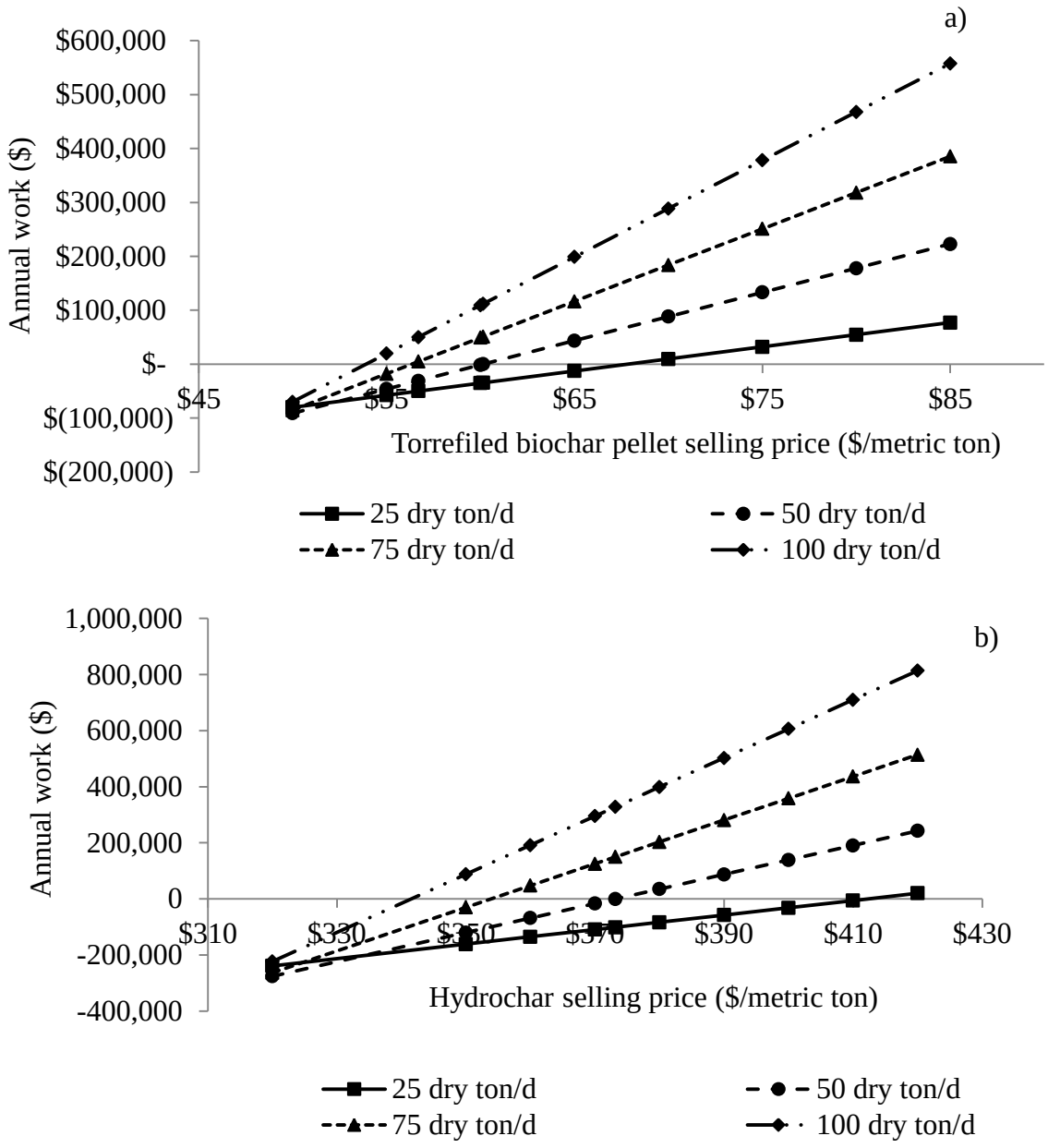
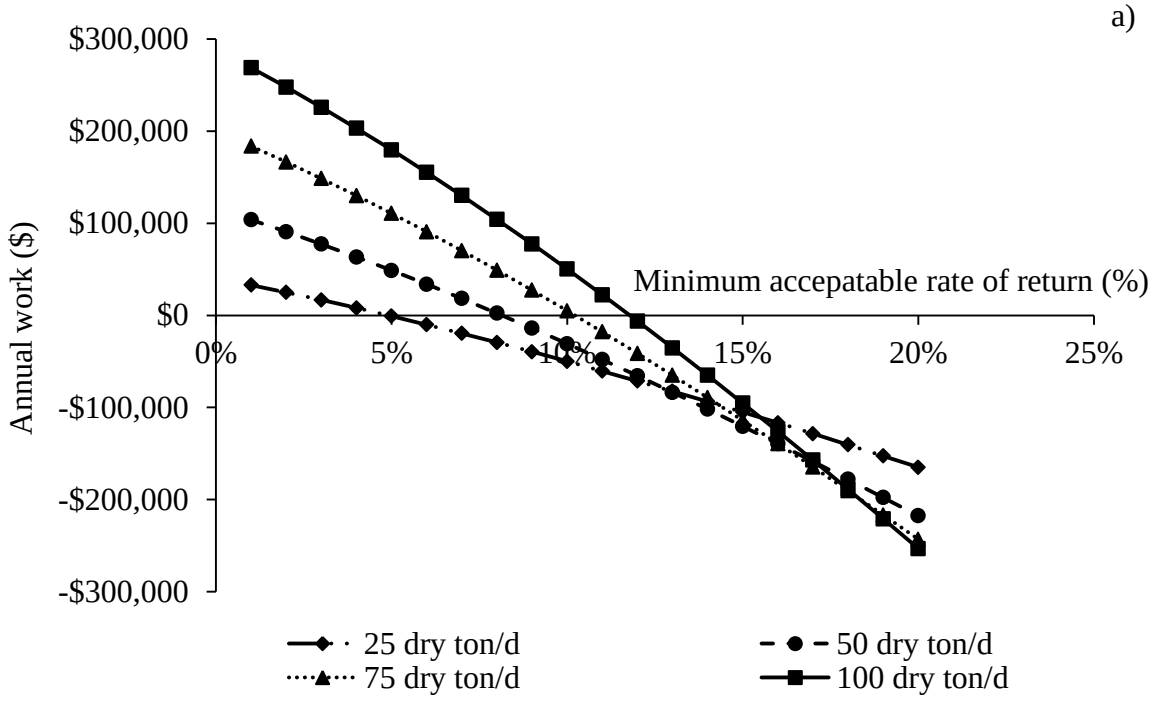


Figure 5.1. Sensitivity analysis of a) Torrefaction process and b) Hydrothermal carbonization process

The break-even selling prices for torrefaction process at 25, 50, 75, and 100 dry ton of Napier grass/day were $67.82, $60.13, $56.33, and $53.89/metric ton of torrefied biochar pellet, respectively. With respect to the results, the plant capacity of the torrefaction process over 75 dry ton of Napier grass/day could result in the positive AW at the current assumed conditions (e.g., selling price of $56.70/metric ton, and MARR of 10%). Nevertheless, the break-even selling prices of the HTC hydrochar pellet were $412.17, $373.17, $353.85, and $341.49/metric ton, for the plant capacity of 25, 50, 75, and 100 dry ton of Napier grass/day, respectively. The selling prices of the hydrochar pellet have to be increased at least 6-fold of the assumed selling price (i.e., $56.70/metric ton) to obtain a positive AW within the range of plant capacity. Moreover, the maximum MARRs which result in positive AW at the selling price of $56.7/metric ton were also evaluated as presented in Figure 5.2.

a)



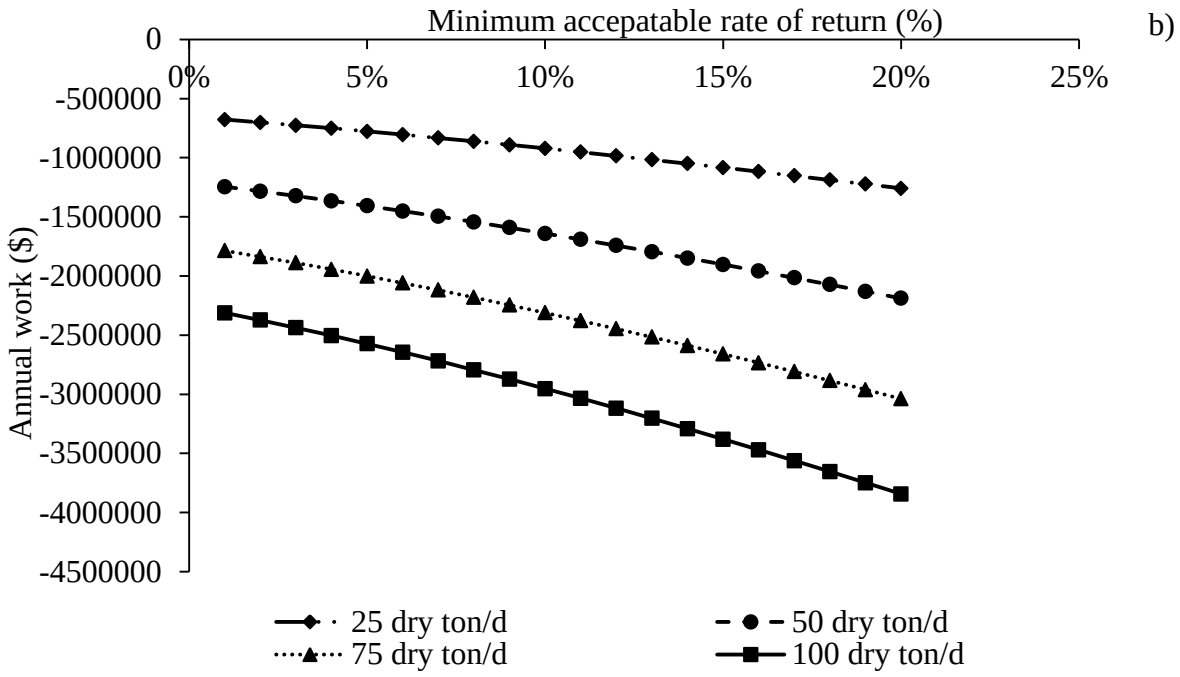


Figure 5.2. The break-even of minimum acceptable rate of return (MARR) of a) Torrefaction process and b) Hydrothermal carbonization process

(Note: assume selling price is $56.70/metric ton)

The minimum MARRs resulting in positive AW of the torrefaction plant with the capacities of 25, 50, 75, and 100 dry ton of Napier grass/day were 4, 8, 10, and 11%, respectively as presented in Figure 5.2. (a). The results from sensitivity analysis showed that the torrefaction plant with the plant capacity of over 75 dry ton of Napier grass/day could result in positive AW. However, for HTC process, even though the MARR was decreased to less than 1%, the AWs were still negative. Thus, it is not possible to get positive AWs of HTC process within the assumed plant capacities.

