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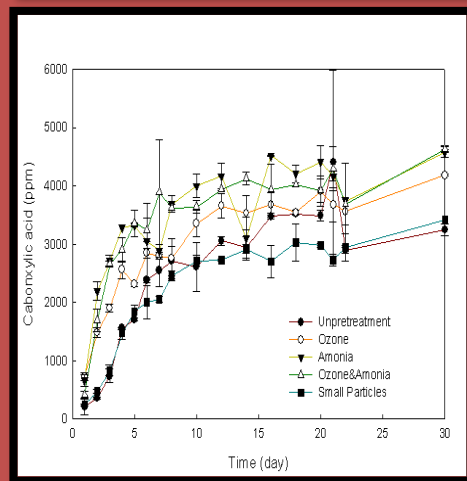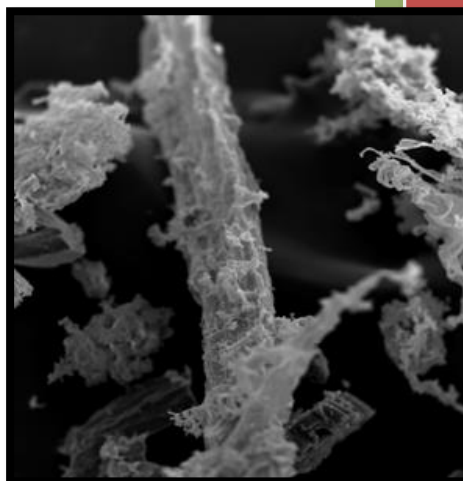*World Class. Face to Face.*

Technology Research and Extension Related to Anaerobic Digestion of Dairy Manure

2015–17 Biennium

Technology Research and Extension Related to Anaerobic Digestion of Dairy Manure

A Project Report
for the

**Washington State University
Agricultural Research Center**

and the

Washington State Department of Agriculture

Applied Bioenergy Research, also known as Appendix A research

In the 2007–09 biennium the Washington State Legislature funded a joint Washington State University (WSU) and Washington State Department of Agriculture (WSDA) program targeting applied bioenergy research. The funds were requested by WSU and WSDA to undertake near-term, applied research needed to successfully implement the Energy Freedom program and bioenergy initiatives enacted in 2006. Examples of projects to be funded were listed in *Appendix A,* which was attached to the funding request.

Since 2007, WSU's Agricultural Research Center and WSDA have collaborated on this research effort. The *Appendix A* funds have been directed to research projects coordinated by the WSU Department of Crops and Soils, as the Biofuels Cropping Systems project, and to research projects coordinated by the WSU Department of Biological Systems Engineering, in the area of energy conversion from agricultural wastes.

In the 2015-17 biennium, Washington State University researchers continued the anaerobic digestion-related research program with support from *Appendix A* funds. This report describes their projects and findings. In many cases, researchers have been able to successfully leverage these funds in support of additional funding. From 2015-2017, this funding was used to leverage funding from the Washington State Department of Ecology, as well as extramural funding competitively awarded by the following federal agencies:

- National Science Foundation (Elucidation and Evaluation of Strategies to Mitigate Secondary Reactions in Cellulose and Lignocellulose Pyrolysis for Enhanced Production of Anhydrosugars, and CAREER - An Integrated research and Educational Plan to Develop Selective Pyrolysis Reactors and Improve the Capacity of Students to Work in Multidisciplinary Environment),
- U.S. Department of Agriculture's National Institute of Food and Agriculture (U.S. dairy adoption of anaerobic digestion systems integrating multiple emerging clean technologies: Climate, environmental, & economic impacts),
- US EPA (Integrated Management of Animal Manure Wastes: Nutrient Recovery, Bio-Fertilizers, Enhanced Bio-Methane Production, and Management Tools, a subcontract to the Water Environment Research Foundation),
- U.S. Department of Agriculture's Natural Resources Conservation Service (Dairy Manure-Derived Fertilizers for Use in Raspberry and Blueberry Cropping Systems: Evaluation for Agronomic, Soil Health, and Food Safety Efficacy).

These complementary funding sources have allowed for much greater accomplishments and impact than would be possible through *Appendix A* funding alone.

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Technology Research and Extension Related to Anaerobic Digestion of Dairy Manure

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Table of Contents

List of Figures and Tables

Figures

vi

Tables

List of Abbreviations

AD	anaerobic digestion
AIL	acid-insoluble lignin
AIRTRAP	Air Induced Recovery Technology for Re-use of Ammonia and Phosphorus
AOP	advanced oxidation process
ASL	acid-soluble lignin
BET	Brunauer–Emmett–Teller
CNG	compressed natural gas
COD	chemical oxygen demand
CSANR	Center for Sustaining Agriculture and Natural Resources
CSTR	continuous stirred tank reactor
DAF	dissolved air flotation
DIC	dissolved inorganic carbon
DOC	dissolved organic carbon
DOH	Department of Health
EPA	Environmental Protection Agency
FTIR	Fourier transform infrared
GTF	ground treated fiber
GUF	ground untreated fiber
HDPE	high-density polyethylene
HRT	hydraulic retention time
HWE	hot water extraction
IC	inorganic carbon
MAD	mesophilic anaerobic digestion
MOE	modulus of elasticity
MOR	modulus of rupture
MPFLR	mixed plug-flow loop reactor
NMR	nuclear magnetic resonance
NR	nutrient recovery
NREL	National Renewable Energy Laboratory
OLR	organic loading rate
OSAA	ozone and aqueous ammonia soaking
PFR	plug flow reactor
PI	profitability index
TAD	thermophilic anaerobic digestion
TAN	total ammonia nitrogen
TC	total carbon
TDC	total dissolved carbon
TGA	thermogravimetric analysis
TN	total nitrogen
TOC	total organic carbon
TS	total solids

UASB	up-flow anaerobic sequencing batch
USDA	United States Department of Agriculture
UUF	unground untreated fiber
VFA	volatile fatty acid
VS	volatile solids
WPC	wood plastic composite
WSDA	Washington State Department of Agriculture
WSU	Washington State University
XPS	X-ray photoelectron spectroscopy

Acknowledgements

Executive Summary

On-farm manure management in the U.S. has traditionally focused on lagoon storage for wastes, followed by application of liquids and solids to forage crops, with amounts and timing controlled to match plant nutrient uptake. Anaerobic digesters provide several important benefits compared to this approach. During anaerobic digestion, naturally-occurring microorganisms working in an oxygen-free environment convert complex organic materials, such as manures or food scraps, into biogas. The process also reduces odors and pathogens, stabilizes waste streams through reduction of solids and organic content, and reduces greenhouse gas releases to the atmosphere. However, adoption of anaerobic digesters in the United States has been slow. As of May 2016, there were 209 operational anaerobic digesters in the U.S., serving an estimated 6% of the U.S. dairy herd. In Washington State, there were seven operational dairy digesters as of mid-2017. One strategy that has been suggested to enhance adoption of anaerobic digestion is to complement the digester with other technologies that allow the system to produce multiple value-added co-products. Marketing of such products could improve the economics of anaerobic digestion, overcoming one of the barriers affecting adoption of these technologies.

While digesters can provide several important benefits, they do not help dairies reduce nutrients. In fact, the amount of nutrients that need to be managed can increase substantially if the digester accepts off-site organics such as pre-processing food wastes (and the nutrients they contain) to improve biogas production and profitability. This is important, as nutrient-related challenges are an increasing issue for dairies, and an increasing public concern in many areas that have significant concentrations of dairy cows. One solution to these concerns that is receiving widespread attention, is to develop viable nutrient recovery opportunities, including those that can be integrated with existing anaerobic digestion technologies. Successful integration would allow dairies to both improve the economic and environmental performance of their manure management operations.

Addressing the twin challenges of improving adoption of anaerobic digesters and providing new tools to improve nutrient management could provide important benefits to the dairy industry and to the public in Washington State. The projects that were part of the Appendix A research during the 2015-2017 biennium focus on these challenges from numerous angles, and address topics including technologies appropriate for large-scale and small-scale dairies, added value bioproducts that could be produced in anaerobic digestion systems to improve economics, and outreach and extension activities. A summary of that work is provided here, with additional detail provided in the chapters that follow.

Section 1: Technologies for an Integrated Large-Scale Anaerobic Digestion System

Anaerobic digestion technologies are commonly applied on large-scale dairies, as larger scales tend to create efficiencies that are needed for improved economics. The three projects in this section all look at new technologies that would be applicable at these larger scales.

Chapter 1: Polishing Effluent from Anaerobic Digestion for Water Reuse describes an investigation into the performance of two technologies: the Fenton process and sand filtration, as well as including a review of the scientific and regulatory context surrounding polishing

anaerobic digestion effluent for water reuse. The researchers investigated options for economically cleaning wastewater to a point where it could be reused within the dairy, or land-applied with a reduced need for managing nutrients. Such reuse options could transform manure management on dairies. These processes were used at the laboratory scale for recovering additional nutrients after using two existing nutrient recovery processes, ammonia stripping for nitrogen recovery and struvite precipitation for phosphorus. Using the four processes—ammonia stripping, struvite precipitation, the Fenton process, and sand filtration—in sequence, over 85% reduction of each of the indicators measured was achieved, including chemical oxygen demand (87.10%), total nitrogen (97.36%), total carbon (92.81%), ammonia nitrogen (98.41%), and total phosphorus (93.74%). These integrated processes also effectively removed color from the wastewater. An economic analysis showed that it could be feasible to integrate the Fenton process and sand filtration technology along with ammonia stripping and struvite if the struvite can be effectively monetized. To further reduce costs, the chemicals used—especially sulfuric acid—should be either optimized or substituted. For more on replacing the sulfuric acid used in ammonia stripping see Chapter 2, below.

Chapter 2: Ammonia Recycling-Based Technology for Pretreatment of Lignocellulose to Enhance Biogas Production focuses on whether agricultural residues, abundant in Washington State, could be co-digested with manure to increase biogas production and improve dairy anaerobic digester profitability (and therefore, hopefully, adoption). The biggest barrier to this is that crop residues (as well as the fiber in manure) require pretreatment to loosen the plant cell wall structure and allow microbes to access and use the material during digestion. In this project, a new integrated process for pretreating crop residues was developed and tested, and the economics were analyzed. In the first step of the process, ammonia was stripped from anaerobic digestion effluent using biogas. Stripping with air would be more common, but that process requires sulfuric acid for fixing the ammonia, adding cost. After stripping, the ammonia was used to pretreat crop residues, in this case, wheat straw. Raw biogas (40% carbon dioxide concentration) achieved greater than 80% ammonia removal at temperatures of 80°C and above, but was not efficient at lower temperatures. At temperatures of 70°C, purified biogas (10% carbon dioxide concentration) also achieved 80% ammonia removal. These results indicate that low concentration ammonia pretreatment is a promising process for the conversion of agricultural residues to bioenergy, but one that would need to be used in combination with thermophilic anaerobic digestion. It is noteworthy that most digesters in the U.S. operate at lower mesophilic temperatures of 30° to 40°C. Among the pretreatment protocols examined, 0.70% ammonia concentration at 105°C resulted in the greatest methane yield from pretreated wheat straw (353.8 mL g^{-1} volatile solids). An economic analysis indicated that thermophilic anaerobic digestion of manure and wheat straw showed improved profitability compared to mesophilic anaerobic digestion without pretreatment, as indicated by a reduced minimum selling price for biogas-derived compressed natural gas. Based on an analysis of the factors affecting the manufacturing cost of biogas-derived compressed natural gas using thermophilic anaerobic digestion, further work to improve profitability should be aimed at increasing compressed natural gas production and developing a market for the organic fertilizer co-product. The analysis also showed that feedstock cost would be an important issue affecting the economic viability of an anaerobic digestion project, for those that purchase feedstocks.

Water characteristics such as color and odor may affect the end uses for wastewater from anaerobic digestion. *Chapter 3: Wet Oxidation for Wastewater Color Removal* investigated

whether advanced oxidation processes could be integrated with nutrient recovery to improve color, odor, and chemical oxygen demand in anaerobic digestion effluent. Different system configurations, ozone concentrations, and sparging[1] times were evaluated at the laboratory scale, and their effects on color, odor, chemical oxygen demand, and dissolved carbon were measured. Up to 88% of true color and up to 40% of chemical oxygen demand were removed. Odor was removed as quickly as within five minutes. Total dissolved carbon content did not drastically change. While ozonation successfully removed color, odor, and chemical oxygen demand, detailed capital and operating costs for implementing this unit process are needed for each system configuration and treatment condition, in order to determine the most viable operation scheme. Further research is also necessary to evaluate ozonation on a continuous basis, for integration with nutrient recovery technologies.

Section 2: Technologies to Enable the Adoption of Anaerobic Digestion in Small Dairy Operations

While current economics limit use of digesters to larger dairies, more than 75% of the dairies in Washington State are small- or mid-sized, with less than 700 mature animals. These dairies have quite different financial, operational, and economic features than larger dairies. Several projects, carried out during the 2015-2017 biennium, specifically aimed to develop digester and nutrient recovery technologies that could be used by small dairies.

In *Chapter 4: Digester Technology for Small- and Medium-Sized Dairy Farms*, researchers reviewed the literature on existing anaerobic digestion technologies, with the goal of identifying simplified and efficient technologies that could be appropriate at smaller scales. Current barriers to applicability of these technologies were then identified, and solutions to overcome these barriers were tested. The review indicated two promising anaerobic digestion technologies for small- and medium-sized dairies. First, anaerobic digestion at thermophilic temperatures is more efficient and leads to a more complete utilization of the coarse fibers in manure. Second, anaerobic digestion conducted at a high level of total solids could be economical for small- and medium-scale anaerobic digestion. However, ammonia inhibition of anaerobic digestion can be a serious concern under thermophilic and high (greater than 10%) total solids conditions. For this reason, the research group developed a technique that simplifies the system by combining ammonia stripping and thermophilic anaerobic digestion, within the same digester. They found that using the optimum stripping rate of 1 min L^{-1} a maximum methane production of 192.3 L kg^{-1} of volatile solids was achieved. Under these conditions, ammonium inhibition was removed, the efficiency of anaerobic digestion was greatly improved, the anaerobic digestion process was simplified, and wastewater emissions were reduced. Overall, this approach has the potential to achieve nitrogen recovery through a simpler and more cost-effective process than other reported options. With further development, this approach may make it possible for small- and medium-scale dairy digesters to digest manure with a high total solids content, improve methane production, and reduce wastewater discharge while recovering nutrients.

Co-products that can be produced in an anaerobic digestion-based biorefinery can directly improve the anaerobic digestion outcomes, and may be applicable to issues faced by small- and mid-size dairies. *Chapter 5: Engineered Industrial Biochar Adsorbents as Alternatives for*

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[1] During sparging, a gas is bubbled through a liquid.

Nutrient Management in Anaerobic Digesters discusses work that advances the design and use of biochar to adsorb and retain nutrients and *Escherichia coli*. Researchers developed an engineered biochar made from anaerobic digestion fiber that fulfills two important roles: adsorbing hydrogen sulfide from biogas, and removing phosphorus from the liquid effluent. Another adsorbent was developed from a woody material with low ash content activated with phosphoric acid for adsorbing ammonia gas. A third adsorbent doped with nitrogen was developed for *E. coli* removal. Biochars produced at high temperatures (above 500°C) from anaerobic digestion fiber adsorbed hydrogen sulfide from biogas at levels that were comparable to those of commercial activated carbon. Phosphate recovery of 97.62% was also observed. The biochar activated with phosphoric acid also showed a high capacity for ammonia removal, adsorbing approximately ten times more ammonia than the activated carbon (170.1 mg of ammonia per g of biochar vs. 16.2 mg of ammonia per g of activated carbon). Biochar engineered by introducing nitrogen functional groups removed up to 82.2 % of *E. coli* from effluent. In addition, new spectroscopic methods (Raman, XPS, and NMR) for biochar characterization were developed through this research, allowing researchers to quantify defects in the biochar structure that could have a major impact on their capacity to adsorb pollutants. This research shows promise for addressing environmental concerns related to anaerobic digester effluent—such as removing hydrogen sulfide, nutrients, and pathogens—and for creating a valuable market for engineered biochars as adsorbents.

Section 3: Co-products from Anaerobic Digestion Systems

While biogas is one major product that is obtained from an anaerobic digestion system, other co-products can be critical to successful project economics, as recent analyses have shown.[2] The first two projects in this section take a closer look at issues relating to these co-products from anaerobic digestion systems.

Chapter 6 examines methods for *Producing Biofertilizer as A Co-Product of Anaerobic Digestion*. Even after processing steps such as ammonia stripping and phosphorus settling, effluent from anaerobic digestion contains considerable nitrogen, which can present challenges related to appropriate use. This project examined the potential of using effluent that has already undergone nutrient recovery for the production of a biofertilizer made from *Azotobacter vinelandii,* a species of nitrogen-fixing bacteria known to exhibit high growth rates while maintaining its nitrogen-fixing abilities. Results showed that *A. vinelandii* growth was promoted with diluted, nutrient-recovered effluent. The 50% nutrient-recovered effluent with supplemented glucose as an energy source was able to produce 6.2 g L^{-1} of biofertilizer. The production of biofertilizer resulted in cleaner effluent, with more than 70% reduction in the residual nitrogen in the effluent. The techno-economic evaluation results suggested that adding a biofertilizer production operation to an integrated biorefinery could improve the economic feasibility of the biorefinery. If the *A. vinelandii* produced provides benefits when applied to crops, it could help reduce crop producers' chemical fertilizer use while improving soil and water quality, in addition to being part of an integrated anaerobic digestion system.

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[2] See for example Galinato et al. (2005), accessible at http://cru.cahe.wsu.edu/CEPublications/EM090E/EM090E.pdf, and a blog article summarizing some of the key messages at http://csanr.wsu.edu/does-ad-cost-too-much/.

Chapter 7 examines the *Production of Composite Materials from Anaerobic Digestion Fiber.* Fiber is already an important co-product from anaerobic digestion, typically used for animal bedding or as a soil amendment. The development of new, value-added products from fiber could further improve the economic viability of anaerobic digesters. Characteristics of this fiber make it suitable for use in composite materials, similar to the wood plastic composites commonly used for decking. Since water resistance is an important characteristic for this composite material, the research team explored and optimized the use of a hot water extraction pretreatment process that removed hemicellulose, reducing the water affinity of composites produced with digested fiber. Composites produced using this pretreatment process had superior mechanical properties to those produced without the pretreatment step. These results confirm the potential to produce composite materials from hot water treated anaerobic digestion fibers.

In addition to research efforts to develop marketable co-products to improve the economic feasibility of anaerobic digestion, the last project in this section focused on another challenge facing dairies that could impact their manure management strategies: changes in the patterns and intensity of precipitation. In western Washington, the seasonality and variability of rainfall patterns and intensity directly impact crop planting and harvest timing, manure storage capacity and holding time, manure application timing, flooding, and the potential for having a runoff event leading to detrimental water quality effects. Anaerobic digestion in combination with nutrient recovery has the potential to *increase* the flexibility a dairy farm has in achieving nutrient management goals given the constraints imposed by precipitation patterns. *Chapter 8* looks at the effluent from anaerobic digestion, and explores a *Proof of Concept for Tools to Evaluate Water Quality Impacts of On-Field Application of Anaerobic Digestion System Nutrient Products*. As a first step to exploring this question, researchers explored the likely future risk of storage-related water quality discharge events due to projected precipitation patterns under climate change. Efforts to model future climate in northwest Washington (Whatcom, Skagit, and Snohomish Counties) project slight increases in the median year precipitation amounts, and larger increases in wet year precipitation amounts for total annual and storage season precipitation, compared to historical years. These results suggest that the recently observed challenge of adequate manure storage capacity in these three northwest Washington counties will likely continue to be a concern in the future. Viable strategies for managing risk need to be assessed, with options that include increasing the capacity of manure storage in the region, developing emergency storage options, or implementing technology solutions such as nutrient recovery to reduce the probability of discharges affecting water quality.

Section 4: Outreach and Extension Activities

The *Commercialization, Technology Transfer, and Extension* component of the Appendix A work described in *Chapter 9* focuses on supporting improved decision making about emerging technologies by dairy industry professionals, the manure management support industry, and others. In order to support the adoption and application of emerging technologies for waste management, the extension team carried out the following outreach activities:

- Provided technical support to regional stakeholders, including answering questions, and pointing them towards appropriate additional resources.

- Participated in a federal advisory panel focused on furthering the adoption of technologies to recover nutrients and to control the production of greenhouse gases.
- Participated in the Washington State Department of Agriculture's Dairy Nutrient Advisory Committee, an effort organized to propose non-regulatory methods for improving dairy nutrient management in Washington State.
- Delivered nine presentations at regional and national conferences, produced two blog articles and five webinars, and published seven formal extension publications, with five additional peer-reviewed publications in progress.
- Summarized existing information on the region's experience with anaerobic digestion systems and Appendix A research, which will be used as the basis for the development of a research roadmap in the summer of 2017.

Outreach work was aimed at 1) increasing awareness of the opportunities and potential surrounding an anaerobic digestion systems approach, and at 2) sharing tools, resources, and successful experiences that can help diverse groups further develop and implement these technologies in their professional fields. Building awareness and making resources available are critical early steps that contribute to improving the economic viability and the environmental footprint of facilities processing organic wastes in Washington State. Through these outreach activities, the team made an estimated 23,880 contacts with scientists, producers, industry professionals, regulators, policy-makers, and other interested parties across the country.