CHAPTER 6

ENGINEERING IMPLICATIONS

A stand-alone decentralized anaerobic digestion biorefinery using energy crops as a sole substrate was developed in this study. The AD process was integrated with thermochemical conversion process, namely torrefaction and hydrothermal carbonization in which the former process acted as a pretreatment for the lignocellulosic biomass, whereas the later converted the pretreated biomass (digestate) into energy-rich solid fuel/clean substrate for downstream processing. The integrated biorefinery approach is illustrated in Figure 6.1.

The selection of end-products and integration technologies (that is thermochemical conversion processes) is one of the key factors for enhancing the economic viability of a process. The selection of end products is governed by several factors such as geographical location, global energy situation, market demand, and government policy among other. The AD of energy crop using horizontal bioreactor to mitigate the scum formation was the center piece of the biorefinery concept developed in this research. A high yielding energy crop as a mono-substrate eliminates the expensive sterilization process especially for utilization of digestate for high-value products and thus enhances the economic feasibility of the system (Paavola and Rintala, 2008). Moreover, it also overcomes the issue of nonavailability of a co-substrate (i.e., animal manure, food waste etc.) in close vicinity, which could affect the quality of the digestate for downstream processing. To maintain the stability of the system, C/N ratio could be alternately adjusted to 20 to 30 by supplementing nitrogen (such as urea fertilizer) without affecting

the system performance or ash content. AD process preferentially degrades the hemicellulose component of biomass, which is subsequently converted into methane via AD process as discussed in section 3.2.3. Thus, the digestate obtained is enriched in cellulose and lignin contents. The produced biogas could be converted onsite into electricity and heat using by CHP unit. Part of the produced electricity could be used within the facility for AD plant and for various biomass processing units operations (Phanphanich and Mani, 2011). The cellulose- and lignin-rich digestate following dewatering can serve as an ideal feedstock to produce high-value products representing a true anaerobic biorefinery concept (Sawatdeenarunat et al., 2016). Traditionally, digestate has been used as a biofertilizer for agricultural applications (Monlau et al., 2015b). Our research identified applications of dewatered digestate via thermochemical processes (torrefaction and HTC) to produce energy-rich solid fuels. The solid fuels, generated from the digestate via thermochemical processes, had the heating value comparable to that of the raw Napier grass with low ash content. The pelletization process is required to obtain high density energy to pellets to facilitate storage and transport (Reza et al., 2012). The waste heat from the CHP could be used for drying the digestate prior to pelletization to lower energy input (Monlau et al., 2015a). The produced high-energy and high-density fuels could be used within the country or exported (similar to coal) to other countries to be co-fired with coal for thermal-based power plants. Moreover, digestate could be further pretreated on-site to remove the lignin, and the cellulose-rich fiber could serve as a feedstock to produce plethora of high-value products (i.e., nano-cellulose, bioplastic, biopolymer, substrate for producing liquid biofuels etc.) (Surendra et al., 2015). The liquid effluents from both AD and HTC processes could be recycled back into the

digesters as dilution water. The proposed approach envisioned in Figure 7 opens up new paradigm for many developing countries in the tropical regions where high yielding energy can be grown at much lower cost. In one hand, it creates job opportunity and contributes to rural development with overall revenue generation for the developing/least developed countries; on the other hand, it supplies clean and renewable energy to meet the clean energy mandate and to curtail the air pollution associated with the use of fossil fuels in developed and/or emerging nations.

For the VFAs production, the liquid effluent after solid-liquid separation might contain various aqueous organic compounds (i.e. VFAs, aldehydes, and alcohols among others) (Khanal, 2008). The mixtures need to be purified in the downstream process to obtain the VFAs as a sole product. Many separation technics have been reported including precipitation, distillation, adsorption, solvent extraction, and membrane separation (Zacharof and Lovitt, 2013). The mixed VFAs after being separated from AD effluent could also be used as carbon source to replace conventional expensive chemical (i.e. methanol) in the biological denitrification process. In addition, the individual acids could be used in many fields. Acetic acid, propionic acid, and butyric acid could be utilized as a food additives and favoring agents in the food industries. Moreover, acetic acid and butyric acid could be used to produce biodegradable plastics and used in pharmaceutical industries, respectively (Zacharof and Lovitt, 2013) The market size and price of VFAs are illustrated in table 4.9.

It should be noticed that acetic acid and propionic acid are the dominant species of produced individual VFAs in this study as presented in section 4.2.

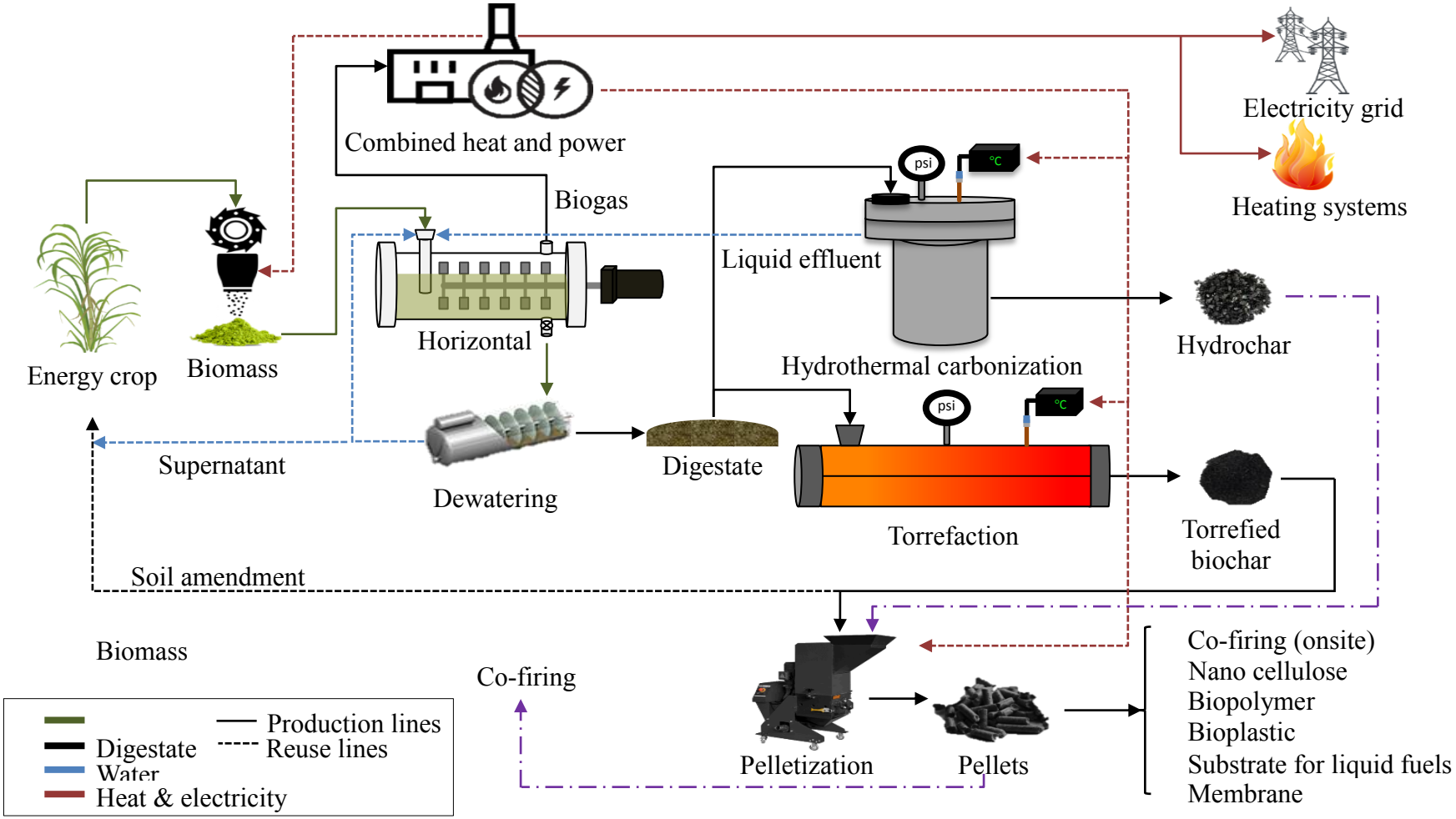


Figure 6.1. The schematic of decentralized anaerobic biorefinery of energy crop by integrating anaerobic digestion platform with thermochemical platform

Moreover, these acids have the highest global market size among others, thus, the VFAs production during AD of energy crop as a feedstock has a high potential to produce organic acids to supply to the market.

Table 6.1. The volatile fatty acids market size and price (adopted from Zacharof and Lovitt, 2013).

Volatile fatty acid	Global market size (metric ton/year)	Unit price (USD/metric ton)	Market value (Million USD/year)
Acetic acid	3500000	400-800	1400-2800
Propionic	180000	1500-1650	270-297
Butyric	30000	2000-2500	60-75

CHAPTER 7

CONCLUSIONS

VFAs production from Napier grass was examined using different inocula, micro-oxygenation dosages, and incubation times. Using anaerobically digested cattle manure as an inoculum showed significantly higher VFAs yield than the anaerobically digested waste activated sludge as an inoculum. There was significant interaction between micro-oxygenation and incubation time, which played important role to enhance VFAs yields from Napier grass. Thus, micro-oxygenation could be an environmental-friendly strategy to enhance VFAs yields from lignocellulosic feedstocks for high-value chemicals and bioenergy production. The bench-scale experiments also confirmed the enhancement of VFAs yields during AD of Napier grass using micro-aeration. The strict anaerobe methanogen was inhibited by injected air. Reversely, hydrolysis and acidogenesis were enhanced during micro-aeration resulted higher SCOD and VFAs compared with anaerobic condition.

Decentralized biorefinery for high-yielding lignocellulosic biomass, integrating AD with thermochemical conversion, was successfully developed in this study. Mono-digestion of high yielding energy crop was successfully carried out using horizontal bioreactors during long-term operation of over a year. The reactor performance was stable with high OLR of 6 kgVS/m^3-d. In addition, the digestate consisting of cellulosic-rich fiber and lignin was thermochemically converted into the energy-rich solid biofuels, which could be used for co-firing with coal in power plants in decentralized locations.

With respect to the techno-economic analysis, the torrefaction with the plant capacity more than 75 dry ton of Napier grass/day might be the appropriate digestate utilization technology following the developed biorefinery concept. However, the torrefaction and hydrothermal carbonization are emerging technologies and are still not fully understood (Batidzirai et al., 2013; Suwelack et al., 2016). Thus, the research and technology development are required to either cut down the capital and operating costs or increase the efficiency (i.e. mass and energy recoveries) to enhance the economic viability of the processes.

CHAPTER 8

FUTURE WORKS

In this study, an innovative decentralized anaerobic biorefinery approach was successfully developed to produce plethora of high-value products (i.e., VFAs and high-energy-density solid fuels) using Napier grass, a lignocellulosic biomass as the feedstock. Some of the further research needs are outlined below.

- In this study, only one air flow rate and frequency was applied in the bench-scale study. The objective was to confirm the positive effect of micro-aeration on the AD of Napier grass. However, there is a need to optimize air flow rate and/or frequency to maximize VFA yield during a long-term operation.
- There is a need of detailed and thorough study on thermochemical conversion processes, such as temperature and residence time to maximize the energy content of solid-fuels produced via torrefaction and hydrothermal carbonization of AD digestate.
- The digestate from acid bioreactor might not be an ideal substrate for biological conversion processes (e.g. enzymatic saccharification) due to the need for expensive chemical neutralization. However, such digestate could be thermochemically processed into high-energy-density solid fuels. There is need to conduct further detailed research on integration of VFAs production via anaerobic digestion with thermochemical conversion.
- The other high-solid substrates (i.e., agri-residues, organic fraction municipal solid waste, ruminant animal manures, and yard waste among others) could also

serve as potential feedstocks for a decentralized biorefinery process. Such feedstocks should also be examined with respect to the quality of digestate and its conversion into solid fuels via thermochemical conversion.

- There is a need to conduct a pilot-scale study to identify key operating parameters with respect to the proposed biorefinery approach and to conduct a thorough techno-economic analysis for various scenarios.
- The life cycle analysis (LCA) of the developed concept could be performed.

APPENDIX A

STATISTICAL ANALYSES OF EXPERIMENTAL DATA

Table A.1. ANOVA table of VFAs yields from batch study

Source	DF	SS	MS Num	F Ratio	Prob>F
Inoculum	1	59066.40	59066.40	3238.96	<.0001
O_2 dosage	2	4.10	2.05	0.11	0.8939
Incubation time	2	3610.89	1805.45	99.00	<.0001
Inoculum*O_2 dosage	2	115.87	57.94	3.18	0.0543
Inoculum*Incubation time	2	4408.91	2204.45	120.88	<.0001
O_2 dosage*Incubation time	4	802.65	200.66	11.00	<.0001
Inoculum*O_2 dosage*Incubation time	4	2116.46	529.12	29.01	<.0001
Replicate	2	72.39	36.20	1.98	0.1530
Errors	34				
Total	53				

Note: Replicate is random effect, DF: degree of freedom, SS: sum of squares, MS Num: numerator mean square, Prob>F: *p*-value

Table A.2. The post-hoc Tukey's test of VFAs yields from batch study

Level								Least Sq Mean
ADCM,15,3	A							107.25563
ADCM,30,3	A							106.00217
ADCM,0,3		B						81.20060
ADCM,0,1		B	C					79.31558
ADCM,0,5			C	D				66.25879
ADCM,15,1				D				61.96851
ADCM,15,5				D				59.26566
ADCM,30,5				D				57.94994
ADCM,30,1				D				53.64285
ADWAS,30,1					E			24.22094
ADWAS,15,1					E	F		14.75359
ADWAS,0,5						F	G	8.10570
ADWAS,0,3						F	G	8.08008
ADWAS,30,3						F	G	6.29291
ADWAS,15,3						F	G	5.92721
ADWAS,0,1						F	G	5.80246
ADWAS,15,5						F	G	3.29104
ADWAS,30,5							G	1.07189

Note: The levels are presented in inoculum, oxygen dosage (mL/gVS_{added}), incubation time (day) and the levels not connected by the same letter are significantly different.

Table A.3. ANOVA table of methane yield from batch study

Source	DF	SS	MS Num	F Ratio	Prob>F
Inoculum	1	10403.80	10403.80	441.34	<.0001
O2 dosage	2	373.06	186.53	7.91	0.0015
Incubation time	2	56052.80	28026.40	1188.93	<.0001
Inoculum*O2 dosage	2	24.02	12.01	0.51	0.6053
Inoculum*Incubation time	2	3617.89	1808.94	76.74	<.0001
O2 dosage*Incubation time	4	139.81	34.95	1.48	0.2291
Inoculum*O2dosage *Incubation time	4	106.32	26.58	1.13	0.3599
Replicate	2	71.78	35.89	1.52	0.2327
Errors	34				
Total	53				

Note: Replicate is random effect, DF: degree of freedom, SS: sum of squares, MS Num: numerator mean square, Prob>F: *p*-value

Table A.4. The post-hoc Tukey's test of methane yields from batch study

Level						Least Sq Mean
ADWAS,15,5	A					101.47097
ADWAS,0,5	A					100.11628
ADWAS,30,5	A					97.23264
ADCM,0,5		B				73.24017
ADWAS,0,3		B				71.67947
ADCM,15,5		B				67.22913
ADWAS,15,3		B				64.56461
ADWAS,30,3		B				63.46664
ADCM,30,5		B				59.32180
ADCM,0,3			C			27.05451
ADCM,30,3			C	D		21.70973
ADCM,15,3			C	D	E	16.95581
ADWAS,0,1				D	E	F	9.61153
ADWAS,15,1				D	E	F	7.07501
ADWAS,30,1					E	F	4.28597
ADCM,0,1					E	F	2.69157
ADCM,30,1						F	0.75271
ADCM,15,1						F	0.70257

Note: The levels are presented in inoculum, oxygen dosage (mL/gVS_{added}), incubation time (day) and the levels not connected by the same letter are significantly different.

Table A.5. ANOVA table of fiber composition from batch study using ADCM as inoculum compared with raw Napier grass

a) Hemicellulose

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	128.83730	25.7675	5.7917
Error	6	26.69411	4.4490	Prob > F
C. Total	11	155.53140		0.0270*

b) Cellulose

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	95.07509	19.0150	6.2260
Error	6	18.32467	3.0541	Prob > F
C. Total	11	113.39975		0.0228*

Note: DF: degree of freedom, Prob>F: *p*-value

Table A.6. The post-hoc Tukey's test of fiber composition from batch study using ADCM as inoculum compared with raw Napier grass

a) Hemicellulose

Sample			Least Sq Mean
Raw grass	A		25.178562
ADCM,15,3		B	19.019104
ADCM,0,1		B	17.713222
ADCM,30,1		B	16.827838

b) Cellulose

Sample			Least Sq Mean
Raw grass	A		39.372043
ADCM,30,1	A		38.616823
ADCM,0,1	A	B	36.341471
ADCM,15,3		B	32.335317

Note: The levels are presented in inoculum, oxygen dosage (mL/gVS_{added}), incubation time (day) and the levels not connected by the same letter are significantly different.

Table A.7. ANOVA table of fiber composition from batch study using ADWAS as inoculum compared with raw Napier grass

a) Hemicellulose

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	70.73847	14.1477	2.3744
Error	6	35.75037	5.9584	Prob > F
C. Total	11	106.48884		0.1612

b) Cellulose

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	104.94652	20.9893	1.8443
Error	6	68.28446	11.3807	Prob > F
C. Total	11	173.23098		0.2386

Note: DF: degree of freedom, Prob>F: *p*-value

Table A.8. The post-hoc Tukey's test of fiber composition from batch study using ADWAS as inoculum compared with raw Napier grass

a) Hemicellulose

Sample		Least Sq Mean
Raw grass	A	39.372043
ADWAS,30,5	A	35.952789
ADWAS,30,1	A	35.732766
ADWAS,30,3	A	32.486583

b) Cellulose

Sample		Least Sq Mean
Raw grass	A	39.372043
ADWAS,30,3	A	35.952789
ADWAS,30,1	A	35.732766
ADWAS,30,5	A	32.486583

Note: The levels are presented in inoculum, oxygen dosage (mL/gVS_{added}), incubation time (day) and the levels not connected by the same letter are significantly different.

Table A.9. ANOVA table of VFAs and SCOD productions and VFAs/SCOD ratio from aerated horizontal bioreactor

a) VFAs production

Source	DF	DFDen	F Ratio	Prob > F
Condition	1	17	93.0350	<.0001*

b) SCOD production

Source	DF	DFDen	F Ratio	Prob > F
Condition	1	17	12.9358	0.0022*

c) VFAs/SCOD ratio

Source	DF	DFDen	F Ratio	Prob > F
Condition	1	17	10.3098	0.0051*

Note: DF: degree of freedom, Den: denominator, Prob>F: *p*-value

Table A.10. The post-hoc Tukey's test of VFAs and SCOD productions and VFAs/SCOD ratio from aerated horizontal bioreactor

a) VFAs production

Level			Least Sq Mean
Micro-aeration	A		3524.8568
Anaerobic		B	2831.9083

b) SCOD production

Level			Least Sq Mean
Micro-aeration	A		8839.6333
Anaerobic		B	8017.3667

c) VFAs/SCOD ratio

Level			Least Sq Mean
Micro-aeration	A		0.39987840
Anaerobic		B	0.35947375

Note: The levels not connected by the same letter are significantly different.

Table A.11. ANOVA table of fiber compositions of the digestate from aerated horizontal bioreactor compared with raw Napier grass

a) Hemicellulose

Source	DF	DFDen	F Ratio	Prob > F
Condition	2	2	152.1716	0.0065*

b) Cellulose

Source	DF	DFDen	F Ratio	Prob > F
Condition	2	2	15.3544	0.0611

Note: DF: degree of freedom, Den: denominator, Prob>F: *p*-value

Table A.12. The post-hoc Tukey's test of fiber compositions of the digestate from the aerated horizontal bioreactor compared with raw Napier grass

a) Hemicellulose

Level				Least Sq Mean
Raw Napier grass	A			29.585000
Micro-aeration		B		27.179425
Anaerobic			C	25.913281

a) Cellulose

Level		Least Sq Mean
Anaerobic	A	46.989067
Micro-aeration	A	46.605196
Raw Napier grass	A	39.775000

Note: The levels not connected by the same letter are significantly different.