Conclusion

Taken together, these diverse projects explore multiple avenues through which Washington State could improve adoption of anaerobic digestion and dairy nutrient management. If adopted, these developments could contribute to further economic, environmental, and social benefits for the dairy industry, the communities they are part of, and the residents of Washington.

SECTION 1

TECHNOLOGIES FOR AN INTEGRATED, LARGE-SCALE ANAEROBIC DIGESTION REFINERY

1. Polishing Effluent from Anaerobic Digestion for Water Reuse

Liang Yu, Ping Ai, Alia Nasir, and Shulin Chen

1.1 Background

One of the key issues for dairy farms is the capacity to store manure and wastewater until it can be land-applied. The volume of washwater can occupy 25% to 50% of the total lagoon volume. This washwater adds little nutrient value and can cause manure nutrients to run off or infiltrate into groundwater if not properly applied. Hauling costs vary greatly depending on the farm and the location of fields, but typically average $0.01 to $0.03 per gallon (Cullens, 2011). Therefore, the reuse of washwater is important for improving the economics of wastewater treatment on dairy farms.

As environmental regulations become more stringent and water resources become scarcer, a new generation of large-scale digesters (including those integrated with dairy farms) will face challenges related to water use and effluent discharge. From an environmental perspective, the ideal scenario is a zero-discharge system, where any effluent produced is reused within the system; in the case of dairy farms, as washwater.

Although various treatments are available in the wastewater treatment industry, most of these technologies are not designed for use in dairy operations. Tailoring these processes for use with anaerobic digestion (AD) and nutrient recovery processes developed at Washington State University (WSU) will help meet the emerging needs of the dairy industry. To achieve widespread adoption, the cost of this technology must also be significantly reduced so that its adoption is economically feasible for Washington dairy farms.

1.2 Objectives

The goal of this project was to develop a waste management system that enables water recycling in dairy operations. This system includes four major components: (1) an anaerobic digester for converting organic wastes to biogas, (2) a post-digestion processing module that converts solids and nutrients in the effluent into fertilizers, (3) a polishing module that reconditions the effluent to be reused, and (4) an operational procedure that optimizes the performance of the system. The first two components have been addressed by the research team through other projects that received Appendix A funding in earlier biennia (Appendix A, 2015). The scope of this project will focus on the components of (3) and (4): reconditioning of the effluent, and optimizing system performance.

This project includes three parts. Part 1 is a review of existing information pertinent to reclaiming water for dairy use. Part 2 describes laboratory-scale tests for efficacy of the various components of the integrated system. And Part 3 focuses on modeling the integrated system to assess its economic feasibility.

1.3 Methods

The methods for Part 1 (review) and Part 3 (modeling) of this project are integrated into sections 1.4.1 and 1.4.3, respectively. Methods for Part 2 (laboratory-scale tests) are described below.

The strategy investigated for AD effluent treatment for water reuse was developed by Dr. Shulin Chen's research team at WSU. This method includes ammonia stripping and phosphorus (P) settlement technologies developed at WSU (AIRTRAP: Air Induced Recovery Technology for Re-use of Ammonia and Phosphorus) with the Fenton process and sand filtration steps (Figure 1.1).
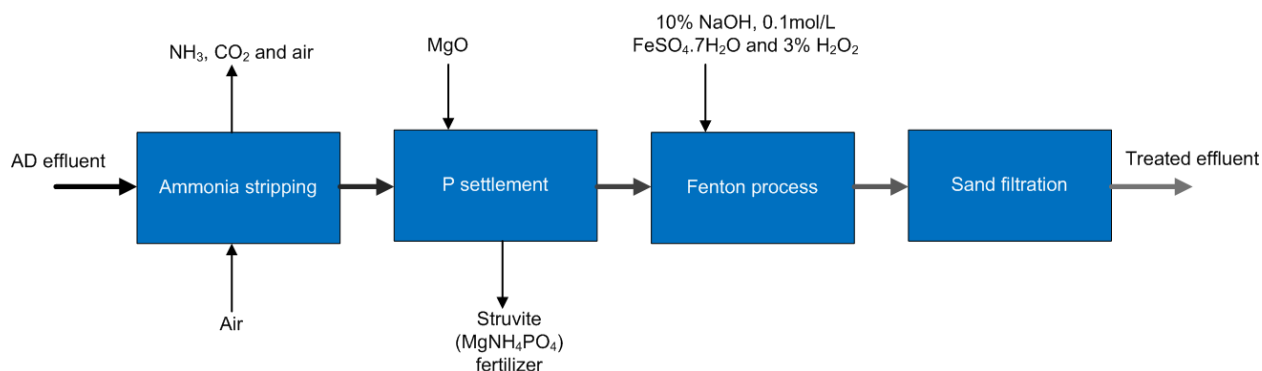
Figure 1.1: AD effluent treatment methods for water reuse

1.3.1 Ammonia stripping
Ammonia stripping was undertaken to remove the ammonia dissolved in the AD effluent. Air was used as the stripping agent. The air flow rates were 300 and 400 L hour^{-1}. The stripping temperatures were 35, 50, and 70°C. Each temperature and flow rate combination was tested. The stripping time was 12 hours.

1.3.2 Phosphorus settlement
Removal of P by precipitation of struvite has been used in management of animal manures and municipal wastewater. Struvite (magnesium ammonium phosphate) is a mineral which has value as a fertilizer. Struvite precipitates as a compact crystal, generating a small amount of easily settled solids (Fiesinger et al., 2006). Because of its lower cost, we used MgO in place of other Mg^{2+} compounds (such as $MgSO_4$) in the P settlement step. In this study, 1 mg MgO was added to 100 mL of AD effluent after ammonia stripping, providing a sufficient Mg source for the P removal process.

1.3.3 Fenton process

The Fenton process is one of a set of advanced oxidation processes (AOPs), which are alternative wastewater treatment processes that can be used to degrade biorefractory organic compounds (organic compounds that are resistant to biodegradation). Advanced oxidation processes have the advantage of typically operating with a lower energy requirement than direct oxidation (Babuponnusami and Muthukumar, 2014).

The Fenton process involves reactions of peroxides (usually hydrogen peroxide, or H_2O_2) with iron ions to form active oxygen species that oxidize organic or inorganic compounds. Besides the energy-related advantages, the Fenton process can remove some heavy metals in forms of precipitated metal hydroxides: $M(OH)_x$. Disinfection can also be achieved, which makes the Fenton process an integrated solution to some water quality problems. Since the complete reduction product of $\cdot OH$ is H_2O, the Fenton process theoretically does not introduce any new hazardous substances into the water. However, integrating the Fenton process in a way that reduces the necessary input of chemical reagents (peroxides) is critical to reducing the process costs. The presence of bicarbonate ions (HCO_3^-) can appreciably reduce the concentration of $\cdot OH$ due to scavenging processes that yield H_2O and a much less reactive species, $\cdot CO_3^-$ (Munter, 2001). In our process, bicarbonate has been eliminated in the ammonia stripping step shown in Figure 1.1.

The Fenton process can effectively decrease total nitrogen (TN) and total carbon (TC) in the effluent. Total carbon includes total organic carbon (TOC) and inorganic carbon (IC). This is a process that involves a low energy consumption process. First, 10% NaOH solution, 0.1 mol L^{-1} $FeSO_4 \cdot 7H_2O$ solution and 3% H_2O_2 solution were prepared. Second, the pH of the AD effluent was adjusted to 3 to 5 with H_2SO_4. Then $FeSO_4 \cdot 7H_2O$ and H_2O_2 were added. The amount of $FeSO_4 \cdot 7H_2O$ was 200-800 mg L^{-1}, and the amount of H_2O_2 was 0.5-1.2 ml L^{-1}. The mixture was agitated and the reaction time was 35 minutes. Finally, after the reaction, NaOH was used to adjust the pH to 8 and to remove Fe^{2+} from the AD effluent.

1.3.4 Sand filtration

Sand filtration is a frequently used, robust method to provide further polishing and reduction of nutrients, such as nitrogen (N) and suspended solids, from an effluent. Sand filtration was selected for this purpose because sand is readily available at a low cost in most areas. A sand filter has a dirt holding capacity of 3 to 6 kg of total suspended solids (TSS) m^{-2} of sand surface. The filtration medium consists of multiple layers of sand, varying in particle size and specific gravity. In this study, sand with a particle size of 1-2 mm was used.

Before the experiments, sand size was selected by sifting sand through a fine mesh screen. The sand was washed to remove dust and dried for packing into a glass tube with a diameter of 30 cm. Glass tubes were filled to heights of 50, 100, and 150 cm. The AD effluent was filtered through the sand-filled glass tubes.

1.4 Results and discussion

1.4.1 Part 1 – Review of literature and regulatory framework

A large-scale AD biorefinery can produce large volumes of digestate and has the potential to be profitable. Digestate is the by-product of methane and heat production in a biogas plant processing organic waste. Depending on the biogas technology, digestate may include solid and liquid material. Solid digestate can be used as organic fertilizer or as a construction material. Liquid digestate, also called effluent from AD, contains N, P, and other macro- and micronutrients. Generally, it is not economical to transport liquid digestate great distances due to its high water content (>97%) and the high costs of trucking and underground pipes. The effluent is therefore generally land-applied to nearby fields. Long-term manure application on these fields has resulted in excess N and P accumulation. Of the dairies in Washington, 36% and 55% are in a state of N and P overload, respectively. This has led to issues regarding the ultimate fate of nutrients applied to these fields, in particular, their potential to contribute to nitrate leaching, eutrophication, ammonia toxicity, and nitrite carcinogenesis (Dvorak and Frear, 2014). The practical utilization of these effluents (liquid digestate) is also limited. This is because the agricultural need for fertilizer is seasonal, and the fact that biogas plants often have limited agricultural land around them (Gong et al., 2013).

One of our research team's major efforts is the development of AD co-products, most notably nutrient recovery through ammonia stripping and P harvesting (Jiang et al., 2014). Overcoming ammonia inhibition problems requires dilution of waste through the addition of a significant amount of fresh water, and an associated facility to hold the effluent. Our technology allows removal of ammonia from AD effluent by reducing the inhibition effect using reclaimed water. Moreover, with this process, excess N can be recovered to produce a fertilizer, converting a liability into a revenue source for the farm operators. We have commercialized an AD-based nutrient recovery process on both dairy and poultry farms called AIRTRAP. This technology recovers ammonia N in the form of $(NH_4)_2SO_4$. Installed on the Vander Haak dairy farm (Lynden, Washington), the system removed over 50% of N and 80% of P from the effluent and produced 7 L m^{-3} d^{-1} of 40% $(NH_4)_2SO_4$ solution, and 8 kg m^{-3} d^{-1} of dry, P-rich (3% P) solids. Although major amounts of N and P in the AD effluent have been recovered through the AIRTRAP technology, higher standards for the reclaimed water must be achieved for irrigation and other uses related to the production of agricultural products (e.g., drinking water for animals, or aquaculture) (McCauley, 2015; Schlender et al., 1997).

Biological, physical, and chemical methods, used alone or in combination, have been reported to remove N, P, heavy metals, and other macro- and micronutrients from wastewater. Some of these methods include struvite formation (Song et al., 2011), ammonia stripping (Jiang et al., 2014), biological membrane reactors, sequential combination of aerobic and anaerobic batch reactors (Frison et al., 2013; Skouteris et al., 2012), adsorption processes with activated carbon, ion exchange resins, zeolites (Huang et al., 2010; Sica et al., 2014), AOPs (Xu et al., 2012), reverse osmosis (Gong et al., 2013), the Fenton process (Babuponnusami & Muthukumar, 2014), and constructed wetlands (Wu et al., 2016; Zhang et al., 2014). Despite the reported success of these methods, challenges remain, including the high cost of raw materials for chemical and physical processes, and the unbalanced C:N ratio for biological processes. Therefore, development of a

relatively cost-effective and operationally robust system is critical for the adoption of these technologies.

To ensure the reclaimed water is safe for the designated uses, the source water must be adequately and reliably treated to meet specific guidelines (USEPA, 2012). Sources for reclaimed water can include stormwater and greywater, in addition to wastewater. More rigid performance standards are required for a reclaimed water facility than a wastewater treatment facility. For every unit treatment process, a reclaimed water treatment facility must have a fully operational and functional backup component, or bypass, to temporary or long-term storage, to avoid delivery to the users when a component fails to provide adequate treatment.

While the use of reclaimed water typically poses greater financial, technical, and institutional challenges than the use of traditional sources of water (e.g., groundwater or surface water), the range of treatment options available make it feasible to achieve any level of water quality. While reclaimed water was once only used for land application for groundwater recharge or crop production, today's advanced treatment processes make it possible to achieve potable water quality standards for indirect potable reuse, that is, the blending of advanced treated, recycled, or reclaimed water into a natural water source (groundwater basin or reservoir) that could be used for drinking (potable) water after further treatment.

There are numerous case studies that demonstrate the challenge of balancing treatment costs with the intended use of reclaimed water. In many of these cases, reuse treatment is developed with the goals of replacing the use of drinking water for non-potable applications and meeting future water demands as high-quality sources, such as groundwater, are depleted. As technologies are now advanced enough to treat wastewater to the water quality required for the intended use, the concept of "Fit for Purpose" provides a framework for cost-effective water treatment (USEPA, 2012). By selecting an appropriate treatment based on the needs of specific applications, water supply costs can be controlled and the cost for improvements to wastewater treatment technologies can be delayed until they are needed.

State and federal governmental agencies in the United States have adopted a wide variety of guidelines and standards regarding the use of reclaimed water for crop irrigation. These specifications have been developed primarily to protect public health and water resources, and specific crop water quality requirements must be developed with the end users. In general, Class A, B, C, and D reclaimed water can be used to irrigate non-food crops including trees, fodder, fiber, and seed crops. Only Class A reclaimed water is usually allowed for use as spray irrigation for all food crops, unless food crops undergo physical or chemical processing sufficient to destroy all pathogenic agents, in which case all Classes are allowed. Class B reclaimed water can be used for surface irrigation (not spray) of food crops as long as there is no contact with the edible portion of the crop. When considering the use of reclaimed water in agriculture, it is critical to understand the factors that determine whether a farming operation can successfully use reclaimed water for irrigation. Several factors, including soil-plant-water interactions, irrigation water quality, plant sensitivity and tolerance, soil characteristics, irrigation management practices, and drainage are important to consider in crop production settings.

Washington State has authorized use of reclaimed water for non-potable uses. However, strict specifications have been implemented to ensure the health and safety of all Washington citizens

and the protection of the environment. In 1997, the Washington State Departments of Health (DOH) and Ecology (Ecology) developed a Standards document for reclaimed water (Schlender et al., 1997). In May 2010, a Draft Rule for reclaimed water (WAC, 2010) was developed, then in October 2014 it was revised to create a Preliminary Draft Rule (WAC, 2014) and is awaiting adoption. Although the 1997 Standards are in place until the new rule adoption, it is advisable to plan for the content and specifications of the new rule.

For instance, the 1997 Standards document only uses total coliform in its definition of four reclaimed water classes (A, B, C, and D) but the Preliminary Draft Rule is much more specific, and includes oxygen demand, suspended solids, turbidity, pH, chlorine residual, total coliform, and virus removal specifications. In addition, the Preliminary Draft allows only two reclaimed water classes (A and B). Sampling requirements vary from 24-hour composites for five-day biochemical oxygen demand (BOD5) and total suspended solids (TSS) to daily grab sampling for total coliform. Class A and B reclaimed water must be less than 2.2 total coliforms per 100 mL based on a seven-day average at the effluent outlet.

Ecology and DOH have worked cooperatively to review and permit wastewater facility projects. According to the 1997 Standards, reclaimed water used for the irrigation of trees or fodder, fiber, and seed crops shall be at all times Class D reclaimed water or better, and reclaimed water used for the irrigation of sod, ornamental plants for commercial use, or pasture to which milking cows or goats have access shall be at all times Class C reclaimed water or better. The preliminary Rule will require Class B reclaimed water or better to irrigate nonfood crops (McCauley, 2015; WAC, 2014). All classes of reclaimed water should be treated by oxidization and disinfection methods at a minimum. Sampling is to be performed daily and Class A requires the highest level of treatment and has the most use potential and the least restrictions on its use. The major difference between Class A and Class B reclaimed water is that Class A water is filtered. Even though Class A reclaimed water may meet drinking water quality standards for potable water, regulations do not permit direct human consumption. Class A reclaimed water is, however, approved for human contact (the public and municipal employees).

Ecology's Water Quality Program and DOH provide guidance on specific requirements for ensuring adequate technology-based treatment of reclaimed water. Two methods of treatment are recognized in regulations for Class A reclaimed water. One is a traditional four-step treatment and the other is membrane filtration and membrane bioreactor treatment plus disinfection. The traditional four step treatment includes oxidation, coagulation, filtration, and disinfection steps. In the membrane filtration and membrane bioreactor process, the same oxidation process and treatment technology standards apply to membrane filtration processes as for biological oxidation. The membranes replace the coagulation and filtration process. Both these membrane techniques are followed by disinfection. Production of Class B reclaimed water requires biological oxidation followed by disinfection. Filtration and the associated treatment steps are not required for this class. Biological oxidation performance standards are identical to those required for Class A reclaimed water. The Preliminary Draft Rule allows the lead agency to authorize alternative treatment processes that the lead agency determines to be equivalent to the processes required in the Rule (McCauley, 2015). The applicant should demonstrate in the engineering report that the alternative treatment method will consistently achieve water quality

limits through proper design, operation, and maintenance of each component of the treatment method.

Currently there is still a need to develop and select low-cost and efficient treatment technologies for dairy wastewater. This need is especially great because many dairies cannot make large investments in wastewater treatment. The previously mentioned technologies (e.g. membrane filtration and membrane bioreactor treatment) are usually used in large-scale wastewater treatment plants with a capacity of over 10,000 tons per day. In these large-scale wastewater treatment plants, high capital investment is required. Operating costs can be significantly reduced with an increase in scale. However, dairy wastewater production is usually much less than 1,000 tons per day. The traditional wastewater treatment methods and technologies cannot be directly selected and used to treat dairy wastewater, even though they can efficiently meet Class A reclaimed water standards. Based on Washington Ecology and DOH guidance, the research team will continue to develop AD and nutrient recovery technologies to establish a low-cost and efficient system for dairy wastewater reuse and recycle.

1.4.2 Part 2 – Laboratory-scale tests

1.4.2.1 Ammonia stripping
The experiments were operated at the flow rate of 300 L hour^{-1}. There were significant differences ($p < 0.05$) across the selected temperature range. Stripping carried out at 70°C reached the stable phase of ammonia removal at around 180 minutes, while stripping carried out at 35°C required almost 720 minutes to achieve that level of ammonia removal (Figure 1.2).
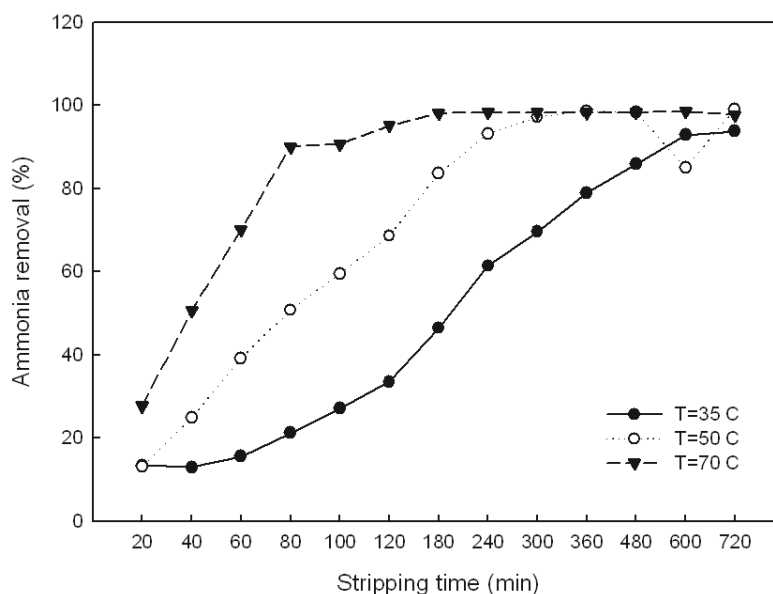
Figure 1.2: Effect of temperature on ammonia removal

The subsequent ammonia removal experiments evaluating different air flow rates were conducted at a temperature of 50°C. There were *no* significant differences ($p > 0.05$) between the 300 L hour^{-1} and the 400 L hour^{-1} flow rates. About 360 minutes of stripping time was required to reach the stable phase of ammonia removal (Figure 1.3).

8
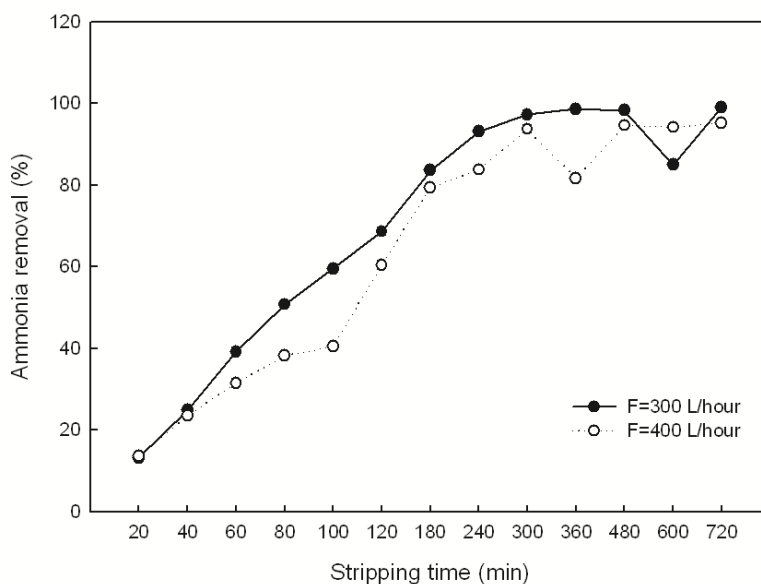
Figure 1.3: Effect of air flow rate on ammonia removal

In these experiments, temperature had a greater effect on ammonia removal than air flow rate. Higher temperatures significantly reduced the time needed for ammonia removal. According to the Wilderness Medical Society, water temperatures above 160°F (70°C) kill all pathogens within 30 minutes (Curtis, 1998). However, higher temperatures require greater energy consumption. Therefore, the selection of operational conditions will depend on the design requirements, which are based on considerations including water quality goals, investment levels, and heat source.