Table A.13. ANOVA table of methane yield from horizontal bioreactor at different OLRs

Source	DF	SS	F Ratio	Prob > F
Rep	1	101.26391	0.7425	0.3953
Day	6	488.87267	0.5975	0.7301
OLR	2	554.86094	2.0343	0.1473

Note: Replicate is random effect, DF: degree of freedom, SS: sum of squares, Prob>F: *p*-value

Table A.14. The post-hoc Tukey's test of methane yield from horizontal bioreactor at different OLRs

Organic loading rate (kg/m^3-d)		Least Sq Mean
6	A	112.48268
5	A	106.81951
4	A	103.70167

Note: The levels not connected by the same letter are significantly different.

Table A.15. ANOVA table of fiber compositions of the digestate from the horizontal bioreactor at different OLRs compared with raw Napier grass

a) Hemicellulose

Source	DF	DFDen	F Ratio	Prob > F
OLR	3	3	4.8895	0.1125

b) Cellulose

Source	Nparm	DF	DFDen	F Ratio	Prob > F
OLR	3	3	3	3.4965	0.1656

Note: DF: degree of freedom, Den: denominator, Prob>F: *p*-value

Table A.16. The post-hoc Tukey's test of fiber compositions of the digestate from the horizontal bioreactor at different OLRs compared with raw Napier grass

a) Hemicellulose

Organic loading rate (kg/m^3-d)		Least Sq Mean
Raw Napier grass	A	24.413207
6	A	22.696372
5	A	21.283814
4	A	18.949018

b) Cellulose

Organic loading rate (kg/m^3-d)		Least Sq Mean
Raw Napier grass	A	40.388435
6	A	38.547220
5	A	38.224253
4	A	37.793359

Note: The levels not connected by the same letter are significantly different.