1.4.2.2 Phosphorus settlement

pH was measured for this study because it has an important effect on the settlement of P, with the greatest P removal occurring around pH 10. pH was measured over time during ammonia stripping (the step prior to P settlement) at 70°C with air flow rates of 300 and 400 L hour^{-1} (Figure 1.4). During this process, pH increased at first, indicating release of CO_2. The pH then decreased to a low value, indicating that ammonia release dominated the process. Subsequently the pH increased again, to around 9.3, indicating further CO_2 release dominated the process once again. With the addition of alkali in the AD effluent, pH can be adjusted from 8.0 to 10.0. Phosphorus removal increased with an increase in pH and the precipitation reaction was very fast. At a pH of 10.0, about 95% P removal was achieved (Figure 1.5).

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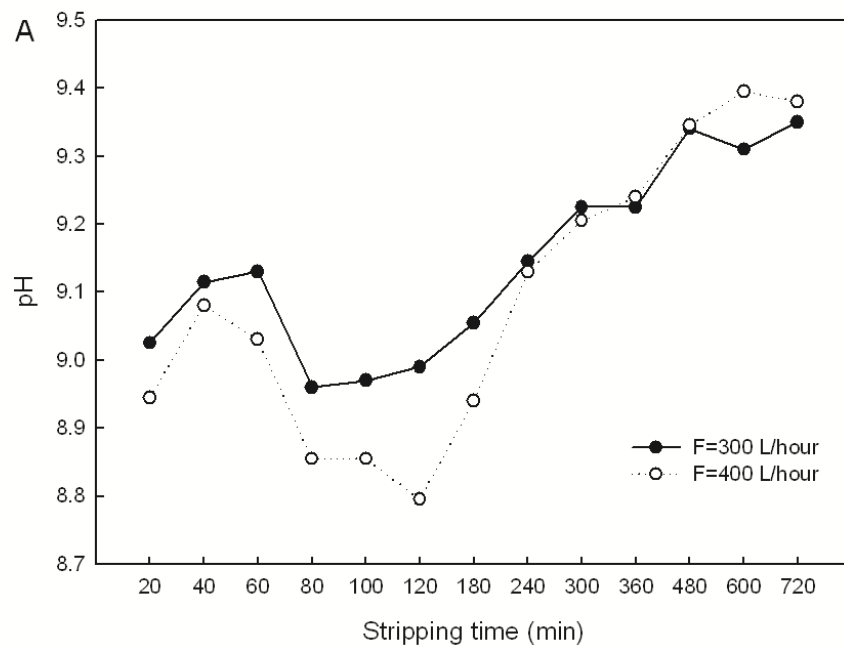
Figure 1.4: Changes of pH through time during ammonia stripping at 70°C
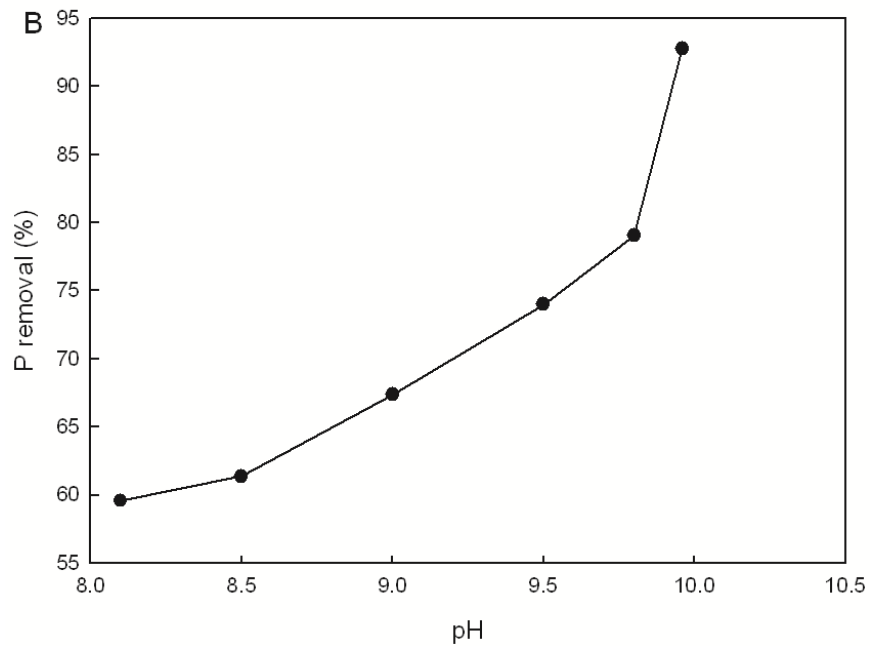
Figure 1.5: Changes in P removal with pH after sufficient MgO addition

1.4.2.3 Fenton process

Comparing the results for different pH levels, the greatest TN and TC removal were observed at a pH of 3.5 (Table 1.1). Before ammonia stripping, the Fenton process removed 47.3% of TN and 41.6% of TC. After ammonia stripping, the Fenton process removed 47.0% of TN and 41.0% of TC. The effects of the Fenton process and sand filtration are shown visually in Figure 1.6. The results indicate that the Fenton process not only can remove TC and TN, but can also remove color. The Fenton process was most effective for color removal at a pH of 3.0 (Figure 1.6).

Table 1.1: Results of the Fenton process on total organic carbon (TOC), inorganic carbon (IC), total carbon (TC), and total nitrogen (TN) before and after ammonia stripping at different pH levels

	TOC (mg L^{-1})	IC (mg L^{-1})	TC (mg L^{-1})	TN (mg L^{-1})
Effluent before stripping	1080	340.6	1430	614.8
pH=3	762	154.9	916.9	412
pH=3.5	608.7	145.2	753.9	359.1
pH=4	747.1	171.1	918.2	439
pH=4.5	767.3	167.3	934.6	431.7
Effluent after stripping	1170	89.09	1250	427.8
pH=3	772.5	59.06	831.6	319.3
pH=3.5	786	58.39	844.4	326.1
pH=4	815.3	55.43	870.7	327.4
pH=4.5	820.8	57.41	878.2	322.4

Figure 1.6: Color of AD effluent after treatment at different pH levels

1.4.2.4 Sand filtration

Removal of TN and TC increased with an increase in the bed height used during sand filtration after ammonia stripping (Table 1.2). Although 150 cm of bed height was observed to remove more TN (62.3%) and TC (57.6%), the corresponding bed pressure was also higher, making the filtration process more difficult to operate.

11

Table 1.2: Effects of sand filtration on total organic carbon (TOC), inorganic carbon (IC), total carbon (TC), and total nitrogen (TN) at different bed heights after ammonia stripping

	TOC (mg L^{-1})	IC (mg L^{-1})	TC (mg L^{-1})	TN (mg L^{-1})
Effluent after stripping	1170	89.09	1250	427.8
Height 50 cm	458.9	287.1	746	309.6
Height 100 cm	384.5	272.7	657.2	281.5
Height 150 cm	295.8	310.3	606.1	231.6

1.4.2.5 Integrated processes

The ammonia stripping, P settlement, Fenton, and sand filtration processes were integrated to treat AD effluent. The different treatment methods showed different effects on the water quality parameters of interest (Table 1.3). Although over 85% of each measured component was removed from the AD effluent, the integrated processes still did not achieve the stringent requirement for reclaimed water in Washington. Compared to NH_3-N, TN was still very high after treatment, suggesting that a significant amount of the organic N did not convert to NH_3-N. Chemical oxygen demand (COD) was also relatively high after treatment. Therefore, a second AD phase will be needed to further decrease TOC and release organic N from the effluent. Further optimization of the Fenton process and of the sand filtration are also needed to achieve water quality results sufficient to meet the requirements for reuse as dairy washwater, drinking water for livestock, or land application.

Table 1.3: Chemical oxygen demand (COD), total nitrogen (TN), total carbon (TC), total organic carbon (TOC), inorganic carbon (IC), ammonia nitrogen (NH$_3$-N), and total phosphorus (TP) after each step of the integrated processes

	Effluent	Ammonia stripping	P settlement	Fenton	Sand filtration	Removal (%)
COD (mg L^{-1})	4790.00	3075.00	1758.20	1008.00	618.58	87.10
TN (mg L^{-1})	2380.00	614.80	536.80	95.90	62.87	97.40
TC (mg L^{-1})	4010.00	1430.00	794.40	388.00	288.20	92.80
TOC (mg L^{-1})	1890.00	1080.00	585.20	353.00	269.90	85.70
IC (mg L^{-1})	2120.00	340.60	209.20	34.97	18.26	99.10
NH$_3$-N (mg L^{-1})	2148.00	30.17	30.13	30.56	34.12	98.40
TP (mg L^{-1})	54.17	54.17	4.12	4.06	3.39	93.70

1.4.3 Part 3 – Modeling to evaluate feasibility

An economic analysis was conducted to evaluate the feasibility of these integrated processes for polishing effluent from anaerobic digestion for water reuse, based on a previous nutrient recovery report (Dvorak and Frear, 2014) and the lab experiments described above. DVO Inc. designed and constructed a pilot nutrient recovery system at Vander Haak Dairy (Lynden, Washington). This system was completed for demonstration of a continuous flow system at a commercial scale on a dairy practicing co-digestion (maximizing the production of biogas in the AD plant by adding substrates beyond manure from the dairy, such as food waste), using a mixed

plug-flow digester made by DVO (Chilton, Wisconsin). The flow rate for this farm and digester was 40,000 gallons per day. This farm had 2,000 cows, with the average effluent discharge for each cow estimated at 20 gallons per day. The previous nutrient recovery report included only ammonia stripping and P-rich solids. This economic analysis expanded on the previous biennium report by including struvite precipitation, the Fenton process, and sand filtration. The total cost per cow per year for nutrient recovery in the previous report (i.e., only ammonia stripping and P-rich solids) was $108.36 while the total revenue per cow per year was $124.89. With the integrated processes (i.e., adding struvite precipitation, the Fenton process, and sand filtration), the total cost per cow per year increased by 7.23% while the total revenue per cow per year increased by 6.09% (Table 1.4). Struvite fertilizer can bring greater revenues that can offset costs for chemicals in the wastewater treatment. Although the net profit per cow per year after the integrated processes were included was reduced by 1.14%, the AD effluent had been polished to an extent that it could be reused to flush manure from dairy barns.

In summary, the integration of the Fenton process and sand filtration with the previous nutrient recovery processes improved the quality of the resulting wastewater and, when the value of struvite fertilizer is considered, did not increase the cost of wastewater treatment. Therefore, it is feasible to use these integrated processes for water reuse. To further reduce costs, the chemicals (including sulfuric acid, $FeSO_4 \cdot 7H_2O$, and H_2O_2) should be either optimized or substituted.

1.5 Conclusions and recommendations

This project involved the review and testing of methods for polishing effluent from anaerobic digestion for water reuse using WSU's AD and nutrient recovery technologies. These methods were selected based on the Washington Departments of Ecology and Health guidance that require that reclaimed water be treated by oxidization and disinfection at a minimum. The Fenton process was selected because it allows both oxidization and disinfection to be completed in one process. Sand filtration was selected because sand is easily available at a low cost in most areas. The integrated process of ammonia stripping, P settlement, the Fenton process, and sand filtration was tested for its effect on several water quality indicators. These integrated processes for treatment of the AD effluent achieved over 85% removal for all indicators, including COD (87.10%), TN (97.36%), TC (92.81%), NH_3-N (98.41%), and TP (93.74%). An economic analysis was conducted to evaluate the feasibility of these integrated processes for polishing effluent from AD for water reuse based on the DVO nutrient recovery report (Dvorak and Frear, 2014) and laboratory experiments. The results showed that it is feasible to integrate some tertiary wastewater treatment processes (Fenton process and sand filtration) with the ammonia stripping and P settlement processes. The production of struvite fertilizer has great potential to improve the profitability of polishing effluent from AD for water reuse. To further reduce costs, the chemicals used (including sulfuric acid, $FeSO_4 \cdot 7H_2O$, and H_2O_2) should be either optimized or substituted, especially sulfuric acid. The research team investigated another method to replace sulfuric acid—biogas stripping and absorption for ammonia recovery—and the feasibility of this method is described in Chapter 2.

Since washwater used on a dairy farm may not need to meet quality standards as high as those required for drinking water, it should be possible to balance economic and environmental considerations to sustainably reuse wastewater. Currently no reclaimed water quality standards

exist for dairy washwater in Washington. In the future, the research team, dairy farmers, and Ecology may collaboratively discuss a reclaimed water quality standard for washwater, to satisfy the washwater needs of dairy farms at a low cost.

Table 1.4: Tentative economic analysis of nutrient recovery and integrated processes

Cost ($ cow^{-1} yr^{-1})		Revenue ($ cow^{-1} yr^{-1})	
Electrical Power: Electrical purchase of 5¢ kwh^{-1}; aeration rate of 20 gallons cfm^{-1}; power need of 20 cfm hp^{-1}; 1.2x for other electrical	$29.78	*Anaerobic Solid (AS) Slurry*: 40% AS by weight; 0.25% influent concentration of NH_3; 80% NH_3 recovery; 3.9 lbs AS: 1 lb NH_3; $80 ton^{-1} slurry; $200 ton^{-1} AS	$69.00
Sulfuric Acid: $175/ton conc. acid; 2.9 lbs of acid: 1lb NH_3 recovered	$56.58	*P-rich Solids*: $175 dry ton^{-1} at 3:1.5:3 NPK; 50% TS; 3.5 wet lbs cow^{-1} day^{-1}	$55.89
Electrical Power: Electrical purchase of 5¢ kwh^{-1}; effluent transport of about 500 m; sand filtration of 1.8 m height and 5 m diameter with 2600 kg m^{-3} density	$2.98	*Struvite:* $925 dry ton^{-1}; 18.14 lbs of production	$7.61
MgO: $210 ton^{-1}; 2.98 lbs of consumption	$0.28	*Credits*: Nutrient Trading; carbon; Renewable Energy Credits—assumed zero for now	$0.00
FeSO$_4$·7H$_2$O: $116 ton^{-1}; dilute to 0.1 mol L^{-1}; 343.42 lbs of consumption	$1.81	*Offset Savings*: Reduction in engine oil with H_2S reduction—zero now	$0.00
H$_2$O$_2$: $445 ton^{-1} 50%; dilute to 3%; 228.95 lbs of consumption	$2.77		
Labor: 0.5 FTE salaried position with salary of $40K yr^{-1}; 2,000 cow farm	$10.00		
O&M: 2% of capital costs at $600 cow^{-1} nutrient recovery only	$12.00		
Total	**$116.20**	**Total**	**$132.50**

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2. Ammonia Recycling-Based Technology for Pretreatment of Lignocellulose to Enhance Biogas Production

Liang Yu, Iin Parlina, Dianlong Wang, and Shulin Chen

2.1 Background

The projects described in this chapter are aimed at developing a new generation of technologies for integrated, large-scale anaerobic digestion (AD) biorefineries that minimize or utilize waste and by-products generated in agricultural and animal production systems, while improving the efficiency of energy and water use. To enable wider adoption of AD technology, there is a need to develop additional co-product options and recover nutrients from the AD digestate. Development of co-product options and nutrient recovery technology will improve the overall public benefit provided by AD systems in terms of managing waste and reducing greenhouse gas emissions.

This project responds to the 2015-2017 Appendix A request for proposals to further develop technologies for an integrated, large-scale anaerobic digestion biorefinery. In large-scale AD, the effluent from animal waste will accumulate excessive ammonia (NH_3). If discharged directly, the AD effluent will cause adverse environment impacts. If the effluent is recycled back to the AD directly, the biogas reactions will be inhibited. Ammonia recycling and reuse are therefore critical to the development of large-scale, sustainable AD biorefineries.

Furthermore, large-scale AD requires sufficient supplies of feedstock. Washington is a leading agricultural state with abundant agricultural residues that can provide a feedstock for the AD process. Compared to animal wastes, agricultural residues are easier to transport. Taking additional residues will enhance the profitability of an anaerobic digester by increasing biogas production. However, the lignocellulosic structure of agricultural residues often hinders their full use in an AD process. For this reason, it is necessary to develop a low-cost and effective pretreatment process to improve AD performance, increase biogas productivity, and improve feasibility.

Scientifically, this project fills the knowledge gap in understanding the action mechanism by which an ammonium hydroxide/ammonium bicarbonate (NH_4OH/NH_4HCO_3) mixture structurally changes lignocellulosic materials. Furthermore, the combination of high temperature pretreatment and thermophilic anaerobic digestion (TAD) can sharply enhance the degradation rate of lignocellulose and increase biogas production. By enhancing the biogas production capacity of agricultural residues by using a novel ammonia recycling system, this project contributes to improving the economic feasibility of large-scale AD in Washington.

2.2 Objectives

The goal of this project is to develop an integrated process for pretreating lignocellulosic biomass to be more easily digested anaerobically, thereby increasing biogas productivity. The novel feature of the process is the use of a recycling strategy for ammonia that is produced on-site by the AD process. In this process, (1) ammonia will be stripped out from the AD effluent of digested dairy manure, (2) the ammonia stream will be used to capture carbon dioxide (CO_2) from biogas, and (3) the ammonia will be used to pretreat lignocellulosic material in feedstock (such as manure fibers and crop residues) to enhance their performance during AD.

The project consists of four parts: Part 1 is a review of scientific information, Part 2 tests unit operations and options for optimizing the process, Part 3 is an evaluation of the pretreatment process, and Part 4 is an economic assessment.

2.3 Methods

The method used to pretreat crop residues using the recycled ammonia from AD effluent is outlined in Figure 2.1. After AD of organic wastes high in nitrogen (N) and phosphorus (P), such as animal manure, most of the effluent is sent to an ammonia stripping column. In the stripping column, biogas with a low CO_2 content is used to remove ammonia from the effluent at high temperature (>50°C). After stripping, the effluent with a reduced ammonia concentration can be reused as dilution water for the high-solids feedstock entering the digester. Separately, the ammonia-bearing biogas and water vapor are sent to a CO_2 and hydrogen sulfide (H_2S) absorber, where ammonia is absorbed under slightly higher pressure, with CO_2, and precipitated into crystalline ammonium bicarbonate (NH_4HCO_3) at low temperature (<30°C). The biogas stream is purified by cycling through the CO_2, NH_3, and H_2S absorber several times, to achieve a target level of purity (e.g., 96%). The crystalline NH_4HCO_3 is sent to the CO_2 regenerator to be heated to a temperature greater than 50°C, causing the NH_4HCO_3 to decompose into ammonium and CO_2. This process allows for separation of the CO_2 into gaseous form, while retaining ammonia in the solution in the form of NH_4OH. The mixture of ammonium hydroxide/ammonium carbonate (NH_4OH/NH_4CO_3) remaining in the solution is then used to pretreat the lignocellulosic feedstocks, such as crop residues. The pretreated solids are then fed to the anaerobic digester. In this process, ammonia is used for both pretreatment of feedstocks and biogas purification.
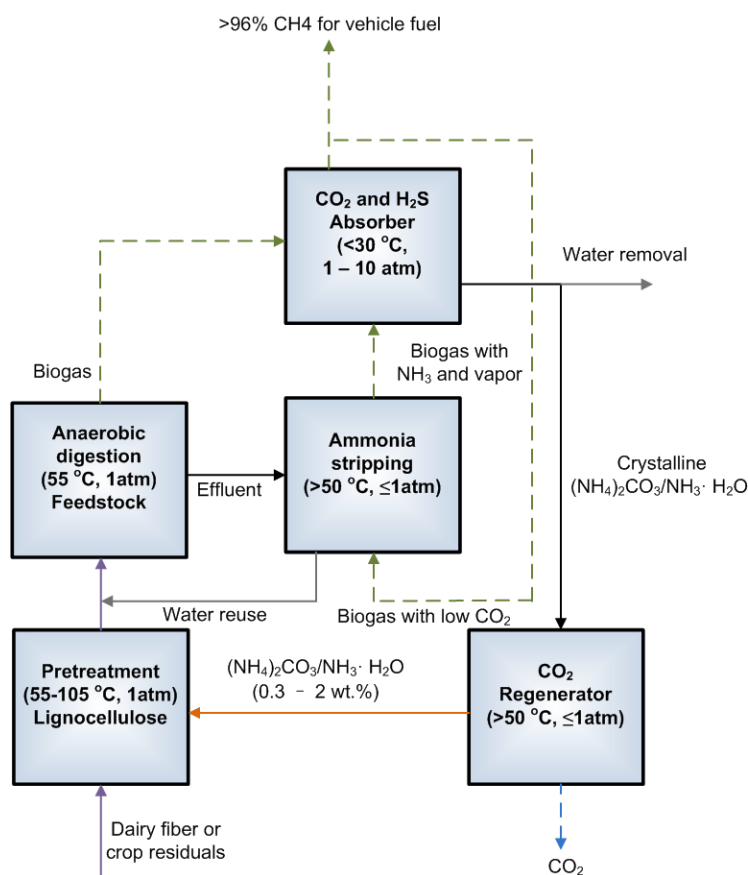
Figure 2.1: Pretreatment of crop residues using recycled ammonia from AD effluent

2.3.1 Materials

Wheat straw (Grange Supply Co., Pullman, Washington) was air dried and then ground using a hammer mill at the Washington State University (WSU) Wood Materials and Engineering Laboratory. The ground straw was passed through a 2-mm aperture standard screen, and then sealed in plastic bags under room temperature for further use. The total solid (TS) and volatile solid (VS) content of the wheat straw were 98.21% and 90.75%, respectively. The wheat straw was composed of 36.21% cellulose, 21.95% hemicellulose, 17.64% acid-insoluble lignin (AIL), 2.27% acid-soluble lignin (ASL), and 8.43% ash. Inoculum for AD was sourced from a mesophilic anaerobic digester at the Pullman Wastewater Treatment Plant in Pullman, Washington. The TS and VS content of the inoculum were 1.19% and 55.56%, respectively. The inoculum was composed of 4.34% cellulose, 2.41% hemicellulose, 21.58% acid-insoluble lignin, 6.83% acid-soluble lignin, and 16.36% ash. Prior to use for thermophilic AD, the inoculum was gradually acclimated to thermophilic conditions (Wang et al., 2016).

2.3.2 Experimental methods

2.3.2.1 Ammonia pretreatment

The ammonium hydroxide solution used in this study was 28-30% (w/w) (JTB-9721-03 ammonium hydroxide). Ammonia pretreatment was carried out using a full factorial

experimental design to study the effects of ammonia concentrations (0.35, 0.70, and 1.05 wt. %) and temperatures (55°C and 105°C) on methane (CH_4) production and degradation of wheat straw lignocellulose. The retention times for pretreatment were 12, 24, and 48 hours. For each run, 5 g of dry wheat straw were treated, and the moisture content was fixed at 85%. The corresponding ratios of ammonia and wheat straw were 2, 4, and 6%, respectively. Treated mixture solutions were vacuum filtered, and the wet solid residues were used for AD experiments. Each run was duplicated and means are reported for each treatment.

2.3.2.2 Anaerobic digestion

Anaerobic digestion experiments were conducted at thermophilic (55°C) conditions for 20 days. The batch experiments of ammonia-pretreated wheat straw and the control (no pretreatment) were conducted in a 0.25 L AD reactor. For each run, 3 g dry feedstock were added and inoculated with 1.5 g sludge. All reactors were capped with rubber stoppers and put into a water bath. Before the fermentation test, the reactors were flushed with nitrogen gas to remove oxygen from the headspace and maintain an anaerobic environment. To minimize errors, each run was conducted in duplicate. The biogas volume was measured by 100 mL syringe, and the biogas samples were stored in 12 mL Labco Exetainer® vials every three days for gas composition analysis. After AD, the solid digestate was collected for composition analysis.

2.3.2.3 Analytical methods

Total solids (TS) and VS were measured using standard methods (APHA, 1998). The pH was measured using a pH meter (AB15). The biogas composition was detected by a Varian CP-3800 Gas Chromatograph equipped with a thermal conductivity detector, a HayeSep Q 80/100 Mesh Silcosteel column, and a SilicaPLOT column (50 m×0.53 mm×4 µm). Analysis of wheat straw samples for carbohydrate, ASL, and AIL content was performed according to the National Renewable Energy Laboratory's (NREL) Laboratory Analytical Procedure (Sluiter et al., 2008).

2.4 Results and discussion

2.4.1 Part 1 – Review of scientific information

A large-scale AD biorefinery is designed for the conversion of large volumes of organic wastes into biogas and other co-products, such as organic fertilizer. Biogas is recovered and used either directly for heating, or transformed into combined power and heat and fed back into the grid. It can also be refined into natural gas suitable for use in vehicles. The development of large-scale anaerobic digesters has occurred mostly in industrialized countries. Many different designs and types of large-scale anaerobic digesters are available. Most of them are high-technology, requiring expert construction, operation, and maintenance skills to run.

Biogas is a form of green energy and has the potential to reduce greenhouse gas emissions. Due to increasing fuel prices and concerns about climate change, large-scale biogas generation from waste and energy crops is gaining interest in developing countries as well (Spuhler, 2016). However, these benefits have not been sufficient to motivate U.S. companies to invest in AD technology widely because the cost required to build and operate a digester is high compared to the value of biogas produced for power generation. The key rate-limiting factors in the AD

process are the recalcitrance of many organic materials, the quantity of functional microorganisms in the digester, the physical and chemical environment within the digester, and the degree of mixing that promotes contact between microbes and substrate.

Agricultural residues represent a significant fraction of biomass production worldwide and include largely unused plant material such as wheat straw, corn stover, and oil palm empty fruit bunch. These materials are not commonly used in chemical or biological conversions due to the difficulties caused by the recalcitrant characteristics of the plant cell wall in their lignocellulosic biomass. Biomass recalcitrance refers to the resistance of the plant cell wall to be naturally degraded for conversion into higher-value products. The primary factors that cause this resistance include the protective layers of lignin and hemicellulose as well as cellulose crystallinity (Chundawat et al., 2011).

Pretreatment methods are needed to reduce this recalcitrance and to increase digestibility of the biomass. Pretreatment processes use physical, mechanical, chemical, or biological means to change the structure of the plant cell wall, through the removal of lignin and hemicellulose or the fragmentation of the cell itself. This results in increased accessibility to the cellulose for the hydrolytic enzymes that convert cellulose into glucose. However, in order to be effective, many of these pretreatment processes require conditions involving extreme chemical concentrations, temperature, and pressure. Due to the heterogeneous nature of biomass, no single pretreatment process is effective on all types of biomass (Behera et al., 2014; Chandra et al., 2007; Gírio et al., 2010).

When choosing a pretreatment process, it is important to consider the entire process, including the subsequent bioconversion step. For example, many pretreatment processes produce inhibitory compounds, including organic acids or furans, that slow down or prevent microbial growth (Zheng et al., 2014). The microorganisms used in the AD system are sensitive to a number of inhibitors, including free ammonia nitrogen at concentrations as low as 150 mg L^{-1} (Yenigün & Demirel, 2013). Despite differences in the overall processes, there are some pretreatment methods for ethanol production that can also be used for biogas production (Hendriks and Zeeman, 2009; Zheng et al., 2014). Optimal pretreatment methods and operational conditions for specific substrates have not yet been identified for either AD or bioethanol production. Alkaline pretreatment is considered the most promising pretreatment method for AD (Montgomery and Bochmann, 2014; Pavlostathis and Gossett, 1985; Yao et al., 2013). However, this method has a relatively slow reaction time, coupled with only a moderate increase in cellulose and hemicellulose digestibility (Di Girolamo et al., 2014).

Alkaline pretreatment using ammonia hydroxide (NH_4OH) has frequently been studied and employed to enhance the biomass digestibility for bioethanol production. However, there are concerns about the safe handling and operational control of ammonia. Another ammonia-based pretreatment method, ammonium carbonate (($NH_4)_2CO_3$), is lower in cost and safer than ammonium hydroxide pretreatment in industrial applications (Kim et al., 2014). Pretreatment using ammonium carbonate has also been found to improve the digestibility of rice straw for ethanol production (Kim et al., 2014). Therefore, substituting some part of the aqueous ammonia with ammonium carbonate may lower operating and pretreatment costs. Furthermore, the mixture of ammonium hydroxide, ammonium carbonate, and ammonium bicarbonate

(NH_4HCO_3) can be generated by integrating AD and the ammonia gas stripping process (Bai and Yeh, 1997; Yeh et al., 2005). Therefore, this proposed integrated system would cost less, since purchasing chemicals from outside the system is unnecessary.

2.4.2 Part 2 – Testing unit operations and optimizing the process

The purpose of this experiment was to develop a low-cost, efficient method that uses biogas with different CO_2 concentrations to strip ammonia from AD effluent. While air can be used to strip ammonia from AD effluent, when air is used, sulfuric acid (H_2SO_4) is required to fix the ammonia after stripping. When biogas is used for ammonia stripping, this eliminates the need for sulfuric acid since CO_2 is used for fixing the ammonia, instead. Eliminating the need for sulfuric acid reduces the cost for this process. Since biogas (composed mostly of CH_4) is combustible gas, it was substituted with nitrogen gas (N_2) for safety in the lab because they have similar solubility (0.023 g gas kg^{-1} water (20°C) for CH_4 and 0.018 g gas kg^{-1} water (20°C) for N_2) (EngineeringToolBox, 2017). In the experimental set-up (Figure 2.2), N_2 and CO_2 are mixed in the gas mixture bottle. The gas mixture flows into the water bath. The three-neck flask is filled with AD effluent. Ammonia is stripped by N_2 and CO_2 and the condenser tube cools the resulting vapor.
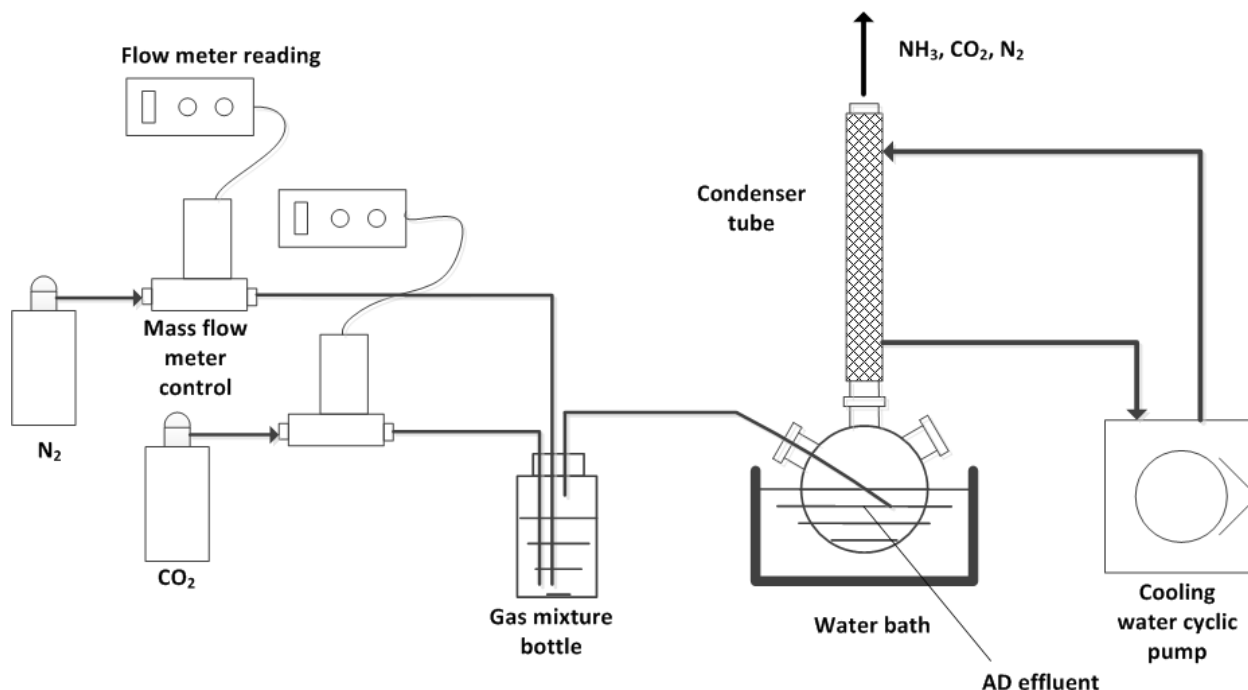
Figure 2.2: Experimental set-up for ammonia stripping using CO_2

2.4.2.1 Effect of temperature and CO_2 concentration on pH

In this study, the effects of temperature and CO_2 concentration on pH were investigated when a CO_2 and N_2 mixture was used as a stripping agent. High temperature and low CO_2 concentration increased the pH of the AD effluent, thus creating more favorable conditions for ammonia stripping. While each combination of CO_2 concentration and temperature resulted in an initial increase in pH, the extent and duration of this increase varied (Figure 2.3). With a 10% CO_2

concentration, pH values for each temperature remained above their initial levels (Figure 2.3A). However, pH decreased to its original value at 70°C and 80°C for 20% and 40% CO_2 concentrations (Figure 2.3B and C). These phenomena occurred because CO_2 is an acidic gas that tends to dissolve or combine with ammonia in the AD effluent. This tendency can reduce the pH of the AD effluent. In addition, high temperature can either prevent CO_2 from bonding to ammonia or can evaporate CO_2 from the AD effluent. These effects can increase the pH of the AD effluent.

2.4.2.2 Effect of temperature and CO_2 concentration on removal of ammonia from AD effluent
The next experiment involved measuring ammonia removal from the AD effluent over time during ammonia stripping. Usually, raw biogas consists of about 60% methane (CH_4) and 40% CO_2 (Sasse, 1988). These results demonstrated that it is not efficient to remove ammonia at 70°C with a 40% CO_2 concentration in the gas mixture used to strip ammonia (Figure 2.4A). Sixty percent ammonia removal was reached with a 20% CO_2 concentration, while over 80% ammonia removal was achieved with a 10% CO_2 concentration. When the temperature was increased to 80°C and 90°C, ammonia removal increased to over 80% (Figure 2.4B and Figure 2.4C). This indicates that high temperature can prevent the bonding of CO_2 and ammonia, which generates ammonium components and, thus, hinders ammonia stripping.

Results obtained showed that it is feasible to use biogas to strip ammonia from AD effluent. Since biogas is already warmer than ambient temperature when it flows out of an anaerobic digester, especially a thermophilic digester (40-60°C), less heating is required for biogas stripping than for air stripping. Furthermore, heat supplied by the exhaust from combined heat and power from anaerobic digesters can significantly reduce the cost of ammonia recovery.
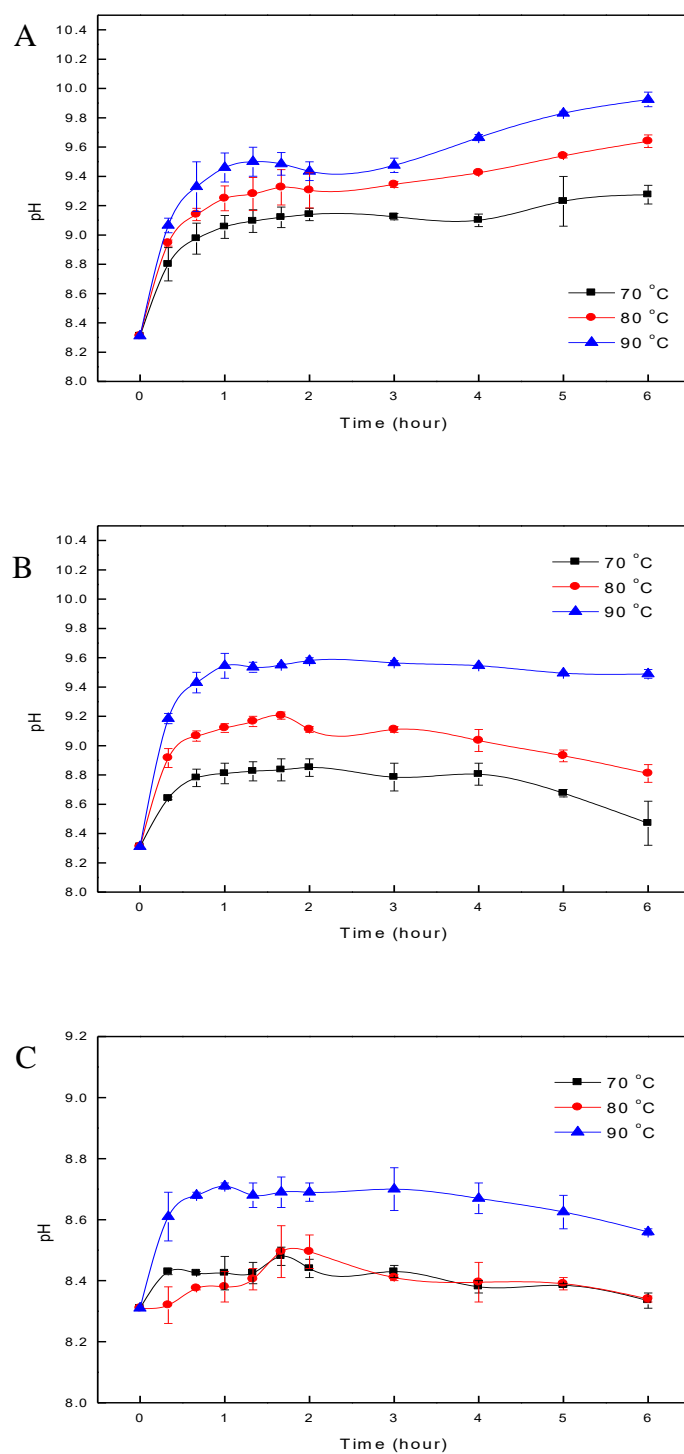
Figure 2.3: pH change with time during ammonia stripping by 10% CO_2 (A), 20% CO_2 (B), and 40% CO_2 (C)
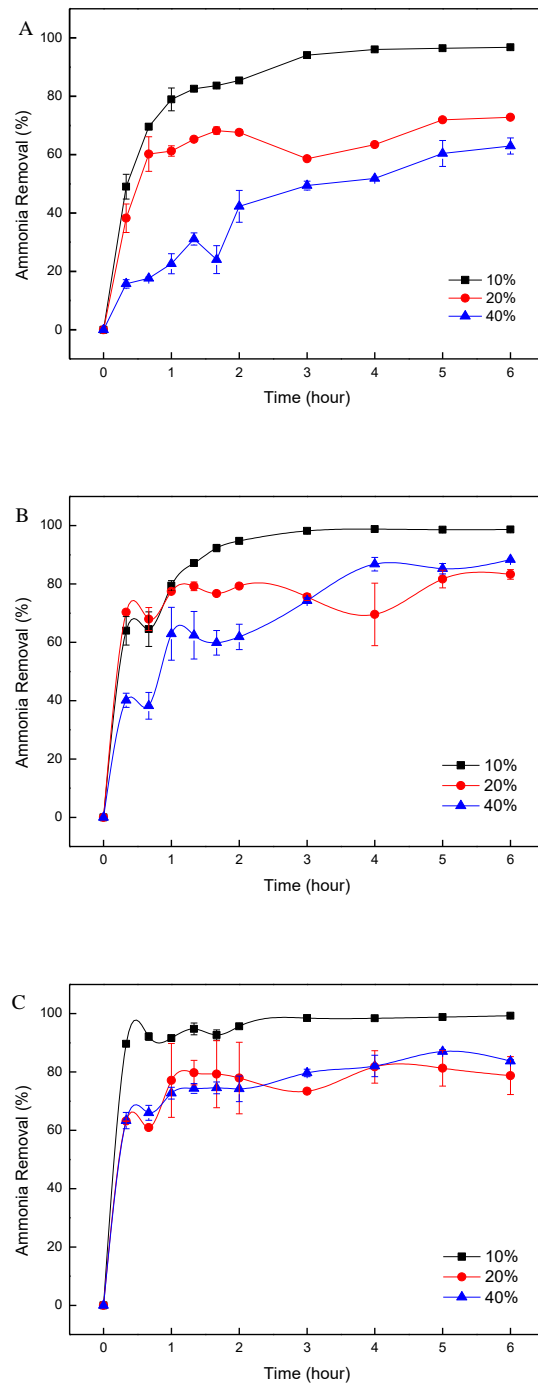
Figure 2.4: Change in ammonia removal using biogas with different CO_2 concentrations over time at 70°C (A), 80°C (B), and 90°C (C)

2.4.2.3 Ammonia stripping and absorption using CO_2

The focus of this study was to test the ability to absorb ammonia in the gas phase using CO_2. The experimental set-up is shown in Figure 2.5. To ensure stable ammonia and water vapor supply to the ammonia absorber, only N_2 was used to strip ammonia from the AD effluent. CO_2 was sent directly to the ammonia absorber to react with ammonia and water vapor. The ammonia that was not absorbed by CO_2 was measured using sulfuric acid (H_2SO_4).
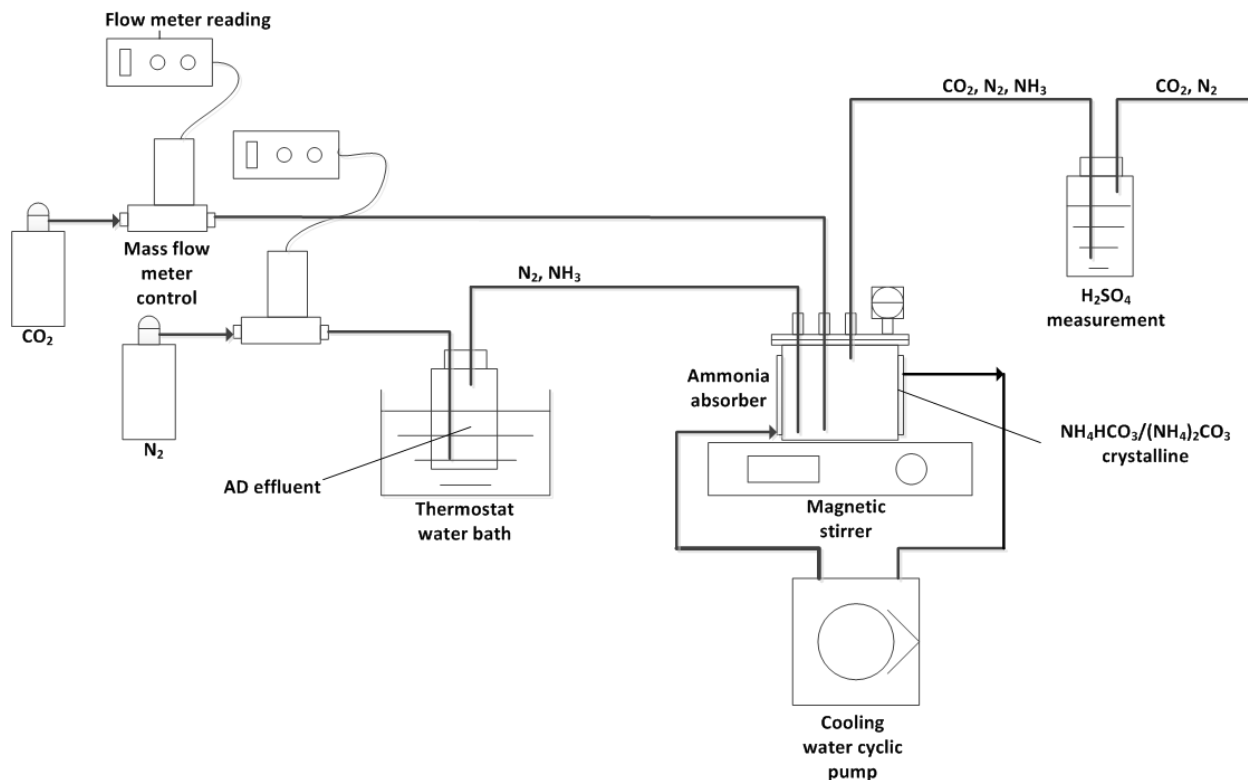
Figure 2.5: Experimental set-up for ammonia stripping and absorption using CO_2

2.4.2.4 Effects of temperature, gas flow rate, and agitation speed on ammonia absorption for crystalline ammonium

The general reactions of NH_3 and CO_2 are as follows:

$$NH_3 + CO_2 + H_2O \rightleftharpoons NH_4HCO_3 \tag{R-1}$$
$$NH_3 + CO_2 \rightleftharpoons NH_2COOH \tag{R-2}$$
$$NH_2COOH + NH_3 \rightleftharpoons NH_2COONH_4 \tag{R-3}$$
$$NH_2COOH + H_2O \rightleftharpoons NH_4HCO_3 + NH_3 \tag{R-4}$$
$$NH_4HCO_3 + NH_3 \rightleftharpoons (NH_4)_2CO_3 \tag{R-5}$$
$$NH_2COONH_4 + CO_2 + H_2O \rightleftharpoons 2NH_4HCO_3 \tag{R-6}$$
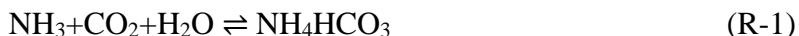
These reaction equations show that the products include three types of crystalline ammonium: ammonium bicarbonate (NH_4HCO_3), ammonium carbonate (($NH_4)_2CO_3$), and ammonium carbamate (NH_2COONH_4). The effect of absorption temperature on each type of crystalline

26

ammonium was clear and significant. Lower temperature led to the increased generation of crystalline ammonium (Table 2.1).