APPENDIX B

PICTURES OF EXPERIMENTAL SETUP

Figure B.1. Biomass processing

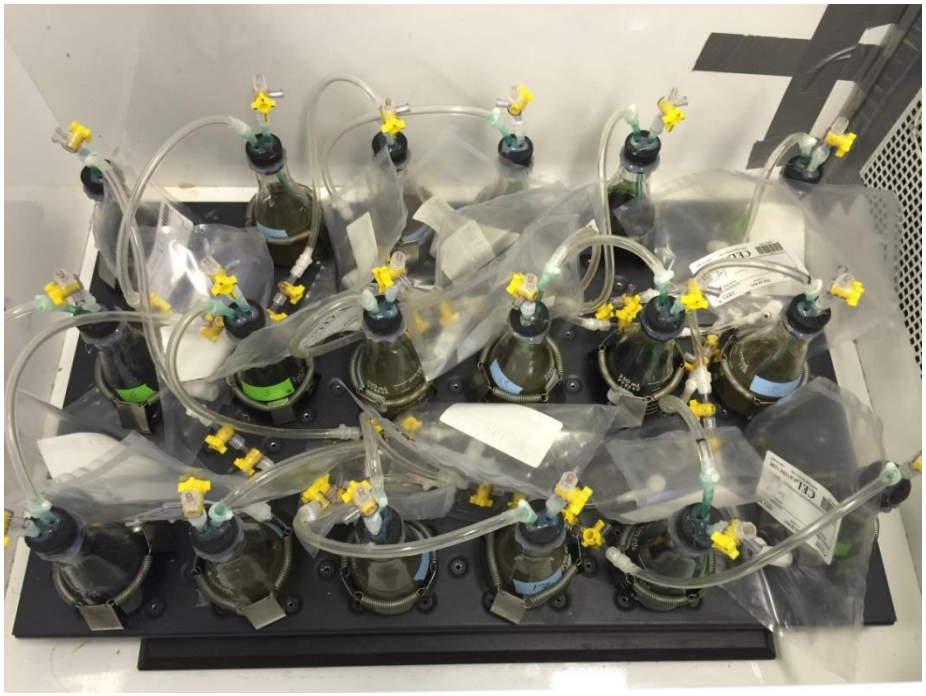


Figure B.2. The series of batch study using 250 mL-Erlenmeyer flasks

Figure B.3. The series of batch study using 2 L-bottles

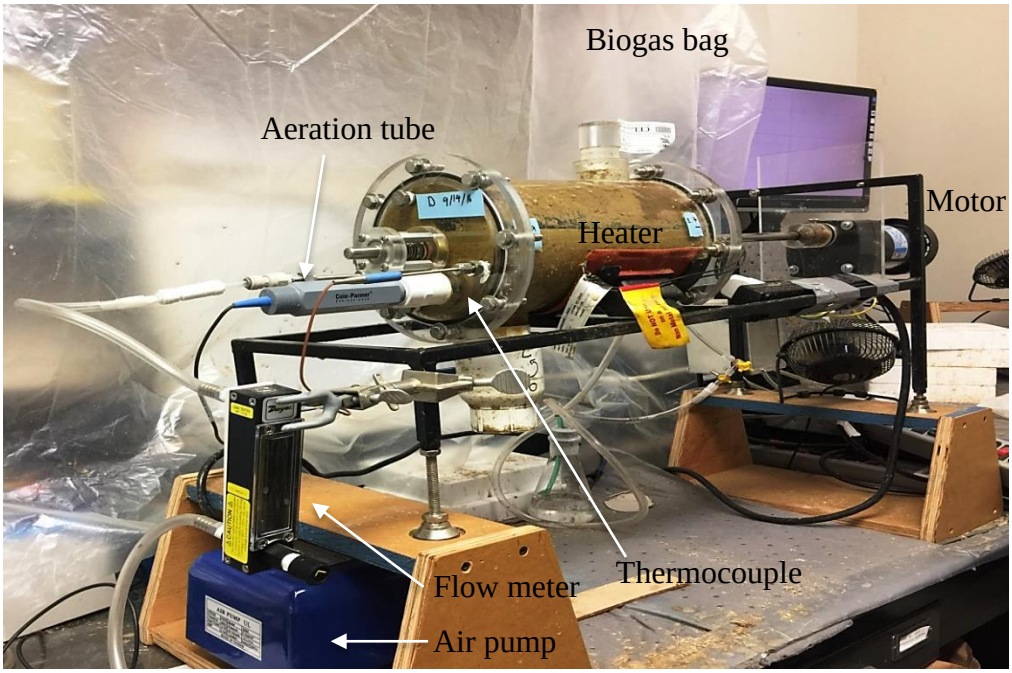


Figure B.4. Aerated horizontal bioreactor for VFAs production

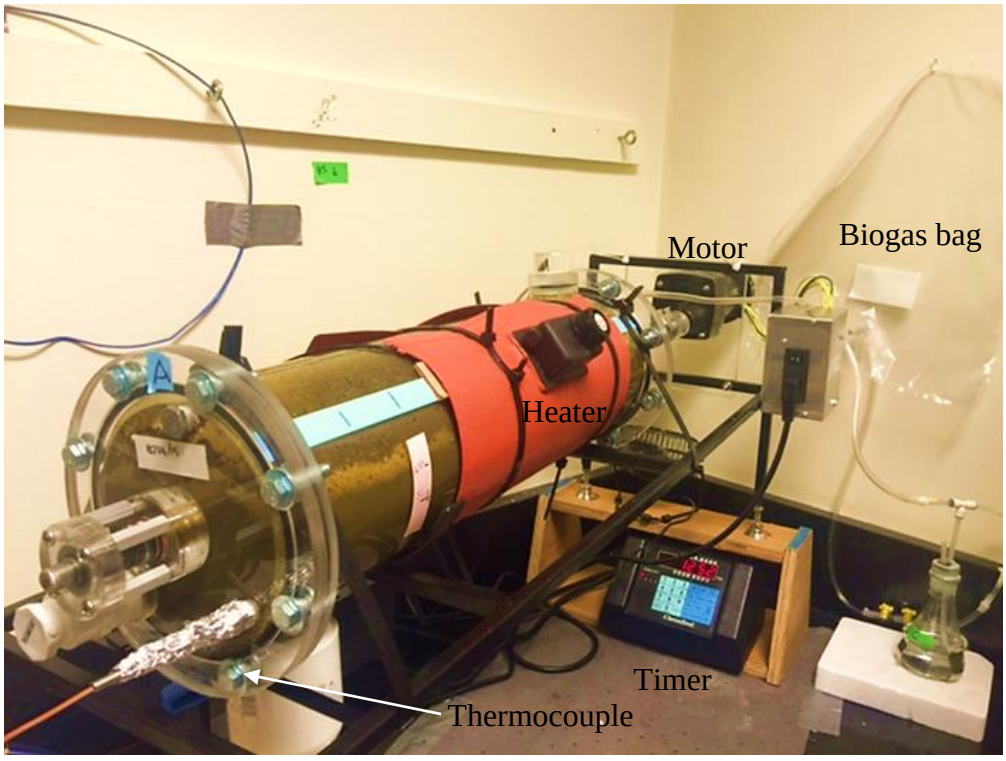


Figure B.5. Horizontal bioreactor for methane production

Figure B.6. Torrefaction reactors

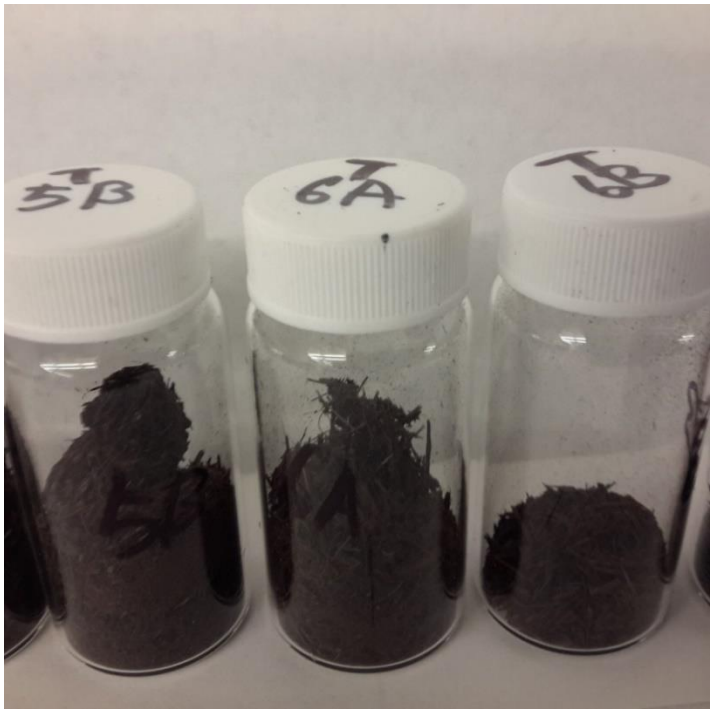


Figure B.7. Torrefied biochars

Figure B.8. Hydrothermal carbonization reactor

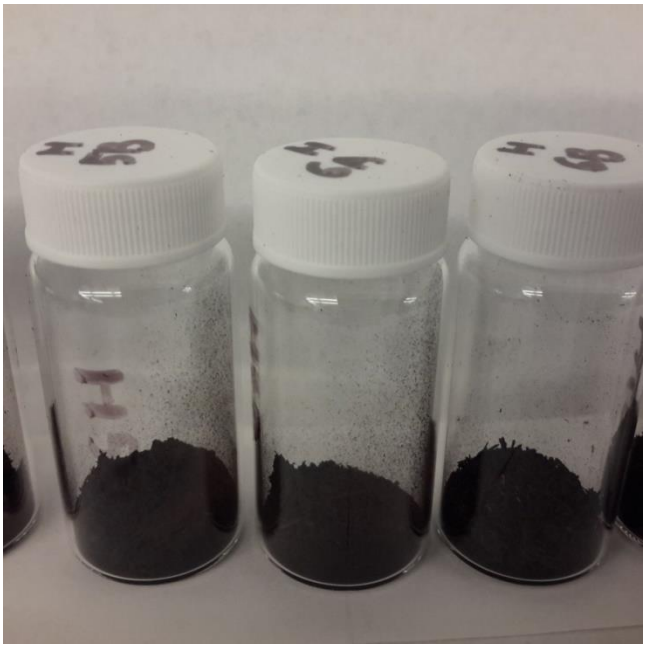


Figure B.9. Hydrothermal carbonized hydrochars

APPENDIX C

LIST OF PUBLICATIONS

Peer-Reviewed Articles

Submitted:

Sawatdeenarunat, C., Nam, H., Adhikari, S., Sung, S., Khanal, S.K., 2017. Decentralized biorefinery for lignocellulosic biomass: Integrating anaerobic digestion with thermochemical conversion. *Environ. Sci. Technol*.

In Press:

Sawatdeenarunat, C., Sung, S., Khanal, S.K., 2017. Enhanced volatile fatty acids production during anaerobic digestion of lignocellulosic biomass via micro-oxygenation. *Bioresource Technology*. (In Press).

Published:

Sawatdeenarunat, C., Surendra, K.C., Takara, D., Oechsner, H., Khanal, S.K., 2014. Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. *Bioresource Technology*. 178, 178–186.

Surendra, K.C., **Sawatdeenarunat, C.,** Shrestha, S., Sung, S., Khanal, S.K., 2015. Anaerobic Digestion-based Biorefinery for Bioenergy and Bio-based Products. *Industrial Biotechnology*. 11(2), 103-112.

Sawatdeenarunat, C., Nguyen, D., Surendra, K.C., Shrestha, S., Rajendran, K., Oechsner, H., Xie, L., Khanal, S.K., 2016. Anaerobic biorefinery: current status, challenges, and opportunities. *Bioresource Technology* 215, 304-313.

Oral & Poster Presentations

In Preparation:

Sawatdeenarunat, C., and Khanal, S.K. Long-term anaerobic mono-digestion of lignocellulosic biomass using horizontal bioreactors with focus on decentralized biorefinery. 15th IWA World Conference on Anaerobic Digestion (AD-15), Oct 17-20, 2017, Beijing, China.

Sawatdeenarunat, C., and Khanal, S.K. Anaerobic biorefinery of lignocellulosic feedstock to produce bioenergy and biobased products. 12th Asian Biohydrogen & Biogas Symposium (The 12th ABBS), Khon Kaen, Thailand, Jul 25-29, 2017.

Presented:

Sawatdeenarunat, C., and Khanal, S.K. Anaerobic digestion of lignocellulosic biomass using horizontal bioreactor: Evaluation of long-term digester performance. International conference on Bioprocessing India 2016, Center of Innovative and Applied Bioprocessing, Dec 15-17, 2016, Mohali, India.

Sawatdeenarunat. C, and Khanal, S.K. Enhanced volatile fatty acids production with micro-oxygenation during anaerobic digestion of lignocellulosic biomass. Oral

presentation. 1st International Conference on Bioenergy, Bioproducts & Environmental Sustainability, Sitges, Spain, 23-26 Oct, 2016.

Sawatdeenarunat, C., and Khanal, S. K. Enhanced volatile fatty acids production with micro-oxygenation during anaerobic digestion of lignocellulosic biomass. Poster Presentation. 28th Annual College of Tropical Agriculture and Human Resources (CTAHR) and College of Engineering (COE) Student Research Symposium, University of Hawai'i at Mānoa, April 8-9, 2016.

Tabatabaie, S.M.H., **Sawatdeenarunat, C.,** Khanal, S.K. and Murthy, G.S. 2016. Techno-economic and life cycle assessment of anaerobic digestion of Napier grass in Hawaii. ASABE Abstract No. 162461826. ASABE, St. Joseph, MI

Sawatdeenarunat, C., and Khanal, S. K. Enhanced volatile fatty acids production with micro-oxygenation during anaerobic digestion of lignocellulosic biomass. Poster Presentation. 27th Annual College of Tropical Agriculture and Human Resources (CTAHR) and College of Engineering (COE) Student Research Symposium, University of Hawai'i at Mānoa, April 10-11, 2015.

REFERENCES

A Joint study sponsored by U. S. Department of Energy (USDOE) and U.S. Department of agriculture, 2005. Biomass as feedstock for a bioenergy and bioproducts industry: The technical feasibility of a Billion-ton annual supply. Oak Ridge, Tennessee.

Agbor, V.B., Cicek, N., Sparling, R., Berlin, A., Levin, D.B., 2011. Biomass pretreatment: fundamentals toward application. Biotechnol. Adv. 29, 675–85. doi:10.1016/j.biotechadv.2011.05.005

Agler, M.T., Wrenn, B. a, Zinder, S.H., Angenent, L.T., 2011. Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. Trends Biotechnol. 29, 70–8. doi:10.1016/j.tibtech.2010.11.006

Alburquerque, J.A., de la Fuente, C., Ferrer-Costa, A., Carrasco, L., Cegarra, J., Abad, M., Bernal, M.P., 2012. Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. Biomass and Bioenergy 40, 181–189. doi:10.1016/j.biombioe.2012.02.018

Alkaya, E., Demirer, G.N., 2011. Anaerobic acidification of sugar-beet processing wastes: Effect of operational parameters. Biomass and Bioenergy 35, 32–39. doi:10.1016/j.biombioe.2010.08.002

Alvira, P., Ballesteros, M., Negro, M.J., 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis : A review. Bioresour. Technol. 101, 4851–4861. doi:10.1016/j.biortech.2009.11.093

Amon, T., Amon, B., Kryvoruchko, V., Machmüller, A., Hopfner-Sixt, K., Bodiroza, V., Hrbek, R., Friedel, J., Pötsch, E., Wagentristl, H., Schreiner, M., Zollitsch, W., 2007a. Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. Bioresour. Technol. 98, 3204–12. doi:10.1016/j.biortech.2006.07.007

Amon, T., Amon, B., Kryvoruchko, V., Zollitsch, W., Mayer, K., Gruber, L., 2007b. Biogas production from maize and dairy cattle manure—Influence of biomass composition on the methane yield. Agric. Ecosyst. Environ. 118, 173–182. doi:10.1016/j.agee.2006.05.007

Anderson, N., Jones, J.G., Page-Dumroese, D., McCollum, D., Baker, S., Loeffler, D., Chung, W., 2013. A comparison of producer gas, biochar, and activated carbon from two distributed scale thermochemical conversion systems used to process forest biomass. Energies 6, 164–183. doi:10.3390/en6010164

AOAC (Association of American Feed Control Officials), 1996. AOAC official method 968.08 - minerals in animal feed and pet food - atomic absorption spectrophotometric method, in: AOAC Official Methods of Analysis. AOAC International, Inc., Gaithersburg, Maryland.

Batidzirai, B., Mignot, A.P.R., Schakel, W.B., Junginger, H.M., Faaij, A.P.C., 2013. Biomass torrefaction technology: Techno-economic status and future prospects. Energy 62, 196–214. doi:10.1016/j.energy.2013.09.035

Bayer, W., 1990. Napier grass: a promising fodder for small holder livestock production

in the tropics. Plant Res. Dev. 31, 103–111.

Bengtsson, S., Hallquist, J., Werker, A., Welander, T., 2008. Acidogenic fermentation of industrial wastewaters: Effects of chemostat retention time and pH on volatile fatty acids production. Biochem. Eng. J. 40, 492–499. doi:10.1016/j.bej.2008.02.004

Bentsen, N.S., Felby, C., Thorsen, B.J., 2014. Agricultural residue production and potentials for energy and materials services. Prog. Energy Combust. Sci. 40, 59–73. doi:10.1016/j.pecs.2013.09.003

Botheju, D., Bakke, R., 2011. Oxygen Effects in Anaerobic Digestion – A Review. Open Waste Manag. J. 4, 1–19. doi:10.4173/mic.2010.2.2

Botheju, D., Samarakoon, G., Chen, C., Bakke, R., 2010. An Experimental Study on the Effects of Oxygen in Bio-gasification – Part 2, in: International Conference on Renewable Energies and Power Quality (ICREPQ'10). Granada, Spain.

Bramono, S.E., Lam, Y.S., Ong, S.L., He, J., 2011. A mesophilic Clostridium species that produces butanol from monosaccharides and hydrogen from polysaccharides. Bioresour. Technol. 102, 9558–63. doi:10.1016/j.biortech.2011.07.077

Bridgeman, T.G., Jones, J.M., Shield, I., Williams, P.T., 2008. Torrefaction of reed canary grass, wheat straw and willow to enhance solid fuel qualities and combustion properties. Fuel 87, 844–856. doi:10.1016/j.fuel.2007.05.041

Brown, R.C., 2003. Biorenewable Resources: Engineering New Products from Agriculture, 1st ed. Iowa State Press, Ames.