Table 2.1: Effect of temperature on ammonia absorption for crystalline ammonium

Temperature (°C)	$(NH_4)_2CO_3$ (g)	NH_4HCO_3 (g)	Other (g)	Total (g)
5	0.3128	0.5069	0.5003	1.3200
10	0.2568	0.0624	0.3309	0.6501
15	0.0758	0	0.0442	0.1200

Although the total amount of crystalline ammonium generated increased significantly with an increase in gas flow rate, $(NH_4)_2CO_3$ did not show such an increase (Table 2.2). The possible reason is that CO_2 was not sufficiently supplied at the low gas flow rate of 0.25 L min^{-1}. As shown in equation R-5, most crystalline ammonium was transformed into $(NH_4)_2CO_3$.

Table 2.2: Effect of gas flow rate on ammonia absorption for crystalline ammonium

Gas flow rate (L min^{-1})	$(NH_4)_2CO_3$ (g)	NH_4HCO_3 (g)	Other (g)	Total (g)
0.25	0.1642	0.0675	0.0533	0.2850
0.5	0.1027	0.3381	0.6291	1.0699
0.75	0.1445	0.5945	0.7660	1.5050

The total crystalline ammonium was increased with an increase in agitation speed (Table 2.3), but these results were not significantly different. One possible reason could be that the agitator used was designed for the liquid phase instead of the gas phase. Therefore, a good gas agitator should be designed to enhance mass transfer between CO_2, NH_3, and H_2O in future research.

Table 2.3: Effect of agitation speed on ammonia absorption for crystalline ammonium

Agitation speed (rpm)	$(NH_4)_2CO_3$ (g)	NH_4HCO_3 (g)	Other (g)	Total (g)
0	0.0552	0.4543	0.6406	1.1501
300	0.1786	0.4898	0.5716	1.2400
600	0.1894	0.4675	0.6582	1.3151

The experiments were conducted in a reactor located in a ventilating hood, under the conditions described above. Crystalline $NH_4HCO_3/(NH_4)_2CO_3$ mixture was observed on the reactor wall (Figure 2.6), suggesting that ammonia can be concentrated in this way. Theoretically, NH_4HCO_3 crystals can reach 48.6% (w/w) ammonia concentration via CO_2 removal. Therefore, it is feasible to obtain the concentration between 0-28% (w/w) $NH_4OH/(NH_4)_2CO_3$ needed for use in lignocellulosic pretreatment.
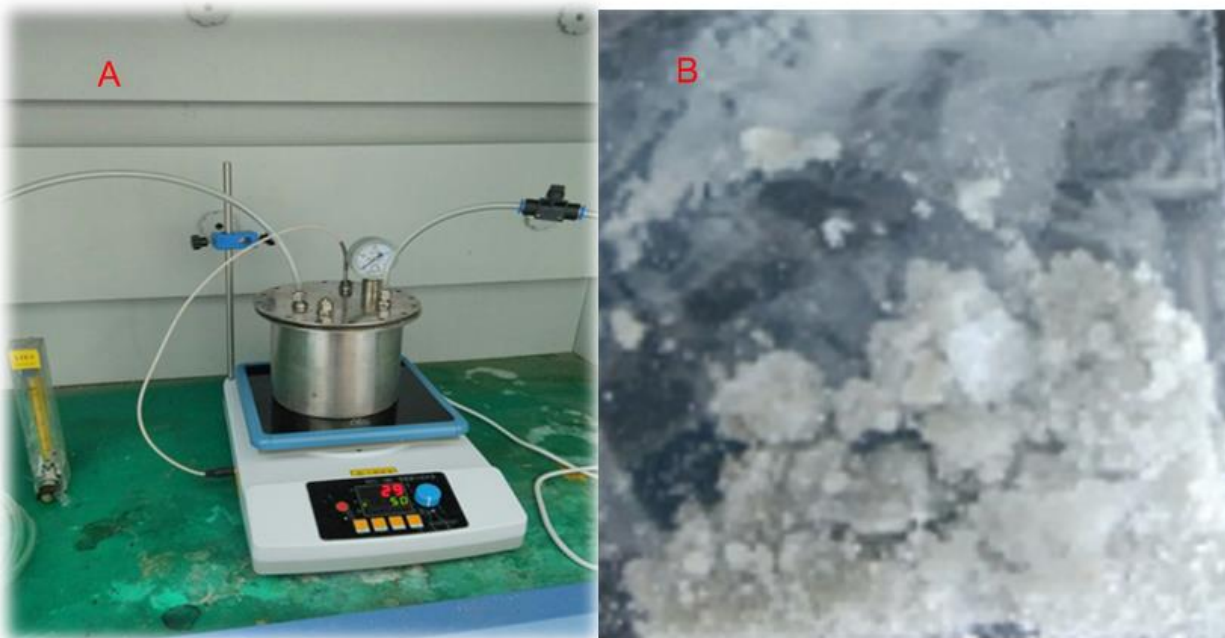
Figure 2.6: Gaseous CO$_2$, NH$_3$, and water vapor reaction to generate crystalline NH$_4$HCO$_3$/(NH$_4$)$_2$CO$_3$ mixture (A) reactor; (B) crystalline NH$_4$HCO$_3$/(NH$_4$)$_2$CO$_3$ mixture

2.4.3 Part 3 – Evaluation of pretreatment

2.4.3.1 Ammonia pretreatment time

Pretreatment time is an important factor to consider when evaluating a pretreatment process. In the ammonia pretreatment process, the pH value reflects ammonia consumption and indicates that the pretreatment is effective and complete. In our experiments, pH value varied with ammonia concentration, temperature and retention time (Figure 2.7). The initial pH value was 10.5 to 10.9. With the processing of ammonia pretreatment, the pH values declined gradually and finally stabilized after 12 hours for the 0.70% and 1.05% ammonia concentrations. For the 0.35% ammonia concentration, the pretreatment at 105°C achieved stability rapidly compared with the pretreatment at 55°C. The changes in pH at 105°C from 12 hours to 48 hours for the 0.35%, 0.70%, and 10.05% ammonia concentrations were 7.4-8.1, 9.3-9.4, and 9.6-9.8, respectively. To prevent acidification during the start-up period of fermentation, the wheat straw pretreated for 24 hours can be used for AD directly, without drying or washing. The ability to use the pretreated biomass directly decreases cost and improves the operating process during pretreatment. For this reason, pretreatment times of 24 hours were used for further investigation of biogas production and yield.
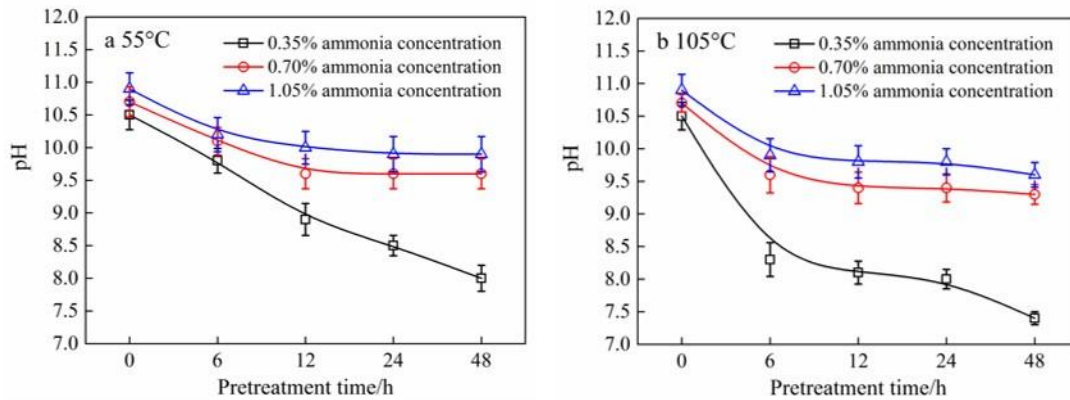
Figure 2.7: Change in pH during pretreatment of wheat straw at 55°C (A) and 105°C (B) with three different concentrations of ammonia (0.35%, 0.70%, and 1.05%)

2.4.3.2 Accumulated biogas production

In a batch experiment, the accumulated biogas production of ammonia-pretreated wheat straw was compared with that of untreated wheat straw (Figure 2.8). Biogas production stabilized after 20 days. Results indicated that the anaerobic fermentation period was shortened by ammonia pretreatment. Moreover, biogas production was significantly affected by ammonia concentration and pretreatment temperature. The biogas production of the control (untreated wheat straw) was only 1,223 mL. After ammonia pretreatment, the biogas production was obviously improved.

After pretreatment at 55°C, biogas production at 0.35% and 1.05% ammonia concentrations were similar (Figure 2.8a). However, the biogas production at 0.70% ammonia concentration was 1,475 mL, which was 9.4% and 9.0% higher than biogas production at ammonia concentrations of 0.35% and 1.05%, respectively. A similar phenomenon occurred during AD of rice straw with ammonia pretreatment at a 4% ammonia concentration (Yuan et al., 2014). As for pretreatment at 105°C (Figure 2.8b), the trend of biogas production was consistent with pretreatment at 55°C. Moreover, for 0.70% ammonia concentration, the biogas production at 105°C was 9.5% and 31.9% higher than that at 55°C and of the control, respectively. This indicated that an increase in pretreatment temperature can strengthen ammonia pretreatment to improve biogas production during AD. The above results show that wheat straw pretreated with 0.70% ammonia concentration at 105°C resulted in the highest anaerobic digestibility.
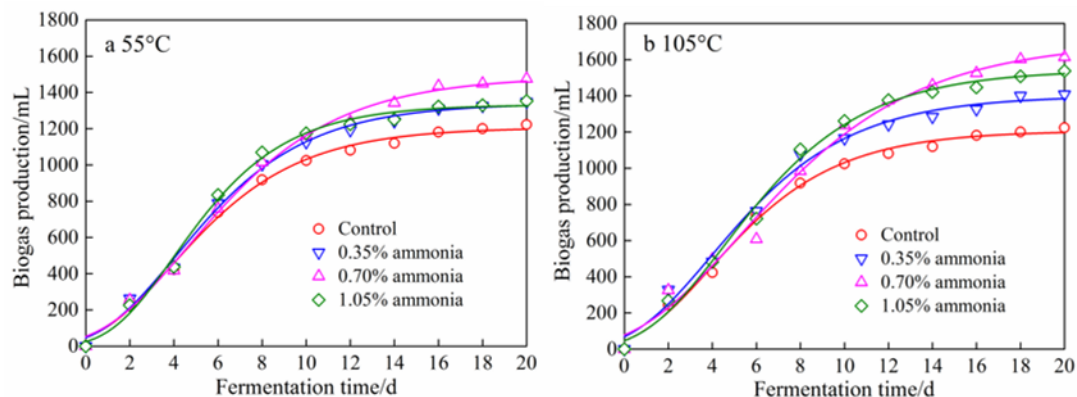
Figure 2.8: Biogas production from wheat straw pretreated at 55°C (A) and 105°C (B) with three different concentrations of ammonia (0.35%, 0.70%, and 1.05%)

2.4.3.3 Methane content and methane yield

Methane yield was calculated based on accumulated biogas production and average methane content of biogas, and was expressed as mL g^{-1} of VS in the digestate. None of the pretreatments had a methane content significantly different from the control's (Figure 2.9). The highest methane content was 61.51% for 105°C and 0.70% ammonia concentration. The methane yield of the control was 249.2 mL g^{-1} VS. After ammonia pretreatment, methane yield increased significantly. For pretreatment at both temperatures, the methane yield from 0.70% ammonia concentration was higher than that from 0.35% and 1.05%. The 0.70% ammonia concentration yielded more methane than 1.05% ammonia concentration because a high concentration of ammonia can lead to ammonia inhibition during AD (Rajagopal et al., 2013). Moreover, an increase in temperature can improve methane yield through the combination of heat and ammonia. A maximum biogas yield of 353.8 mL g^{-1} VS was obtained at 0.70% ammonia concentration and 105°C, which was 29.6% higher than of the yield from untreated wheat straw.
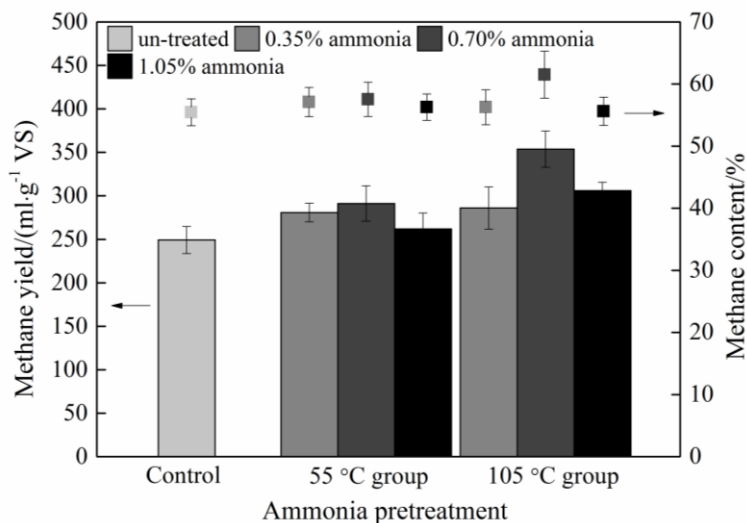
Figure 2.9: Methane content and methane yield after ammonia pretreatment at two temperatures (55°C and 105°C) at three different concentrations of ammonia (0.35%, 0.70%, and 1.05%)

2.4.3.4 Changes to lignocellulose during ammonia pretreatment and AD

To clarify the mechanism of lignocellulose degradation during ammonia pretreatment, the VS, cellulose, and hemicellulose contents were determined, and recovery rates (percent converted to biogas) of these components were calculated (Table 2.4). The VS, cellulose, and hemicellulose contents of pretreated wheat straw were greater than those of untreated wheat straw. This was due to the removal of soluble components, lignin, and ash in the pretreatment (Kim et al., 2008). Pretreatment at an ammonia concentration of 0.70% resulted in a relatively high VS and a low cellulose content at 55°C and 105°C. Hemicellulose content increased at 55°C with increasing ammonia concentration, but decreased at 105°C with increasing of ammonia concentration. This difference was the result of interaction between temperature and ammonia concentration. In terms of recovery rate, the VS, cellulose, and hemicellulose recovery rates were remarkably high, with 97.31% and 93.40% of cellulose recovered for 0.70% ammonia concentration at 55°C and 105°C, respectively. The hemicellulose recovery rate at 105°C was slightly lower than at 55°C. The hemicellulose recovery rate was 76.35% at conditions of 105°C and 0.70% ammonia concentration.

Table 2.4: Changes in volatile solids (VS), cellulose, and hemicellulose after ammonia pretreatment

Pretreatment conditions	VS (%)	Cellulose (%)	Hemicellulose (%)	Recovery rate (%)		
				VS	Cellulose	Hemicellulose
Untreated	90.75	36.21	21.95	-	-	-
0.35%, 55°C	91.43	44.31	22.78	81.61	90.68	87.40
0.70%, 55°C	97.14	40.54	25.84	86.06	97.31	92.54
1.05%, 55°C	96.88	43.82	27.57	84.76	95.92	92.83
0.35%, 105°C	92.31	43.74	28.00	79.14	92.50	88.41
0.70%, 105°C	93.55	43.05	27.22	74.22	93.40	76.35
1.05%, 105°C	93.10	46.97	25.40	74.69	89.26	76.45

In addition, the total lignin, AIL, ASL, and ash removal were calculated (Figure 2.10). After pretreatment, AIL and ASL were removed due to the delignification effect of the ammonia (Wyman et al., 2005). Lignin and ash removal during pretreatment increased with increasing ammonia concentration and temperature (Figure 2.10). Acid-soluble lignin removal was higher than AIL removal because ASL content was low and ASL is more easily degraded than AIL. The pretreatment at 105°C and 0.70% ammonia concentration achieved the greatest total lignin removal of 33.3%. Therefore, pretreatment at 105°C and 0.70% ammonia concentration resulted in the greatest lignin removal and biogas yield. Li et al. (2015) also found that lignin removal was positively related to biogas yield. The ammonia pretreatment used in this study also showed a strong ability to remove ash, though there was no significant difference between pretreatment temperatures or ammonia concentrations.
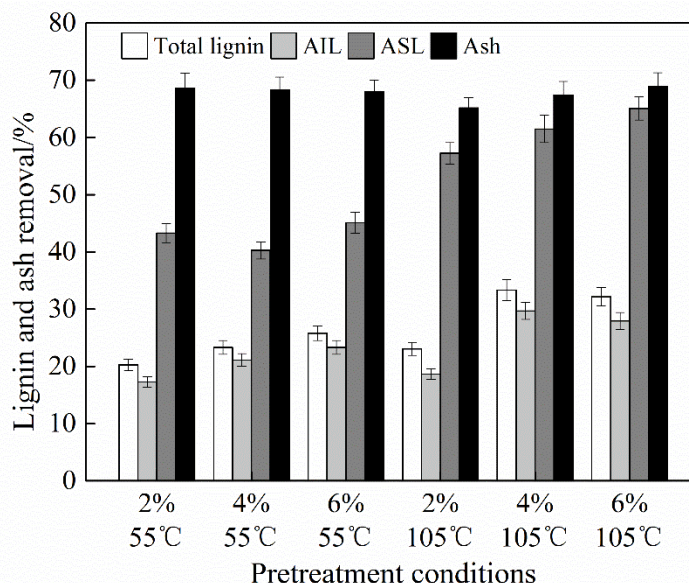
Figure 2.10: Lignin, ASL, AIL, and ash removal during ammonia pretreatment

Volatile solids, cellulose, and hemicellulose degradation rates after AD were also measured (Table 2.5). The VS degradation rate was greater than the control only for the 0.70% and 1.05% ammonia concentrations at 105°C. This was because most VS in wheat straw were converted to volatile fatty acids. At 55°C, the cellulose degradation rate was greatest at 0.70% ammonia concentration, whereas at 105°C, the highest rate was obtained at 1.05% ammonia concentration. This indicated that there were synergistic effects between temperature and ammonia concentration. The hemicellulose degradation rate showed a similar tendency. The rate of hemicellulose degradation was greater than the cellulose degradation rate. Therefore, most cellulose and hemicellulose were successfully recovered during ammonia pretreatment, and utilized for biogas production.

Table 2.5: Changes in volatile solids, cellulose, and hemicellulose after anaerobic digestion

Pretreatment conditions	VS (%)	Cellulose (%)	Hemicellulose (%)	Degradation rate (%)		
				VS	Cellulose	Hemicellulose
Untreated	78.50	19.37	10.83	75.50	75.61	79.35
0.35%, 55°C	80.39	19.83	10.75	69.59	71.98	77.10
0.70%, 55°C	78.57	17.31	9.54	70.09	75.70	79.77
1.05%, 55°C	85.37	19.01	9.81	64.29	72.15	78.52
0.35%, 105°C	82.22	17.76	8.12	70.22	76.32	84.27
0.70%, 105°C	81.25	18.26	5.67	76.91	78.97	91.70
1.05%, 105°C	76.09	15.49	4.58	78.60	82.21	93.73

2.4.3.5 Overall mass balance

The changes in composition of 100 g dry wheat straw during pretreatment and AD are shown in Figure 2.11. The dilute ammonia pretreatment has many advantages over conventional acid and alkali pretreatment. Lignin in the wheat straw was selectively removed, decreasing its content by 33.0%. Most of the cellulose and hemicellulose was recovered from the wheat straw. Thus, the

amounts of glucose and xylose in the liquid after pretreatment were very low, only 2.7 g and 4.3 g, respectively. The treated wheat straw was rich in carbohydrates, including 47.0% cellulose and 25.4% hemicellulose. These results were similar to those of a study involving the pretreatment of rice straw with 15% ammonia at 130°C (48.0% cellulose and 23.6% hemicellulose) (Kim et al., 2011). The wheat straw pretreated using ammonia was further converted to biogas via AD. 47.87 g (39.3 L) of biogas was obtained per 100 g dry wheat straw. It can be assumed that the 0.70% ammonia pretreatment at 105°C greatly increased the porous surface and accessible surface area, which made cellulose and hemicellulose more accessible to anaerobic microorganisms. After AD, most cellulose and hemicellulose were degraded. This indicated that low concentration ammonia pretreatment of wheat straw is an effective strategy to enhance the bioenergy conversion of agricultural by-products such as wheat straw and rice straw. Moreover, the low concentration ammonia pretreatment did not require the use of large amounts of water to wash the pretreated straw or chemicals to adjust the initial pH for AD, which keeps energy requirements and costs low. For these reasons, commercialization of this pretreatment is promising.
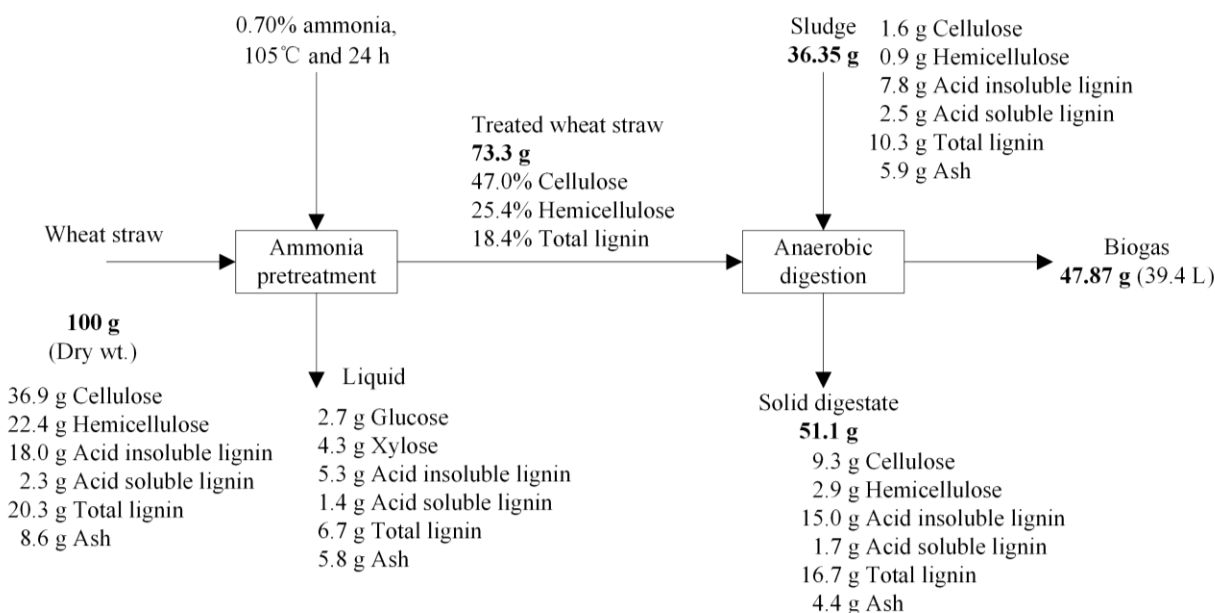
Figure 2.11: Mass balance of overall process including ammonia pretreatment and anaerobic digestion

2.4.3.6 Physical analysis of ammonia-pretreated wheat straw

To reveal the structural changes during low concentration ammonia pretreatment, Fourier transform infrared (FTIR) spectroscopy of untreated and ammonia-treated wheat straw was compared for the wavelength region from 750 to 4,000 cm^{-1} (Table 2.6). The attributions of FTIR absorption were made according to previous literature (Gao et al., 2012). The untreated wheat straw had a strong hydrogen bond (O-H) stretching at 3,354.21 cm^{-1} and C-H stretching vibrations at 2,920.22/2,850.79 cm^{-1}, whereas the same characteristics in the ammonia-treated sample were noted at 3,352.28 and 2,924.08/2,854.64 cm^{-1}, respectively. The peak at 1,734.00 cm^{-1} was ester linkage absorbance, which decreased slightly after treatment. The absorption

bands at 1,602.84 cm^{-1} and 1,506.40 cm^{-1} of untreated wheat straw corresponded to the aromatic skeleton of lignin. They decreased after ammonia pretreatment. Furthermore, the absorbance at 1,462.04/1,421.53 cm^{-1} and 1,454.32/1,423.46 cm^{-1} were attributed to aromatic ring vibrations of lignin. The absorbance at 1,371.38/1,367.53 cm^{-1} became weaker after ammonia pretreatment, indicating the C-H bonds in cellulose and hemicellulose were changed. Absorbance at 1,319.31 cm^{-1} was due to C-O vibration of S-rings, and was strengthened in ammonia-pretreated wheat straw. The intensity of absorption at 1,236.37 cm^{-1} in untreated wheat straw was stronger than in pretreated wheat straw, suggesting a high guaiacyl content in raw wheat straw (Gupta and Lee, 2010). It disappeared after ammonia pretreatment, indicating the removal of guaiacyl lignin. The peaks at 1,201.65 and 1,159.21 cm^{-1} demonstrated the stretching and vibration of C-O-C in cellulose and hemicellulose. For untreated and ammonia-treated samples, the peaks at 1,053.12/898.82 cm^{-1} were characteristic of β-glycosidic linkages between the glucose units, such as C-O stretch and C-H deformation.

Table 2.6: FTIR absorption peaks of untreated and ammonia-treated wheat straw

λ (cm^{-1}) of peaks		Attribution of FTIR absorption
Untreated	Ammonia-treated	
3,354.21	3,352.28	O-H stretching vibration of polymer
2,920.22/2,850.79	2,924.08/2,854.64	C-H stretching vibration (CH$_3$, CH$_2$)
1,732.07	1,734.00	C=O stretching vibration
1,602.84	1,604.77	C=O stretching and aromatic vibrations
1,506.40	1,506.40	Aromatic skeletal vibrations
1,462.04/1,421.53	1,454.32/1,423.46	Aromatic ring vibrations of lignin
1,371.38	1,367.53	C-H deformation of cellulose and hemicellulose
1,317.38	1,319.31	C-O vibration of S ring
1,236.37	none	Methoxyl, C-C and C=O stretching vibrations
1,201.65	1,201.65	Symmetric stretching C-O-C glycoside
1,159.21	1,159.21	C-O-C vibrations in cellulose and hemicellulose
1,053.12	1,053.12	C-O stretch in cellulose
898.82	898.82	C-H deformation in cellulose

Physical characteristics of wheat straw pretreated at 55°C and 105°C with 0.70% ammonia concentration were also analyzed using Brunauer–Emmett–Teller (BET). The isotherm plot showed that adsorption was not limited at high relative pressure (Figure 2.12), an attribute of type H3 hysteresis loop, which indicated that the pore structure was very irregular. Moreover, there were more micropores in pretreated wheat straw. The surface area of untreated wheat straw was 2.41 m² g^{-1}. Ammonia pretreatment at 55°C and 105°C increased the surface area relative to the untreated wheat straw (3.42 m^2 g^{-1} and 4.14 m^2 g^{-1}, respectively). The increase in pretreatment temperature led to further increases in BET surface area. The increase in internal surface area could be related to removal of the hemicellulose and lignin. Imman et al. (2014) reported the diminished shielding effects of hemicellulose and lignin and the formation of additional pores after acid and alkaline pretreatment. In this study, both cumulative and maximum pore volumes were increased after ammonia pretreatment (Table 2.7). Based on the previous biogas production and lignocellulose compositions results, this study indicated that the increase of cellulose accessibility is more important than lignin removal during pretreatment

(Rollin et al., 2011). Therefore, the overall increase in the accessibility of cellulose and hemicellulose to anaerobic microorganisms resulted in greater biogas yield from AD.
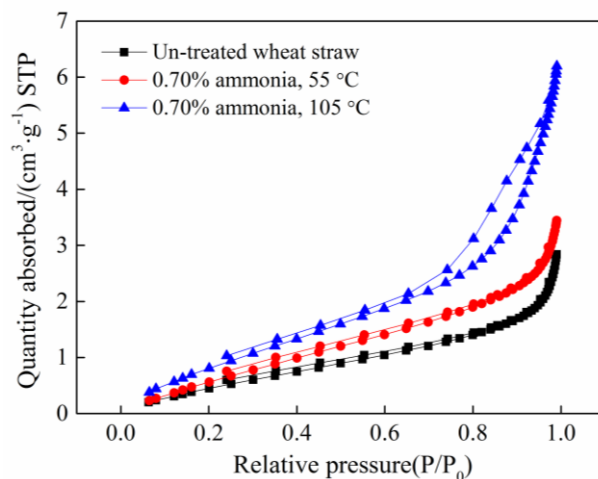
Figure 2.12: The N$_2$ adsorption isotherms of untreated and ammonia-treated wheat straw

Table 2.7: BET surface area and pore characteristics of wheat straw

	Untreated	**0.70%, 55°C**	**0.70%, 105°C**
BET surface area (m² g^{-1})	2.41	3.42	4.14
Cumulative pore volume (cm³ g^{-1})	0.004386	0.005531	0.009778
Maximum pore volume (cm³ g^{-1})	0.000598	0.000734	0.001079
Average pore diameter (nm)	7.79	6.74	8.74

2.4.4 Part 4 – Economic assessment

Based on the pretreatment methods described in the previous sections, a techno-economic analysis was conducted to evaluate the WSU TAD technology for wheat straw. Washington State is one of the major wheat producing states in the U.S. In 2016, Washington wheat growers harvested 2.2 million acres of wheat which had an average yield of 71.5 bushels per acre. Total wheat production in Washington for 2016 was 157,290 million bushels (WGC, 2016). Wheat straw could provide sufficient potential feedstock to build large-scale AD plants.

In this study, we compared the TAD technology with current mesophilic anaerobic digestion (MAD) technology to demonstrate the advantages of the thermophilic technology. Anaerobic digestion occurring in mesophilic (25-40°C) and thermophilic (45-60°C) temperature ranges has different characteristics. Thermophilic fermentation is characterized by rapid digestion, high gas yield, and short retention time. This process is frequently used for disposal of animal waste because it achieves better levels of disinfection. Fermentation in the mesophilic range has the advantage of lower energy consumption as the decomposition of the feedstock is slower. Digestion in the mesophilic temperature range also has the advantage of greater stability of waste in the digester, due to a slower death rate for specific microbes. Mesophilic AD has been widely adopted throughout the world. Thermophilic AD has been the focus of these research efforts

because, as detailed below, it has the advantage of reducing the reactor volume and, thus, reducing capital investment.

2.4.4.1 Design parameters and assumptions

The process simulation software ASPEN Plus (Aspen Technology, Inc., Bedford, Massachusetts) was used in this study. Several conditions were assumed: (1) the anaerobic digesters (TAD and MAD) were the same size (10,000 m^3) and the price of each was $640,000; (2) the feedstock price was $0.061 per kg; (3) the solid digestate fertilizer had a value of $0.046 per kg. The design parameters were based on the literature (Dongyan et al., 2014) and the above experiments. The dry wheat straw TS were diluted to 15% and sent to the digesters. The solid and liquid digestates were separated after discharge from the digesters. The solid digestate had TS of 50% and was used as organic fertilizer. The liquid digestate, also called effluent, was recycled back to the digesters as a dilution agent to reduce water consumption. Design parameters and results for WSU TAD technology and the current MAD technology are shown in Table 2.8.

Table 2.8: Design parameters and results

Design Parameters	MAD	WSU-TAD
Handling capacity (ton day^{-1})	344	602
Biogas yield (m^3 ton^{-1} TS)	370	486
Temperature (°C)	35	55
Retention time (days)	35	20
Results		
Biomethane productivity (m^3 day^{-1})	10,330	25,310
Solid digestate (ton day^{-1})	76	118

2.4.4.2 Block flow diagram

A block flow diagram for the WSU TAD technology, including two separate processes, is shown in Figure 2.13. The first process, pretreatment, uses high temperature dilute ammonia to treat wheat straw. The second process is TAD that converts pretreated wheat straw to produce biomethane at 55°C.
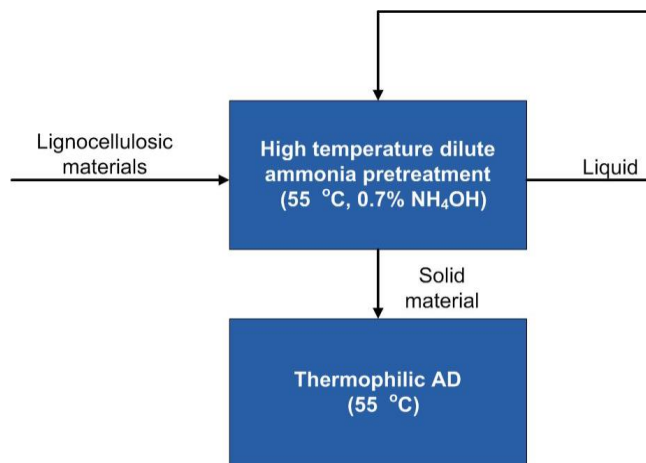
Figure 2.13: Block flow diagram for WSU TAD technology

2.4.4.3 Techno-economic analysis

Biogas, which has a methane content of approximately 60%, can be upgraded to biogas compressed natural gas (CNG), which has a methane content greater than 96% and is stored at a high pressure. Biogas CNG can be used in place of gasoline, diesel fuel, and liquefied petroleum gas. For this study, the biogas CNG price was set at $0.33 per m^3 (see NREL methodology for estimating minimum selling price for profitability, below). Since the WSU TAD technology had a larger handling capacity than MAD technology, the total capital cost, total operating cost, total raw materials cost, and total utility cost were correspondingly increased (Table 2.9). However, because of its larger handling capacity, the WSU TAD technology can bring greater revenues from products including biogas CNG and organic fertilizer. The payout period (time required to recover cost) was 9.19 years for WSU TAD technology. In contrast, it was not possible to recover the cost of MAD technology with a biogas CNG price of $0.33 per m^3. The profitability index (PI) also provides this information. A PI > 1 indicates a profitable project while a PI < 1 indicates an unprofitable project.

Table 2.9: Techno-economic analysis of WSU TAD technology and MAD technology

Biogas CNG ($0.33 m^{-3})	Unit	MAD	WSU-TAD
Total Project Capital Cost	$	8,740,742.67	10,012,881.71
Total Operating Cost	$ year^{-1}	1,647,598.94	2,840,996.17
Total Raw Materials Cost	$ year^{-1}	1,056,297.78	1,848,521.12
Total Utilities Cost	$ year^{-1}	235,256.79	538,430.89
Total Product Sales	$ year^{-1}	2,308,481.61	4,495,978.95
Desired Rate of Return	% year^{-1}	10	10
Payout Period	year	-	9.19
PI (Profitability Index)		0.79	1.03

The National Renewable Energy Laboratory's (NREL) methodology was used to estimate the minimum selling price of biogas CNG for profitability of the WSU TAD technology and the MAD technology (Humbird et al., 2011). Once the total capital investment, variable operating costs, and fixed operating costs had been determined, a discounted cash flow rate of return analysis was used to determine the minimum selling price per cubic meter of biogas CNG produced. This analysis was completed by iterating the selling cost of biogas CNG until the net present value of the project was zero, and requires that the discount rate, depreciation method, income tax rates, plant life, and construction start-up duration be specified. Because this hypothetical plant was equity-financed, some assumptions about the loan terms are also required. These parameters and assumptions were obtained from the NREL report (Humbird et al., 2011). The minimum selling price of biogas CNG required for the WSU TAD technology to break even was $0.33 per m^3, while the MAD technology required a minimum selling price of $0.54 per m^3 to break even.

The factors affecting the manufacturing cost of biogas CNG for the WSU TAD technology are shown in Table 2.10. This table provides information on cost distribution; that is, which costs affected the minimum selling price for biogas CNG. Organic fertilizer had a manufacturing cost of -9.4 cents per m^3 of biomethane, indicating that it provided value, rather than cost. Thus, developing high-value organic fertilizer can enhance profitability of the AD process.

Furthermore, feedstock purchase, transportation, storage and handling were very important factors affecting the manufacturing cost of biogas CNG. In the WSU TAD technology, ammonia can be recycled and reused as a pretreatment agent. This reduced the manufacturing cost of biogas CNG, but not by a significant amount.

Table 2.10: Factors affecting manufacturing cost of biogas CNG for WSU TAD technology

	Manufacturing Costs (cents m^{-3} biomethane)	Manufacturing Costs ($ yr^{-1})
Feedstock + Handling	21.0	$1,800,000
Ammonia	3.1	$300,000
Electricity and process utility	6.1	$500,000
Waste Disposal	0.0	$0
Organic Fertilizer	-9.4	-$800,000
Fixed Costs	3.3	$300,000
Capital Depreciation	2.3	$200,000
Average Income Tax	0.3	$0
Average Return on Investment	5.9	$500,000

2.4.4.4 Sensitivity analysis

To understand how parameter changes can affect the minimum selling price of biogas CNG, a sensitivity analysis was conducted using the techno-economic model described above. In the base case (in which biogas CNG was $0.33 per m^3), the biogas CNG production rate was 8.79 million m^3 per year, the feedstock cost was $61.35 per ton, the organic fertilizer cost was $46.01 per ton, the fixed capital investment was $5.27 million, and the fixed operating cost was $290,000 per year. These parameters were increased and decreased by 20% to analyze their effects on minimum selling price of biogas CNG. The results showed that biogas CNG production rate is the most sensitive parameter (as indicated by the width of the bars in Figure 2.14). If the biogas CNG production rate could be increased by 20%, the minimum selling price of biogas CNG would be reduced to $0.27 per m^3. As with the effects on the manufacturing cost of biogas CNG (Table 2.10), the cost of feedstock and the selling price of organic fertilizer also had a strong effect on the minimum selling price of biogas CNG.

Based on the sensitivity analysis of the minimum selling price of biogas CNG, we recommend that research be aimed at improving profitability of AD projects. If biogas CNG production rate could be increased by 20% (10.55 million m^3 per year) and the organic fertilizer selling price would increase by 320% ($147.23 per ton), the minimum selling price of biogas CNG would be reduced to $0.10 per m^3. The market price of natural gas is about $3.00/MMBtu ($0.11 per m^3 [1 m^3 natural gas = 0.035315 MMBtu]) (EIA, 2017). Based on a search of the Amazon website, organic fertilizer prices are at least $2.31 per lb ($5,100 per ton) (Amazon, 2017), though this price may include other investments. However, this indicates that it may be possible to develop high-value organic fertilizer to offset the low price of natural gas. Further improvement to the biogas CNG production rate is also a major focus for ongoing research.
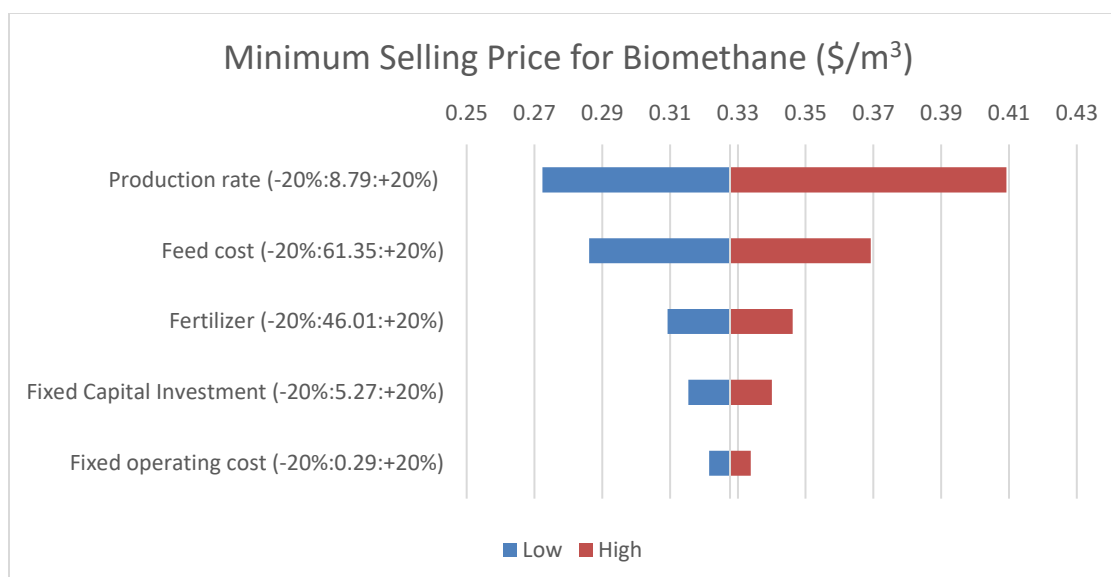
Figure 2.14: Sensitivity analysis of minimum selling price of biogas CNG for WSU TAD technology

2.5 Conclusions and recommendations

This project has explored the major unit operations for a novel ammonia recycling technology. The results show raw biogas (40% CO_2 concentration) can be efficiently used to strip ammonia from AD effluent at over 80°C and 80% ammonia removal can be achieved. Purified biogas (10% CO_2 concentration) showed the same capacity to strip ammonia from AD effluent at 70°C as air stripping. Ammonia can be absorbed by CO_2 and water vapor on the reactor wall to generate crystalline ammonium. The effects of temperature, gas flow rate, and agitation speed on crystalline ammonium formation were investigated. The results indicate that low temperature, high gas flow rate, and high agitation speed increase the total weight of crystalline ammonium formed.