Calhoun, D.S., Prine, G.M., 1985. Response of elephantgrass to harvest interval and method of fertilization in the cooler subtropics, in: Soil & Crop Science Society of Florida. pp. 112–115.

Chae, K.J., Choi, M.J., Kim, K.Y., Ajayi, F.F., Chang, I.S., Kim, I.S., 2010. Selective inhibition of methanogens for the improvement of biohydrogen production in microbial electrolysis cells. Int. J. Hydrogen Energy 35, 13379–13386. doi:10.1016/j.ijhydene.2009.11.114

Charles, W., Walker, L., Cord-Ruwisch, R., 2009. Effect of pre-aeration and inoculum on the start-up of batch thermophilic anaerobic digestion of municipal solid waste. Bioresour. Technol. 100, 2329–35. doi:10.1016/j.biortech.2008.11.051

Chen, H., Meng, H., Nie, Z., Zhang, M., 2013. Polyhydroxyalkanoate production from fermented volatile fatty acids: effect of pH and feeding regimes. Bioresour. Technol. 128, 533–8. doi:10.1016/j.biortech.2012.10.121

Chen, Y., Jiang, S., Yuan, H., Zhou, Q., Gu, G., 2007. Hydrolysis and acidification of waste activated sludge at different pHs. Water Res. 41, 683–9. doi:10.1016/j.watres.2006.07.030

Cherubini, F., 2010. The biorefinery concept: Using biomass instead of oil for producing energy and chemicals. Energy Convers. Manag. 51, 1412–1421. doi:10.1016/j.enconman.2010.01.015

Cherubini, F., Strømman, A., 2011. Chemicals from lignocellulosic biomass: opportunities, perspectives, and potential of biorefinery systems. Biofuels, Bioprod.

Biorefining 3918–3924.

Demirbaş, A., 2005. Estimating of Structural Composition of Wood and Non-Wood Biomass Samples. Energy Sources 27, 761–767. doi:10.1080/00908310490450971

Demirel, B., Scherer, P., 2011. Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane. Biomass and Bioenergy 35, 992–998. doi:10.1016/j.biombioe.2010.12.022

Du, C., Lin, S.K.C., Koutinas, A., Wang, R., Webb, C., 2007. Succinic acid production from wheat using a biorefining strategy. Appl. Microbiol. Biotechnol. 76, 1263–70. doi:10.1007/s00253-007-1113-7

EBA, 2013. Biogas Production in Europe: Biogas Report 2013.

Edgar, K.J., Buchanan, C.M., Debenham, J.S., Rundquist, P. a, Seiler, B.D., Shelton, M.C., Tindall, D., 2001. Advances in cellulose ester performance and application. Prog. Polym. Sci. 26, 1605–1688. doi:10.1016/S0079-6700(01)00027-2

Elefsiniotis, P., Wareham, D.G., 2007. Utilization patterns of volatile fatty acids in the denitrification reaction. Enzyme Microb. Technol. 41, 92–97. doi:10.1016/j.enzmictec.2006.12.006

Fang, H., Yu, H.Q., 2000. Effect of HRT on mesophilic acidogenesis of dairy wastewater. J. Environ. Eng. 126, 1145–1148.

Fei, Q., Chang, H.N., Shang, L., Choi, J., Kim, N., Kang, J., 2011. The effect of volatile fatty acids as a sole carbon source on lipid accumulation by Cryptococcus albidus

for biodiesel production. Bioresour. Technol. 102, 2695–701. doi:10.1016/j.biortech.2010.10.141

FitzPatrick, M., Champagne, P., Cunningham, M.F., Whitney, R.A., 2010. A biorefinery processing perspective: treatment of lignocellulosic materials for the production of value-added products. Bioresour. Technol. 101, 8915–22. doi:10.1016/j.biortech.2010.06.125

Fontanille, P., Kumar, V., Christophe, G., Nouaille, R., Larroche, C., 2012. Bioconversion of volatile fatty acids into lipids by the oleaginous yeast Yarrowia lipolytica. Bioresour. Technol. 114, 443–9. doi:10.1016/j.biortech.2012.02.091

Frigon, J.-C., Guiot, S.R., 2010. Biomethane production from starch and lignocellulosic crops: a comparative review. Biofuels, Bioprod. Biorefining 4, 447–458. doi:10.1002/bbb.229

García-Gusano, D., Espegren, K., Lind, A., Kirkengen, M., 2016. The role of the discount rates in energy systems optimisation models. Renew. Sustain. Energy Rev. 59, 56–72. doi:10.1016/j.rser.2015.12.359

Gerardi, M.H., 2003. The microbiology of anaerobic digesters, 1st ed. John Wiley & Sons, Inc., Hoboken, New Jersey. doi:10.1002/0471468967

GÖMEÇ, Ç.Y., 2006. Behavior of the Anaerobic CSTR in the Presence of Scum During Primary Sludge Digestion and the Role of pH. J. Environ. Sci. Heal. Part A 41, 1117–1127. doi:10.1080/10934520600620519

Gu, Y., Chen, X., Liu, Z., Zhou, X., Zhang, Y., 2014. Effect of inoculum sources on the anaerobic digestion of rice straw. Bioresour. Technol. 158, 149–155. doi:10.1016/j.biortech.2014.02.011

Hawaii Clean Energy Initiative [WWW Document], n.d. URL http://www.hawaiicleanenergyinitiative.org/ (accessed 11.10.16).

Hendriks, A.T.W.M., Zeeman, G., 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour. Technol. 100, 10–18. doi:10.1016/j.biortech.2008.05.027

Hitzl, M., Corma, A., Pomares, F., Renz, M., 2014. The hydrothermal carbonization (HTC) plant as a decentral biorefinery for wet biomass. Catal. Today 257, 154–159. doi:10.1016/j.cattod.2014.09.024

Hu, Z.-H., Yu, H.-Q., 2005. Application of rumen microorganisms for enhanced anaerobic fermentation of corn stover. Process Biochem. 40, 2371–2377. doi:10.1016/j.procbio.2004.09.021

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

Jagadabhi, P.S., Kaparaju, P., Rintala, J., 2010. Effect of micro-aeration and leachate replacement on COD solubilization and VFA production during mono-digestion of grass-silage in one-stage leach-bed reactors. Bioresour. Technol. 101, 2818–2824. doi:10.1016/j.biortech.2009.10.083

Janejadkarn, A., Chavalparit, O., 2013. Biogas Production from Napier Grass (Pak Chong 1) (*Pennisetum purpureum* × *Pennisetum americanum*). Adv. Mater. Res. 856, 327–332. doi:10.4028/www.scientific.net/AMR.856.327

Johansen, J.-E., Bakke, R., 2006. Enhancing hydrolysis with microaeration. Water Sci. Technol. 53, 43. doi:10.2166/wst.2006.234

Kam, L.E., Leung, P.S., Ostrowski, A.C., 2003. Economics of offshore aquaculture of Pacific threadfin (Polydactylus sexfilis) in Hawaii. Aquaculture 223, 63–87. doi:10.1016/S0044-8486(03)00162-5

Kambo, H.S., Dutta, A., 2015. Comparative evaluation of torrefaction and hydrothermal carbonization of lignocellulosic biomass for the production of solid biofuel. Energy Convers. Manag. 105, 746–755. doi:10.1016/j.enconman.2015.08.031

Kaparaju, P., Serrano, M., Thomsen, A.B., Kongjan, P., Angelidaki, I., 2009. Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept. Bioresour. Technol. 100, 2562–8. doi:10.1016/j.biortech.2008.11.011

Karimi, K., 2015. Lignocellulose-Based Bioproducts. doi:10.1007/978-3-319-14033-9

Karthikeyan, O.P., Visvanathan, C., 2012. Bio-energy recovery from high-solid organic substrates by dry anaerobic bio-conversion processes: a review. Rev. Environ. Sci. Bio/Technology 12, 257–284. doi:10.1007/s11157-012-9304-9

Khanal, S.K., 2008. Anaerobic biotechnology for bioenergy production: principles and applications. John Wiley & Sons, Inc., Ames, Iowa.

Kiener, A., Leisinger, T., 1983. Oxygen Sensitivity of Methanogenic Bacteria. Syst. Appl. Microbiol. 4, 305–312. doi:10.1016/S0723-2020(83)80017-4

Kim, D., Lee, K., Park, K.Y., 2014. Hydrothermal carbonization of anaerobically digested sludge for solid fuel production and energy recovery. Fuel 130, 120–125. doi:10.1016/j.fuel.2014.04.030

Klemm, D., Heublein, B., Fink, H.P., Bohn, A., 2005. Cellulose: Fascinating Biopolymer and Sustainable Raw Material. Angew. Chemie - Int. Ed. 44, 3358–3393. doi:10.1002/anie.200460587

Kumar, P., Barrett, D.M., Delwiche, M.J., Stroeve, P., 2009. Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. Ind. Eng. Chem. Res. 48, 3713–3729. doi:10.1021/ie801542g

Kumar, R., Singh, S., Singh, O., 2008. Bioconversion of lignocellulosic biomass: biochemical and molecular perspectives. J. Ind. Microbiol. … 1125, 308–21. doi:10.1196/annals.1419.030

Lebuhn, M., Liu, F., Heuwinkel, H., Gronauer, a, 2008. Biogas production from mono-digestion of maize silage-long-term process stability and requirements. Water Sci. Technol. 58, 1645–51. doi:10.2166/wst.2008.495

Lee, J., 1997. Biological conversion of lignocellulosic biomass to ethanol. J. Biotechnol. 56, 1–24. doi:10.1016/S0168-1656(97)00073-4

Lehtomäki, A., Huttunen, S., Rintala, J.A., 2007. Laboratory investigations on co-

digestion of energy crops and crop residues with cow manure for methane production: Effect of crop to manure ratio. Resour. Conserv. Recycl. 51, 591–609. doi:10.1016/j.resconrec.2006.11.004

Li, Y., Khanal, S.K., 2017. Bioenergy principles and applications. Hoboken : John Wiley & Sons, Inc.