This study demonstrated that most cellulose and hemicellulose in wheat straw can be retained for biogas production after ammonia pretreatment. Low concentration ammonia pretreatment was an effective way to enhance lignin removal and surface area of pretreated wheat straw for improving biogas production and methane yield. Moreover, the increased accessibility of cellulose was more important than lignin removal during pretreatment. Anaerobic digestion of wheat straw pretreated by 0.70% ammonia concentration at 105°C resulted in the highest methane yield (353.8 mL g^{-1} VS). This study also showed that low concentration ammonia pretreatment and TAD are promising processes for the conversion of biomass to bioenergy, achieving effective utilization of the organic carbon in biomass.

A techno-economic analysis was used to evaluate high temperature ammonia pretreatment and thermophilic digestion. The WSU TAD technology was compared with current MAD technology in terms of profitability and minimum selling price of biogas CNG. The results show the WSU TAD technology can increase profitability and reduce the minimum selling price of biogas CNG, when compared to MAD. According to the analysis of manufacturing cost and sensitivity,

feedstock cost is one of the major issues to affect the economic viability of an AD project. Organic fertilizer has great potential to improve the profitability of an AD project and reduce the minimum selling price of biogas CNG. Further research to enhance biogas production rate is also crucial for the development of a profitable AD project.

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3. Wet Oxidation for Wastewater Color Removal

Alex Dunsmoor and Manuel Garcia-Pérez

3.1 Background

As part of their manure management plans, dairy producers often apply manure and parlor washwater to fields. Aerial irrigation is a growing trend for field application of wastewater, as it is easier and more economical than conventional spreading methods (Seely, 2013). However, this type of application aerosolizes some of the liquid, leaving it subject to movement by wind and raising concerns over uncontrolled nutrient addition, public health risks, and constraints on use and enjoyment of neighboring properties (Genskow and Larson, 2016). These concerns have led to conflict between the public and farmers in many areas over the practice of aerial application of wastewater (Bergquist, 2016).

Over the last few decades, anaerobic digestion (AD) and nutrient recovery (NR) have been used to mitigate these concerns by treating waste prior to land application. Pathogens and odor are greatly reduced by AD, while NR recovers phosphorus and ammonia using dissolved air flotation (DAF) and aeration, respectively. Though these processes significantly alter some characteristics of the original waste (Ma et al., 2013), they do not address other physiochemical characteristics, such as color, odor, and chemical oxygen demand (COD). For this reason, aesthetic and biological issues remain concerning application of the effluent.

Advanced oxidation processes (AOPs) have been used to treat livestock manure and have proven successful in abating odor and color, and improvement of other physiochemical characteristics (e.g., COD reduction; Riaño et al., 2014). Advanced oxidation processes are attractive treatment methods because they do not increase the volume of or the salt content of wastewater, nor do they leave any residues (Achwal, 1996). Among three of the most popular AOPs, ozone-based AOP have been shown to be the cheapest and least energy intensive for treating micropollutants (Rosenfeldt et al., 2006). While various treatments are available in the wastewater industry (Johnson et al., 2004), these technologies were not designed for use with wastewater from confined animal feeding operations, they have not been implemented in conjunction with AD or NR, and they require research to tailor these systems for use as a manure management strategy.

3.2 Objectives

The conventional NR scheme recovers phosphorus and ammonia by treating AD effluent with DAF, followed by aeration (Figure 3.1) (Zhao et al., 2014).
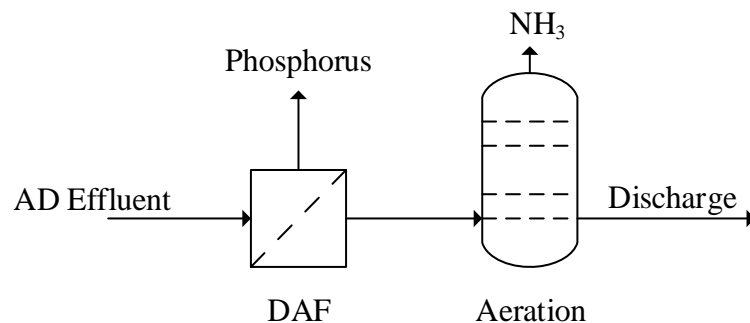
Figure 3.1: Conventional nutrient recovery scheme

For this study, an ozone-based AOP was incorporated into this NR scheme, and its effects on the remaining physiochemical concerns associated with the applied liquid (color, odor, COD) were evaluated. Two system configurations were tried: (1) placing an AOP after the DAF system (Figure 3.2), and (2) placing an AOP after the DAF system and aeration (Figure 3.3). These are referred to throughout this chapter as system configurations 1 and 2, respectively. In addition to color, odor, and COD, dissolved carbon was evaluated through measurement of dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), and total dissolved carbon (TDC). These types of dissolved carbon have been shown to influence AOP treatment (Peyton et al., 1997).
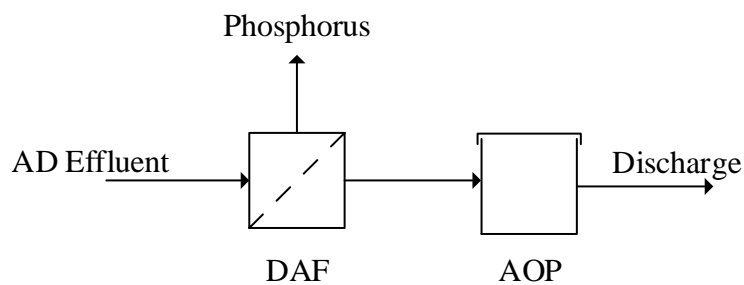
Figure 3.2: System configuration 1
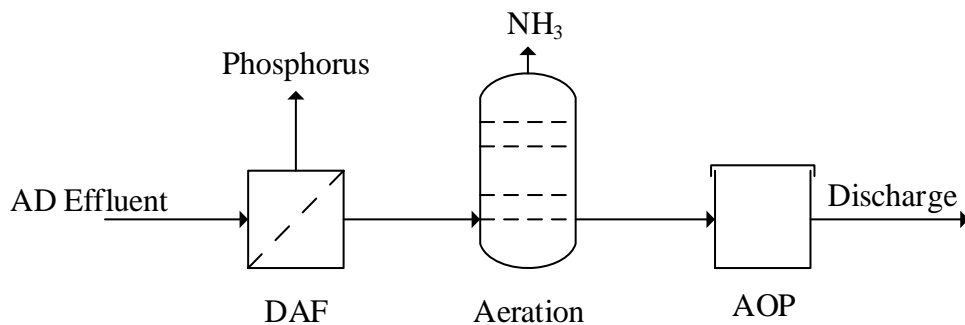
Figure 3.3: System configuration 2

3.3 Methods

An ozone/oxygen mixture was generated using a Pacific Ozone™ L11 Generator supplied with 99.8% oxygen. The ozone/oxygen mixture was sparged through 450 mL of effluent sample using a Pyrex® ASTM 40-60 12C sintered glass gas diffuser, at a rate of 500 mL per minute. A 200-mesh stainless steel (316L grade) screen was secured above the liquid to act as a sieve plate for mechanically knocking down foam while sparging. The ozone reactor was a 2-foot long, 1 5/8-inch inner diameter borosilicate tube containing the AD effluent, glass diffuser, and mesh. Jacketing the glass tube was a 2-foot section of 4-inch schedule 80 clear PVC (polyvinyl chloride) pipe, the annulus of which contained water to maintain the reactor temperature at $50°C$ using a water bath.

The off-gas from the reactor was conditioned to reduce moisture for downstream analysis. This was accomplished by flowing the off-gas through a 40-inch long section of 1/16-inch I.D. Nafion™ membrane, to which 2 L per minute of dry nitrogen gas flowed counter-current to the off-gas stream. The conditioned gas was analyzed for ozone using an ultraviolet photometer (INUSA H1-X V6.0 Single Channel Gaseous Ozone Analyzer). These measurements were validated by the standard iodometric wet-chemistry method (Kerwin et al., 1996). The experimental setup in its entirety is illustrated in Figure 3.4.
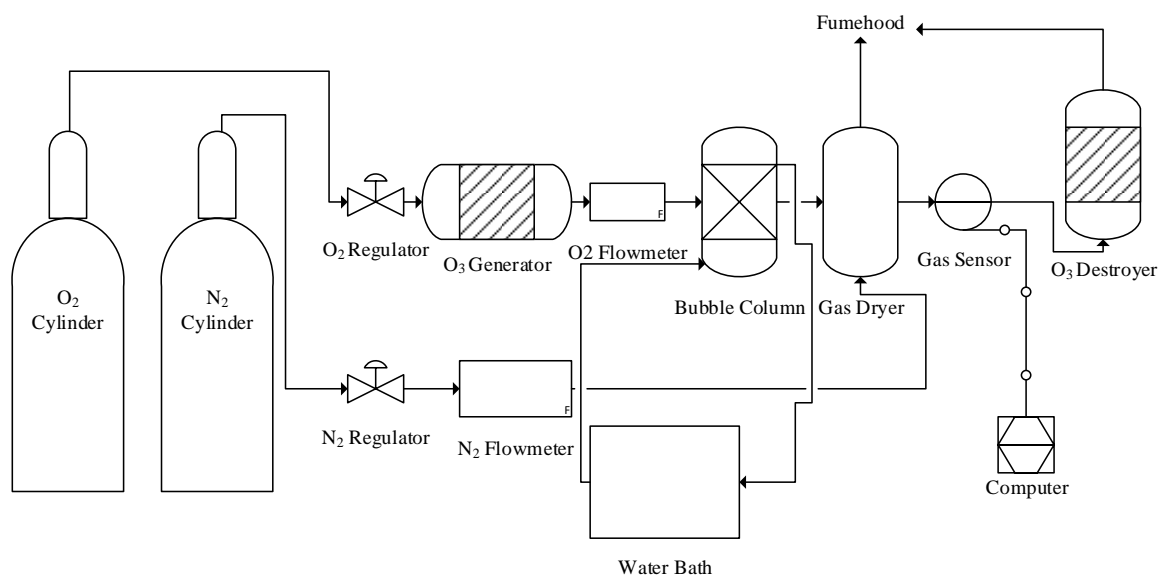
Figure 3.4: Experimental setup used in AD effluent ozonation studies

Six contact times (5, 10, 15, 20, 25, and 30 minutes) were investigated at four different ozone dosages (2, 4, 6, and 8 wt. %). These trials were performed in triplicate for each of the two system configurations. Color was measured in terms of absorption units using a Spectronic 20 Genesys where the visible light absorption at 475 nm was measured through 1.5 cm path length cells. Chemical oxygen demand was measured in accordance with USEPA Standard Method 5220 D (ASTM, 1995). Total dissolved carbon, DOC, and DIC was measured with a Shimadzu TOC-5000A analyzer.

3.4 Results and discussion

The physiochemical characteristics of the effluent in conventional NR prior to ozonation are detailed in Table 3.1. The DAF plus aeration effluent contained significantly less TDC as a result of lower DIC. This is expected as per patent US 2014/0314657A1 (Zhao et al., 2014), because aeration removes carbon dioxide, which is responsible for DIC content, from the solution.

Table 3.1: Physiochemical characteristics of AD effluent after nutrient recovery stages

Effluent*	Color (AU**)	COD (ppm)	TDC (ppm)	DIC (ppm)	DOC (ppm)
DAF	0.895±0.002	2,900±56	2,160±18	1,060±2	1,100±20
DAF plus aeration	0.916±0.003	3,030±32	1,570±2	410±9	1,160±10

* DAF effluent is the liquid between DAF and aeration, while DAF plus aeration is the liquid after DAF and aeration (see steps in Figure 3.1).
** AU = absorption units

Color removal for each effluent treatment is shown in Figure 3.5. For both configurations, increasing ozone dosage and sparging time increased color removal, as demonstrated in other experiments (Riaño et al., 2014). Color removal occurred more quickly for configuration 2. Color removal stabilized at approximately 85% and 90% for configuration 1 and 2, respectively. This trend may be the result of diminishing concentration of readily oxidizable chromophores as treatment progressed. Oxidation rates of organic solutions are known to be rapid during early stages of ozonation, but slow considerably with increased treatment time as more refractory carbon compounds are formed (Rice and Browning, 1980). The difference in color removal between system configurations may be due to differences in DIC content (Figure 3.8). At the temperature (50°C) and pH (8.5-9.0) conditions present during these tests, ozone is not stabilized in solution and decomposes to the hydroxyl radical (Sonntag et al., 2012), which is scavenged by carbonate ions, forming the carbonate radical (Beltrán, 2004).

While subtle differences between colors of effluents from the two different configurations were picked up easily with the spectrometer, effluents that received similar sparge time and ozone dosage were indistinguishable to the naked eye. For example, the color resulting from a 25 minute, 4 wt. % ozone dosage under configuration 1 appeared identical to the color resulting from a 25 minute, 4 wt. % ozone dosage under configuration 2. A more qualitative representation of color removal is show in Figure 3.6. The sample in the upper left corner is identical to the effluent color prior to ozonation, and should be used as a reference for comparing treatments. Sparge time increases from left to right, while ozone dosage increases from top to bottom.

Samples to the right of the red line did not have a bile odor, based on the researcher's experience handling them (Figure 3.6). Rather, ammonia was the predominant olfactory signature with a slight fruit/citrus hue. This latter smell is best described as similar to the smell of the orange variety of Windex®.
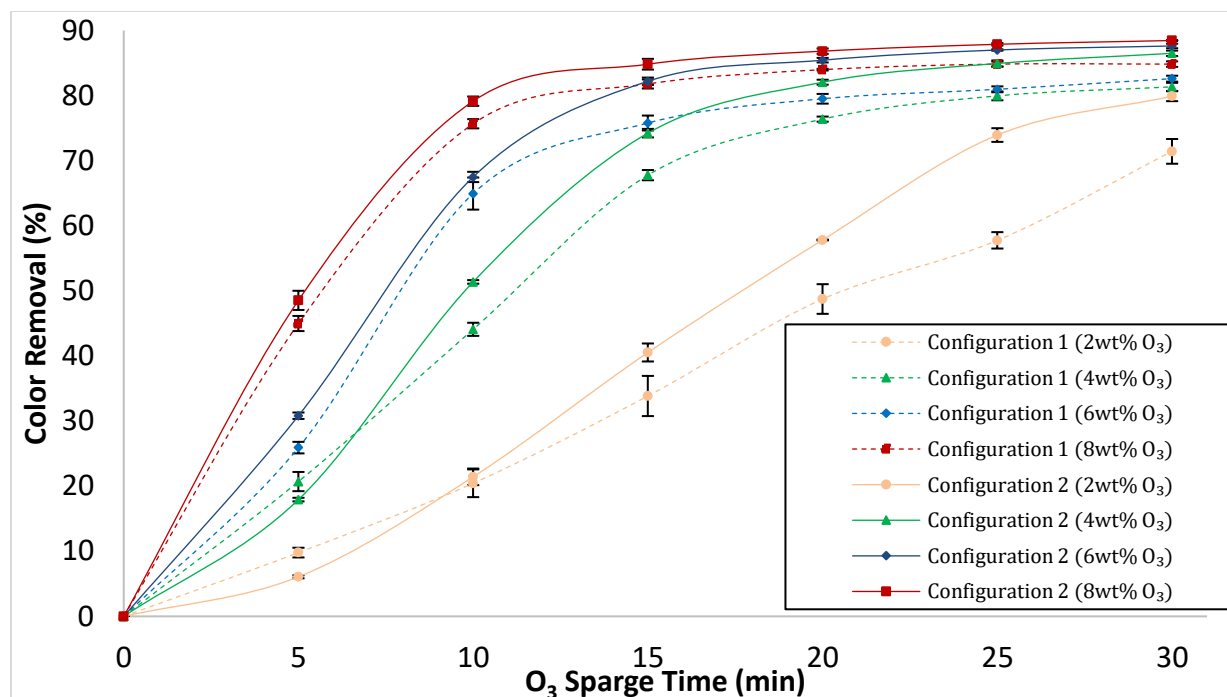
Figure 3.5: Color removal from ozonated effluents

Total dissolved carbon for both effluents after ozonation are shown in Figure 3.7. Total dissolved carbon is the sum of DIC and DOC, both of which are discussed later in this chapter. Both configurations initially showed increases in TDC, then tended to decrease as sparge time increased, regardless of ozone dosage. A carbon balance of dissolved carbon showed that at 5 minutes, additional carbon was introduced to the aqueous phase in both system configurations in the form of DIC. This additional carbon likely resulted from suspended solids solubilizing into the aqueous phase. Suspended solid measurements were not performed to verify this because particulates would adhere to the stainless-steel mesh in the bubble column, resulting in inaccurate measurements. The greater DIC during the first 5 minutes of sparging suggests complete mineralization during initial oxidation. This effect was more profound in system configuration 1, possibly due to its higher DIC content (Figure 3.8).