Li, Y., Zhang, R., He, Y., Zhang, C., Liu, X., Chen, C., Liu, G., 2014. Anaerobic co-digestion of chicken manure and corn stover in batch and continuously stirred tank reactor (CSTR). Bioresour. Technol. 156, 342–347. doi:10.1016/j.biortech.2014.01.054

Libra, J. a, Ro, K.S., Kammann, C., Funke, A., Berge, N.D., Neubauer, Y., Titirici, M.-M., Fühner, C., Bens, O., Kern, J., Emmerich, K.-H., 2011. Hydrothermal carbonization of biomass residuals: a comparative review of the chemistry, processes and applications of wet and dry pyrolysis. Biofuels 2, 71–106. doi:10.4155/bfs.10.81

Lim, J.W., Chiam, J.A., Wang, J.Y., 2014. Microbial community structure reveals how microaeration improves fermentation during anaerobic co-digestion of brown water and food waste. Bioresour. Technol. 171, 132–138. doi:10.1016/j.biortech.2014.08.050

Lim, J.W., Wang, J.Y., 2013. Enhanced hydrolysis and methane yield by applying microaeration pretreatment to the anaerobic co-digestion of brown water and food waste. Waste Manag. 33, 813–819. doi:10.1016/j.wasman.2012.11.013

Liu, Z., Quek, A., Kent Hoekman, S., Balasubramanian, R., 2013. Production of solid biochar fuel from waste biomass by hydrothermal carbonization. Fuel 103, 943–949. doi:10.1016/j.fuel.2012.07.069

Maclellan, J., Chen, R., Kraemer, R., Zhong, Y., Liu, Y., Liao, W., 2013. Anaerobic treatment of lignocellulosic material to co-produce methane and digested fiber for ethanol biorefining. Bioresour. Technol. 130, 418–23. doi:10.1016/j.biortech.2012.12.032

McKendry, P., 2002. Energy production from biomass (Part 1): Overview of biomass. Bioresour. Technol. 83, 37–46.

Mislevy, P., Blue, W.G., Roessler, C.E., 1989. Productivity of Clay Tailings from Phosphate Mining: III. Grain Crops. J. Environ. Qual. 18, 95–100. doi:10.2134/jeq1991.00472425002000040013x

Monlau, F., Barakat, A., Trably, E., Dumas, C., Steyer, J.-P., Carrère, H., 2013. Lignocellulosic Materials Into Biohydrogen and Biomethane: Impact of Structural Features and Pretreatment. Crit. Rev. Environ. Sci. Technol. 43, 260–322. doi:10.1080/10643389.2011.604258

Monlau, F., Sambusiti, C., Antoniou, N., Barakat, A., Zabaniotou, A., 2015a. A new concept for enhancing energy recovery from agricultural residues by coupling anaerobic digestion and pyrolysis process. Appl. Energy 148, 32–38. doi:10.1016/j.apenergy.2015.03.024

Monlau, F., Sambusiti, C., Barakat, A., Guo, X.M., Latrille, E., Trably, E., Steyer, J.-P.,

Carrere, H., 2012. Predictive models of biohydrogen and biomethane production based on the compositional and structural features of lignocellulosic materials. Environ. Sci. Technol. 46, 12217–25. doi:10.1021/es303132t

Monlau, F., Sambusiti, C., Ficara, E., Aboulkas, A., Barakat, A., Carrere, H., 2015b. New opportunities for agricultural digestate valorization: current situation and perspectives. Energy Environ. Sci. 8, 2600–2621. doi:10.1039/C5EE01633A

Mumme, J., Eckervogt, L., Pielert, J., Diakité, M., Rupp, F., Kern, J., 2011. Hydrothermal carbonization of anaerobically digested maize silage. Bioresour. Technol. 102, 9255–9260. doi:10.1016/j.biortech.2011.06.099

Mussoline, W., Esposito, G., Lens, P., Garuti, G., Giordano, A., 2012. Design considerations for a farm-scale biogas plant based on pilot-scale anaerobic digesters loaded with rice straw and piggery wastewater. Biomass and Bioenergy 46, 469–478. doi:10.1016/j.biombioe.2012.07.013

Na, C.-I., Sollenberger, L.E., Erickson, J.E., Woodard, K.R., Vendramini, J.M.B., Silveira, M.L., 2015. Management of Perennial Warm-Season Bioenergy Grasses. I. Biomass Harvested, Nutrient Removal, and Persistence Responses of Elephantgrass and Energycane to Harvest Frequency and Timing. BioEnergy Res. 8, 581–589. doi:10.1007/s12155-014-9541-6

Nam, H., Capareda, S., 2015. Experimental investigation of torrefaction of two agricultural wastes of different composition using RSM (response surface methodology). Energy 91, 507–516. doi:10.1016/j.energy.2015.08.064

Nguyen, P.H.L., Kuruparan, P., Visvanathan, C., 2007. Anaerobic digestion of municipal solid waste as a treatment prior to landfill. Bioresour. Technol. 98, 380–7. doi:10.1016/j.biortech.2005.12.018

Nizami, a. S., Orozco, a., Groom, E., Dieterich, B., Murphy, J.D., 2012. How much gas can we get from grass? Appl. Energy 92, 783–790. doi:10.1016/j.apenergy.2011.08.033

Nyambati, E.M., Muyekho, F.N., Lusweti, C.M., Onginjo, E., 2010. Production , characterization and nutritional quality of Napier grass [Pennisetum Purpureum (Schum)] cultivars in western Kenya. African J. Plant Sci. 4, 496–502.

Obeta Ugwuanyi, J., Harvey, L.M., McNeil, B., 2005. Effect of aeration rate and waste load on evolution of volatile fatty acids and waste stabilization during thermophilic aerobic digestion of a model high strength agricultural waste. Bioresour. Technol. 96, 721–730. doi:10.1016/j.biortech.2004.06.028

Octave, S., Thomas, D., 2009. Biorefinery: Toward an industrial metabolism. Biochimie 91, 659–64. doi:10.1016/j.biochi.2009.03.015

Orozco, a M., Nizami, a S., Murphy, J.D., Groom, E., 2013. Optimizing the thermophilic hydrolysis of grass silage in a two-phase anaerobic digestion system. Bioresour. Technol. 143, 117–25. doi:10.1016/j.biortech.2013.05.118

Osgood, R. V, Dudley, N.S., Jakeway, L. a, 1996. A Demonstration of Grass Biomass Production on Molokai 1986, 1–5.

Paavola, T., Rintala, J., 2008. Effects of storage on characteristics and hygienic quality of digestates from four co-digestion concepts of manure and biowaste. Bioresour. Technol. 99, 7041–7050. doi:10.1016/j.biortech.2008.01.005

Pala, M., Kantarli, I.C., Buyukisik, H.B., Yanik, J., 2014. Hydrothermal carbonization and torrefaction of grape pomace: A comparative evaluation. Bioresour. Technol. 161, 255–262. doi:10.1016/j.biortech.2014.03.052

Patzek, T.W., Croft, G.D., 2010. A global coal production forecast with multi-Hubbert cycle analysis. Energy 35, 3109–3122. doi:10.1016/j.energy.2010.02.009

Pérez, J., Muñoz-Dorado, J., De La Rubia, T., Martínez, J., 2002. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: An overview. Int. Microbiol. 5, 53–63. doi:10.1007/s10123-002-0062-3

Perlack, R.D., Wright, L.L., Turhollow, A.F., Graham, R.L., Stokes, B.J., Erbach, D.C., 2005. Biomass as feedstock for a bioenergy and bioproducts industry: The technical feasibility of a billion-ton annual supply, DOE/GO-102995-2135, U.S. Department of Energy. doi:10.1017/CBO9781107415324.004

Phanphanich, M., Mani, S., 2011. Impact of torrefaction on the grindability and fuel characteristics of forest biomass. Bioresour. Technol. 102, 1246–1253. doi:10.1016/j.biortech.2010.08.028

Poudel, J., Ohm, T.I., Oh, S.C., 2015. A study on torrefaction of food waste. Fuel 140, 275–281. doi:10.1016/j.fuel.2014.09.120

Prachand Shrestha, Mary Rasmussen, Samir K. Khanal, Anthony L. Pometto III, A.J. (Hans) V.L., 2008. Solid-Substrate Fermentation of Corn Fiber by Phanerochaete chrysosporium and Subsequent Fermentation of Hydrolysate into Ethanol. Agric. Food Chem. 56, 3918–3924.

Prine, G.M., Woodard, K.R., 1994. Herbaceous energy crops in humid lower South USA, in: First Biomass Conference of the Americas: Energy, Environment, Agriculture and Industry. pp. 278–283.

Prochnow, a, Heiermann, M., Plöchl, M., Linke, B., Idler, C., Amon, T., Hobbs, P.J., 2009. Bioenergy from permanent grassland--a review: 1. Biogas. Bioresour. Technol. 100, 4931–44. doi:10.1016/j.biortech.2009.05.070

Rabelo, S.C., Carrere, H., Maciel Filho, R., Costa, A., 2011. Production of bioethanol, methane and heat from sugarcane bagasse in a biorefinery concept. Bioresour. Technol. 102, 7887–95. doi:10.1016/j.biortech.2011.05.081

Ren, S., Lei, H., Wang, L., Bu, Q., Chen, S., Wu, J., 2013. Thermal behaviour and kinetic study for woody biomass torrefaction and torrefied biomass pyrolysis by TGA. Biosyst. Eng. 116, 420–426. doi:10.1016/j.biosystemseng.2013.10.003

Reza, M.T., Lynam, J.G., Vasquez, V.R., Coronella, C.J., 2012. Pelletization of Biochar from Hydrothermally Carbonized Wood. Environ. Prog. Sustain. Energy 31, 225–234.

Reza, M.T., Werner, M., Pohl, M., Mumme, J., 2014. Evaluation of integrated anaerobic digestion and hydrothermal carbonization for bioenergy production. J. Vis. Exp. 1–

9. doi:10.3791/51734

Samson, R., Mani, S., Boddey, R., Sokhansanj, S., Quesada, D., Urquiaga, S., Reis, V., Ho Lem, C., 2005. The Potential of C4 Perennial Grasses for Developing a Global BIOHEAT Industry. CRC. Crit. Rev. Plant Sci. 24, 461–495. doi:10.1080/07352680500316508

Sawatdeenarunat, C., Nguyen, D., Surendra, K.C., Shrestha, S., Rajendran, K., Oechsner, H., Xie, L., Khanal, S.K., 2016. Anaerobic biorefinery: Current status, challenges, and opportunities. Bioresour. Technol. 215, 304–313. doi:10.1016/j.biortech.2016.03.074

Sawatdeenarunat, C., Sung, S., Khanal, S.K., 2017. Enhanced volatile fatty acids production during anaerobic digestion of ligno- cellulosic biomass via micro-oxygenation. Bioresour. Technol. doi:10.1016/j.biortech.2017.02.029

Sawatdeenarunat, C., Surendra, K.C., Takara, D., Oechsner, H., Kumar Khanal, S., 2015. Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. Bioresour. Technol. 178, 178–186. doi:10.1016/j.biortech.2014.09.103

Schittenhelm, S., 2008. Chemical composition and methane yield of maize hybrids with contrasting maturity. Eur. J. Agron. 29, 72–79. doi:10.1016/j.eja.2008.04.001

Scoma, A., Bertin, L., Fava, F., 2013. Effect of hydraulic retention time on biohydrogen and volatile fatty acids production during acidogenic digestion of dephenolized olive mill wastewaters. Biomass and Bioenergy 48, 51–58. doi:10.1016/j.biombioe.2012.10.028

Sevilla, M., Maciá-Agulló, J.A., Fuertes, A.B., 2011. Hydrothermal carbonization of biomass as a route for the sequestration of CO 2: Chemical and structural properties of the carbonized products. Biomass and Bioenergy 35, 3152–3159. doi:10.1016/j.biombioe.2011.04.032

Sheng, C., Azevedo, J.L.T., 2005. Estimating the higher heating value of biomass fuels from basic analysis data. Biomass and Bioenergy 28, 499–507. doi:10.1016/j.biombioe.2004.11.008

Sims, R.E.H., Hastings, A., Schlamadinger, B., Taylor, G., Smith, P., 2006. Energy crops: current status and future prospects. Glob. Chang. Biol. 12, 2054–2076. doi:10.1111/j.1365-2486.2006.01163.x

Spoljaric, S., Genovese, A., Shanks, R. a., 2009. Polypropylene–microcrystalline cellulose composites with enhanced compatibility and properties. Compos. Part A Appl. Sci. Manuf. 40, 791–799. doi:10.1016/j.compositesa.2009.03.011

Surendra, K.C., Khanal, S.K., 2014. Effects of crop maturity and size reduction on digestibility and methane yield of dedicated energy crop. Bioresour. Technol. 178, 187–193. doi:10.1016/j.biortech.2014.09.055

Surendra, K.C., Sawatdeenarunat, C., Shrestha, S., Sung, S., Khanal, S.K., 2015. Anaerobic Digestion-Based Biorefinery for Bioenergy and Biobased Products. Ind. Biotechnol. 11, 103–112. doi:10.1089/ind.2015.0001

Suwelack, K., Dostert, N., Wüst, D., Kruse, A., 2016. Economics of hydrothermal carbonization of biogas digestate in a hybrid AD-HTC plant, in: EUBCE, 24th

European Conference. doi:10.5071/24thEUBCE2016-3DO.9.4

Takara, D., Khanal, S.K., 2011. Green processing of tropical banagrass into biofuel and biobased products: an innovative biorefinery approach. Bioresour. Technol. 102, 1587–92. doi:10.1016/j.biortech.2010.08.106

Teater, C., Yue, Z., MacLellan, J., Liu, Y., Liao, W., 2011. Assessing solid digestate from anaerobic digestion as feedstock for ethanol production. Bioresour. Technol. 102, 1856–62. doi:10.1016/j.biortech.2010.09.099

Thamsiriroj, T., Murphy, J.D., 2010. Difficulties Associated with Monodigestion of Grass as Exemplified by Commissioning a Pilot-Scale Digester. Energy & Fuels 24, 4459–4469. doi:10.1021/ef1003039

Trevisan, V., Monteggia, L.O., Delabary, H.D.S., 2014. A short-term test for the evaluation of hydrogen and volatile fatty acids production from industrial effluents. Int. J. Hydrogen Energy 39, 7730–7734. doi:10.1016/j.ijhydene.2014.03.139

Triolo, J.M., Sommer, S.G., Møller, H.B., Weisbjerg, M.R., Jiang, X.Y., 2011. A new algorithm to characterize biodegradability of biomass during anaerobic digestion: influence of lignin concentration on methane production potential. Bioresour. Technol. 102, 9395–402. doi:10.1016/j.biortech.2011.07.026

Tsavkelova, E.A., Netrusov, A.I., 2012. Biogas production from cellulose-containing substrates: A review. Appl. Biochem. Microbiol. 48, 421–433. doi:10.1134/S0003683812050134

U.S. Energy Information Administration (EIA), 2017. Hawaii Natural Gas Prices [WWW Document]. URL https://www.eia.gov/dnav/ng/ng_pri_sum_dcu_SHI_m.htm (accessed 3.10.17).

U.S. Energy Information Administration (EIA), 2016. Coal Data Browser - Aggregate coal mine production for all coal [WWW Document]. URL https://www.eia.gov/beta/coal/data/browser/ (accessed 3.10.17).

Usack, J.G., Spirito, C.M., Angenent, L.T., 2012. Continuously-stirred Anaerobic Digester to Convert Organic Wastes into Biogas: System Setup and Basic Operation. J. Vis. Exp. 1–9. doi:10.3791/3978

USEPA (United States Environmental Protection Agency), 1994. Method 200.7, Revision 4.4: Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry [WWW Document]. URL https://www.epa.gov/sites/production/files/2015-08/documents/method_200-7_rev_4-4_1994.pdf (accessed 4.4.17).

Wan, C., Li, Y., 2012. Fungal pretreatment of lignocellulosic biomass. Biotechnol. Adv. 30, 1447–57. doi:10.1016/j.biotechadv.2012.03.003

Wang, K., Yin, J., Shen, D., Li, N., 2014. Anaerobic digestion of food waste for volatile fatty acids (VFAs) production with different types of inoculum: Effect of pH. Bioresour. Technol. 161, 395–401. doi:10.1016/j.biortech.2014.03.088

Wang, Y., Zhang, Y., Wang, J., Meng, L., 2009. Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. Biomass and Bioenergy

33, 848–853. doi:10.1016/j.biombioe.2009.01.007

Weiland, P., 2003. Production and energetic use of biogas from energy crops and wastes in Germany. Appl. Biochem. Biotechnol. 109, 263–74.

Wilkie, A., Goto, M., Bordeaux, F., Smith, P., 1986. Enhancement of anaerobic methanogenesis from napiergrass by addition of micronutrients. Biomass 11, 135–146.

Woodard, K.R., Prine, G.M., 1993. Dry Matter Accumulation of Elephantgrass, Energycane, and Elephantmillet in a Subtropical Climate. Crop Sci. 33, 818. doi:10.2135/cropsci1993.0011183X003300040038x

Woodard, K.R., Prine, G.M., 1991. Forage yield and nutritive value of elephantgrass as affected by harvest frequency and genotype. Agron. J. 83, 541–546.

Xu, F., Linnebur, K., Wang, D., 2014. Torrefaction of Conservation Reserve Program biomass: A techno-economic evaluation. Ind. Crops Prod. 61, 382–387. doi:10.1016/j.indcrop.2014.07.030

Xu, S., Selvam, A., Wong, J.W.C., 2014. Optimization of micro-aeration intensity in acidogenic reactor of a two-phase anaerobic digester treating food waste. Waste Manag. 34, 363–9. doi:10.1016/j.wasman.2013.10.038

Yan, W., Acharjee, T.C., Coronella, C.J., Squez, V.R.V., 2009. Thermal Pretreatment of Lignocellulosic Biomass. Environ. Prog. Sustain. Energy 28, 435–440. doi:10.1002/ep

Yao, Y., Gao, B., Inyang, M., Zimmerman, A.R., Cao, X., Pullammanappallil, P., Yang, L., 2011. Biochar derived from anaerobically digested sugar beet tailings: Characterization and phosphate removal potential. Bioresour. Technol. 102, 6273–6278. doi:10.1016/j.biortech.2011.03.006

Yin, J., Wang, K., Yang, Y., Shen, D., Wang, M., Mo, H., 2014. Improving production of volatile fatty acids from food waste fermentation by hydrothermal pretreatment. Bioresour. Technol. 171, 323–9. doi:10.1016/j.biortech.2014.08.062

Yoo, C.G., Zhang, S., Pan, X., 2017. Effective conversion of biomass into bromomethylfurfural, furfural, and depolymerized lignin in lithium bromide molten salt hydrate of a biphasic system. RSC Adv. 7, 300–308. doi:10.1039/C6RA25025D

Yu, H., Fang, H., 2002. Acidogenesis of dairy wastewater at various pH levels. Water Sci. Technol. 45, 201–206.

Yuan, Q., Sparling, R., Oleszkiewicz, J. a, 2011. VFA generation from waste activated sludge: effect of temperature and mixing. Chemosphere 82, 603–7. doi:10.1016/j.chemosphere.2010.10.084

Yue, Z., Teater, C., Liu, Y., Maclellan, J., Liao, W., 2010. A sustainable pathway of cellulosic ethanol production integrating anaerobic digestion with biorefining. Biotechnol. Bioeng. 105, 1031–9. doi:10.1002/bit.22627

Yue, Z., Teater, C., MacLellan, J., Liu, Y., Liao, W., 2011. Development of a new bioethanol feedstock – Anaerobically digested fiber from confined dairy operations using different digestion configurations. Biomass and Bioenergy 35, 1946–1953.

doi:10.1016/j.biombioe.2011.01.035

Zacharof, M.P., Lovitt, R.W., 2013. Complex effluent streams as a potential source of volatile fatty acids. Waste and Biomass Valorization 4, 557–581. doi:10.1007/s12649-013-9202-6

Zeikus, J.G., Jain, M.K., Elankovan, P., 1999. Biotechnology of succinic acid production and markets for derived industrial products. Appl. Microbiol. Biotechnol. 51, 545–552. doi:10.1007/s002530051431

Zewdu, T., Baars, R.M.T., Yami, A., 2002. Effect of plant height at cutting , source and level of fertiliser on yield and nutritional quality of Napier grass (Pennisetum purpureum (L .) Schumach .). African J. range forage Sci. 19, 123–128.

Zhang, Y.-H.P.;, Ding, S.-Y.;, Mielenz, J.R.;, Cui, J.-B.;, Elander, R.T.;, Laser, M.;, Himmel, M.E.;, McMillan, J.R.;, Lynd, L.R., 2007. Fractionating recalcitrant lignocellulose at modest reaction conditions. Biotechnol. Bioeng. 97, 214–223. doi:10.1002/bit.21386

Zhu, M., Lü, F., Hao, L.-P., He, P.-J., Shao, L.-M., 2009. Regulating the hydrolysis of organic wastes by micro-aeration and effluent recirculation. Waste Manag. 29, 2042–50. doi:10.1016/j.wasman.2008.12.023