Higher DIC could cause a greater degree of mineralization if the DOC consists largely of small organic molecules that are almost mineralized. This is due to carbonate radicals predominating in the solution, which selectively oxidize these compounds, while not reacting with larger organic molecules (Peyton et al., 1997). For system configuration 2, DIC was initially very low, so the non-selective hydroxyl radical predominated in solution, reacting with both large and small organic molecules. Reacting with large organic molecules would oxidize these molecules to higher states, but further oxidation would be required for complete mineralization. For this reason, less mineralization occurred in system configuration 2 than in system configuration 1. Molecular weight distribution and testing for functional groups would be needed to verify this mechanism, which was beyond the scope of this study. Following the logic of this mechanism, we would expect more DOC consumed in system configuration 1 as the greater DIC concentration leads to more scavenging by hydroxyl radicals, creating carbonate radicals, and selectively oxidizing smaller, nearly mineralized organics to completion (Figure 3.9).
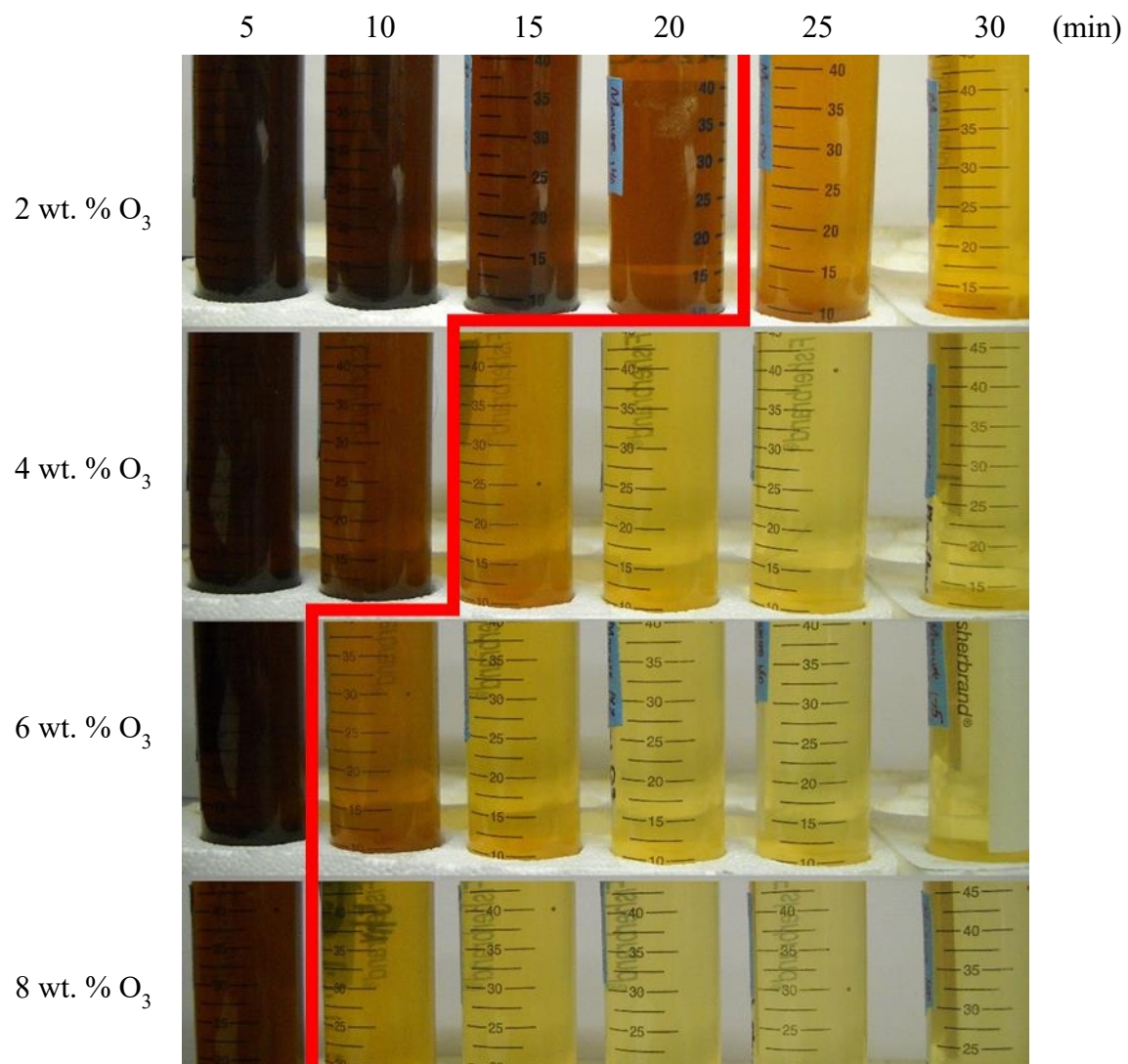
Figure 3.6: Actual color after treatment using different sparge times and ozone dosages. Samples to the right of the red line did not have a bile odor
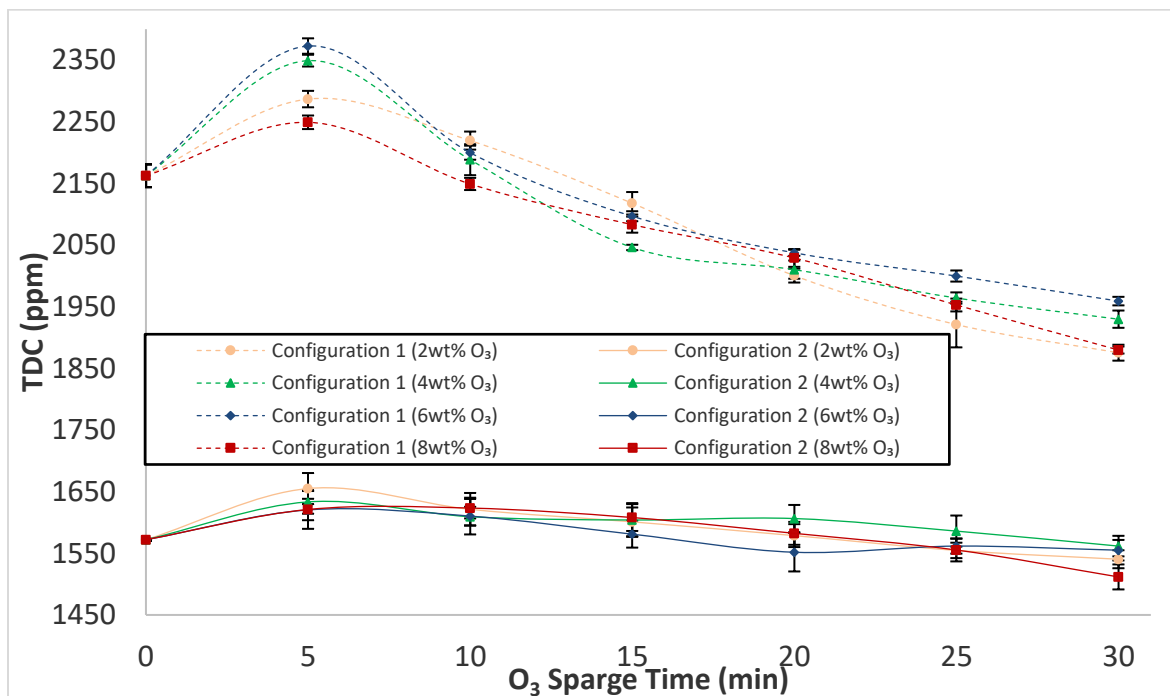
Figure 3.7: Total dissolved carbon (TDC) of ozonated effluents
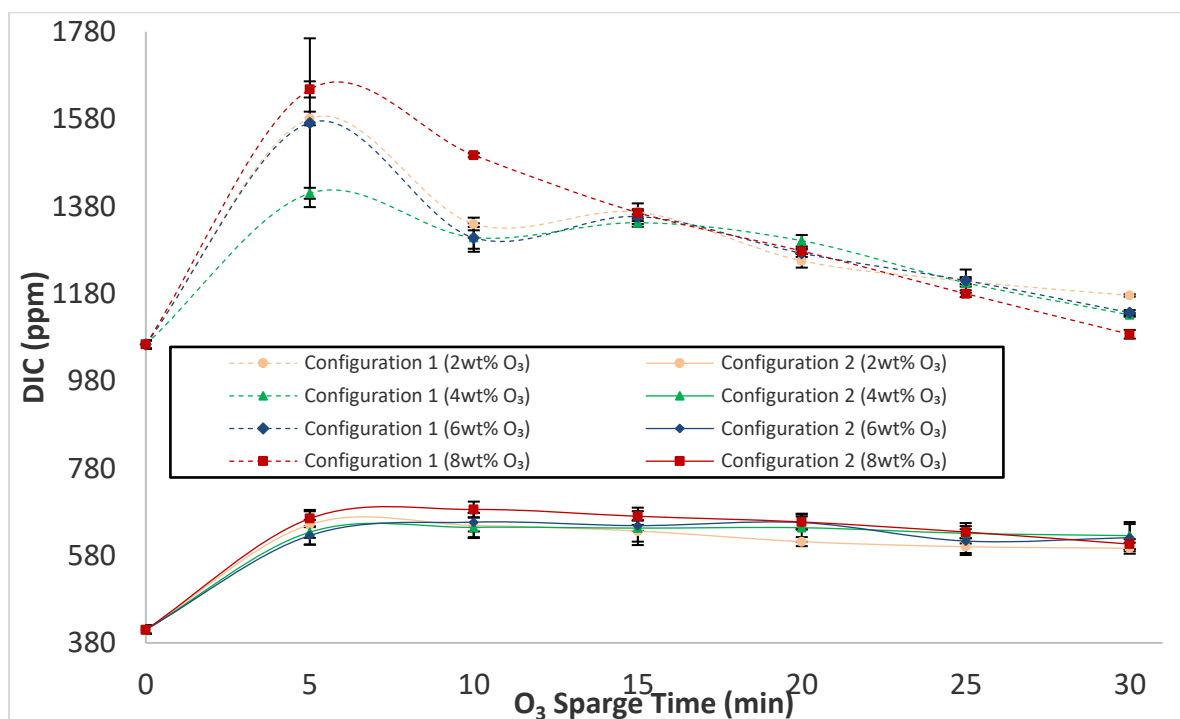
Figure 3.8: Dissolved inorganic carbon (DIC) of ozonated effluents
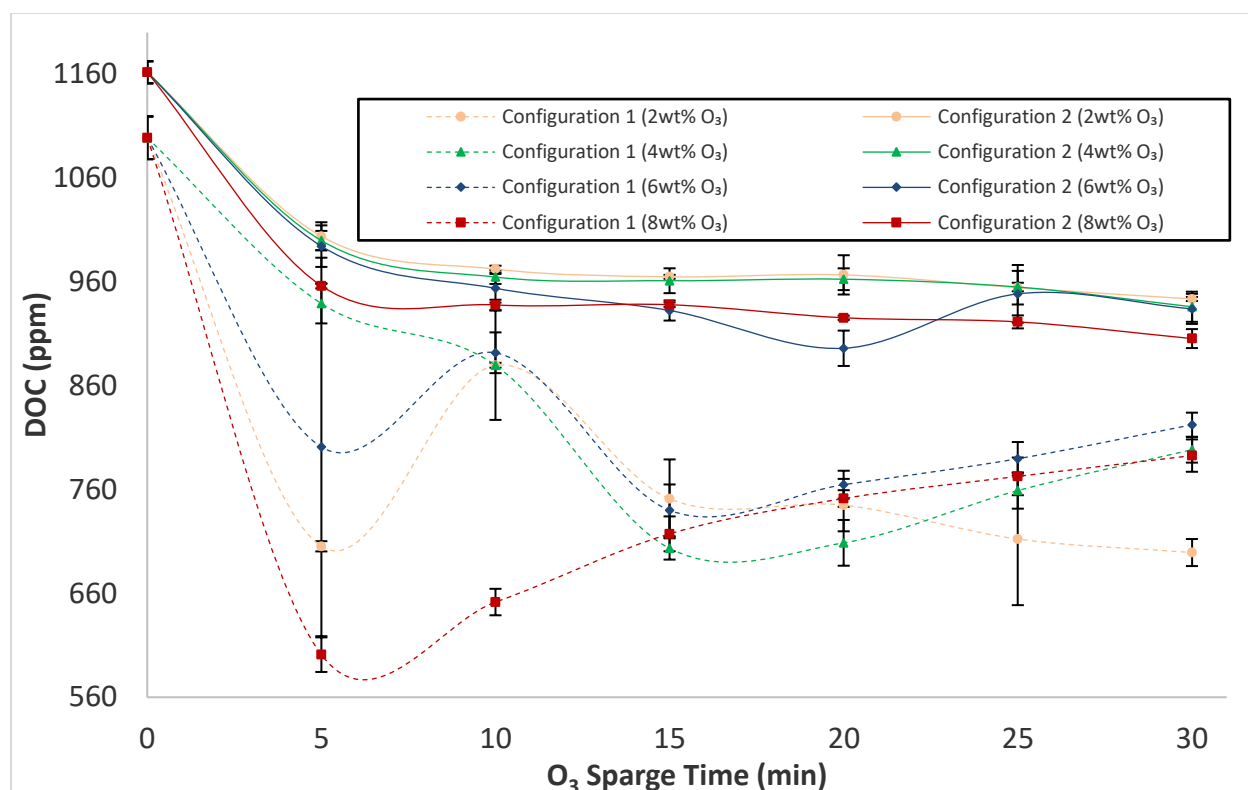
Figure 3.9: Dissolved organic carbon (DOC) of ozonated effluents

After five minutes, system configuration 1 varied greatly, with higher ozone dosages increasing DOC removal. After 10 minutes, the DOC level resulting from all ozone dosages except the 8 wt. % in system configuration 1 increased to roughly the same level. This increase may have been due to organics solubilizing into solution, because at 10 minutes DIC dropped for all ozone dosages except 8 wt. %. A lower DIC content scavenges fewer hydroxyl radicals, allowing the oxidation of larger organic molecules, while high DIC in the 8 wt. % scavenged hydroxyl radicals and converted them to carbonate radicals, inhibiting the oxidation potential. By 15 minutes, all ozone dosages had roughly the same DOC and DIC and the oxidation driving forces were roughly equivalent among ozone dosages.

Regardless of ozone dosage, DOC levels in system configuration 2 declined and remained relatively constant after five minutes. This may have occurred because the original DOC was progressively oxidized to higher states and remained in solution, or DOC may have been mineralized and off-gassed as CO_2 at the same rate suspended solids were solubilized to the aqueous phase.

Effluents from both system configurations showed greater COD removal with increasing ozone dosage and sparge time (Figure 3.10). The moderate COD removal relative to color removal may be attributed to more recalcitrant organic by-products after treatment; carboxylic acids are the second most oxidized form of carbon prior to complete oxidation, and are known to react slowly with ozone compared to other oxidation states of carbon (Etsuo et al., 1983). System configuration 1 may have leveled off earlier and had greater COD reduction than the system

50

configuration 2 because the initial DOC was so much lower, hence less theoretical COD from non-mineralized carbon.
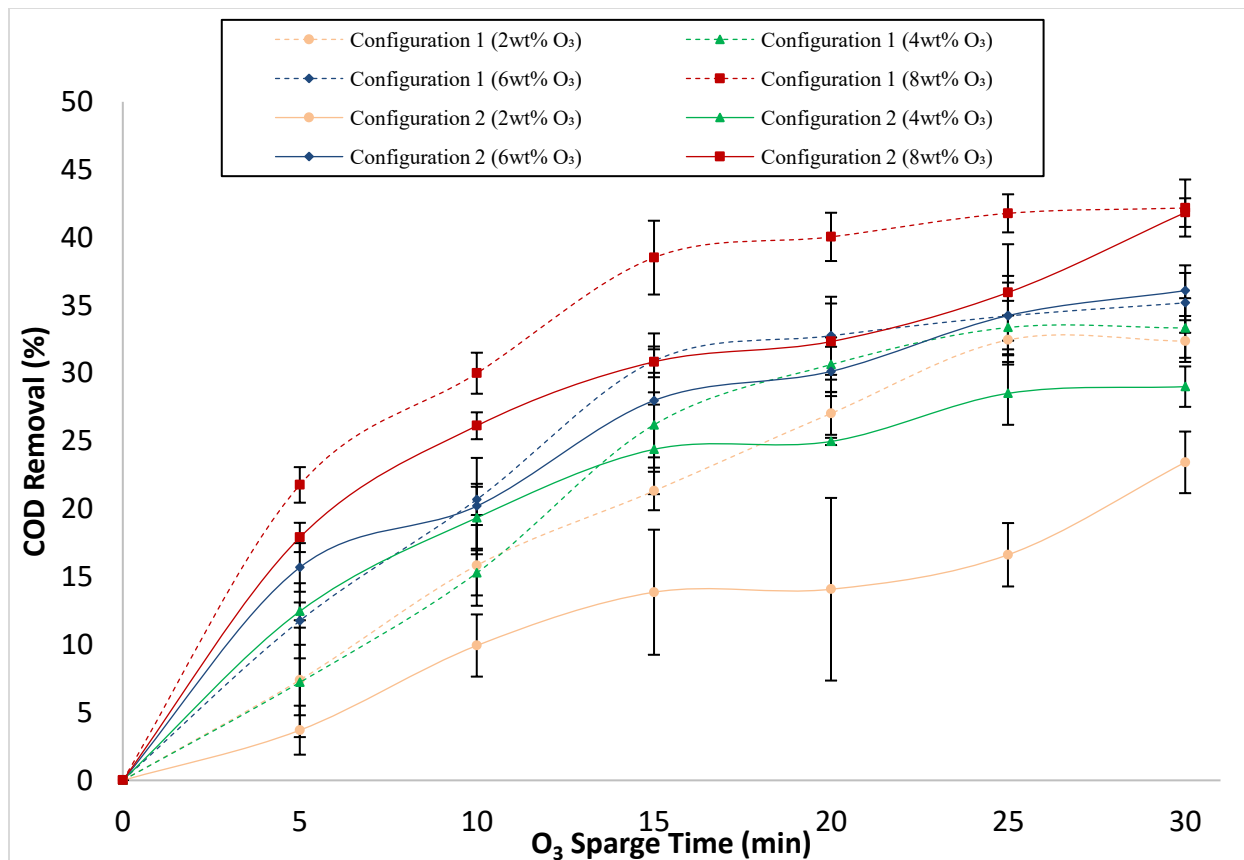
Figure 3.10: Chemical oxygen demand (COD) removal of ozonated effluents

3.5 Conclusions

Depending on the desired characteristic of the final effluent, one can use ozonation in either system configuration.

- Both color and odor significantly improved with ozone treatment in as little as five minutes regardless of the system configuration. However, color removal was slightly faster when ozonation occurred after DAF and ammonia stripping (system configuration 2).

- If greater ammonia recovery is desired, it may be optimal to perform ozonation for five minutes on the DAF effluent (system configuration 1). Under these conditions DIC was greatest, resulting in a higher pH during the aeration process, favoring a shift in equilibrium from dissolved ammonia to the gaseous phase. Further research is needed to determine recovery benefits from this treatment.

- Removal of COD was faster for system configuration 1, where approximately 40% was removed in 15 minutes with an 8 wt. % ozone dosage.

While ozonation within an NR system was shown to abate color, odor, and COD, detailed capital and operating costs for implementing this unit process are needed for each system configuration and treatment condition, to determine the most viable operation scheme. Further research is also necessary to evaluate ozonation on a continuous basis, for integration with the described NR technology.

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SECTION 2

TECHNOLOGIES TO ENABLE THE ADOPTION OF ANAEROBIC DIGESTION IN SMALL DAIRY OPERATIONS

4. Digester Technology for Small and Medium Sized Dairy Farms

Yiqing Yao and Shulin Chen

4.1 Background

Environmental and economic concerns faced by government agencies and farmers have created considerable interest in the utilization of livestock manure as a renewable energy source (Demirer and Chen, 2005). Animal manure is typically disposed of through land application near confined feeding operations in order to minimize cost. This can have severe environmental effects: contamination of surface and groundwater with pathogens such as *Escherichia coli*, odor emissions, buildup of excess phosphate in the soil, and deterioration of soil biology. In addition, disposing of manure through land application means losing the potential for a source of renewable energy. Alternatively, technology to produce biomethane through anaerobic digestion (AD) may provide an environmentally acceptable approach with potential financial benefits and fertilizer by-products. This commercially proven technology is widely used for treating livestock manure (Krishania et al., 2013b).

Over the past decade, AD on U.S. dairy farms has been promoted as a technology with both environmental and economic benefits. As one of the nation's top ten dairy states, Washington is considered a prime market for dairy digesters. With each cow producing 27 tons of manure per year, Washington's commercial dairies produce more than 6.9 million tons of manure annually. Based on 2010 registration data from the Washington State Department of Agriculture's (WSDA) Dairy Nutrient Management Program, Washington has 443 commercial dairy farms with more than 250,000 mature dairy cows. Of these, 175 dairies, or 40%, are classified as small, with 1-199 mature cows; 165 dairies, or 37%, are classified as medium size, with 200-699 mature cows; and 103 dairies, or 23%, are classified as large, with 700 or more mature animals, including 16 dairies that have 2,500 or more cows.

On an annual basis, Washington's dairy digesters capture 2,500 tons of methane, equivalent to more than 50,000 tons of carbon dioxide that would otherwise be released through conventional manure management. The greatest economic issue facing small- and medium-scale AD is the uncertainty in non-market factors, and the availability of AD in small and medium dairy farms is low due to the low profit margins (Lansing and Klavon, 2012). In other words, the low efficiency of digesters prevents the further development and application of this approach. For this reason, it is necessary to develop a new and efficient small- and medium-scale AD approach for manure treatment to enhance its applicability in these settings.

Anaerobic digestion of dairy manure typically takes place under thermophilic conditions with a high percentage of total solids (TS). This approach is taken to ensure a high efficiency of energy

recovery. However, serious accumulation of ammonia usually occurs under these conditions, which lead to instability in the AD process and even the cessation of methane production. Therefore, in addition to the traditional digester configuration as previously described, ammonia stripping coupled with thermophilic AD at high TS is suggested as an alternative approach for efficient treatment of dairy manure. This approach removes the accumulated ammonia, and enhances substrate utilization, thereby improving methane production.

4.2 Objectives

The objectives of this study were: (1) to assess the suitability of existing small AD technologies for small and medium dairies, (2) to identify the major technical challenges and opportunities for cost reduction in AD technologies for small and medium dairies, and (3) to devise and test technical solutions to these challenges.

4.3 Methods

The methods described below are those for Objective 3, above, which involved testing technical solutions to the challenges of AD technology. In this case, the technical solution being tested was thermophilic AD coupled with simultaneous ammonia stripping to overcome the issue of ammonia inhibition in AD of manure with a high TS content.

4.3.1 Feedstock and inoculum

Dairy manure was collected from the Washington State University (WSU) Dairy Center in Pullman, Washington and stored at 4°C prior to use. The inoculum was obtained from an anaerobic digester at a wastewater treatment facility in Pullman, Washington. Characteristics of the dairy manure and the inoculum are shown in Table 4.1.

Table 4.1: Characteristics of dairy manure and inoculum

Parameter	Dairy manure	Inoculum
Total solids (%)	12.5 ± 0.4	1.9 ± 0.0
Volatile solids (% of total solids)	80.9 ± 0.1	74.4 ± 0.1
pH	8.4 ± 0.0	7.6 ± 0.0
Total carbon (%)	40.7 ± 0.1	35.5 ± 0.0
Total nitrogen (%)	1.8 ± 0.0	5.5 ± 0.0
Total hydrogen (%)	5.7 ± 0.0	5.3 ± 0.0
Total ammonia nitrogen (g N L^{-1})	1.4 ± 0.0	0.6 ± 0.0

4.3.2 Anaerobic digester design

4.3.2.1 Thermophilic anaerobic digestion of dairy manure at different total solids concentrations

To better understand ammonia accumulation of dairy manure, we first conducted digestion experiments to measure ammonia accumulation in samples with different levels of TS. The amount of dairy manure and inoculum required for each digester were 400 g and 100 g, respectively, based on wet weight. The total solids (TS) and volatile solids (VS) for each digester were 52 g and 42 g, respectively. Deionized water was added to bring the mixture to 6, 8, and 10% TS. This test was conducted in batch mode at the laboratory scale. The volume of each digester was 1 L. The headspace of digesters was flushed with nitrogen gas (N_2) for about 5 minutes to obtain anaerobic conditions, after which they were capped tightly with rubber stoppers, incubated at 55°C, and shaken at a speed of 120 rpm (Lin et al., 2011; Zheng et al., 2009). Digestion experiments were conducted in triplicate for each condition.

4.3.2.2 Simultaneous ammonia stripping for thermophilic AD of dairy manure at the bottleneck solids concentration

The next experiment involved simultaneous ammonia stripping in a 3.0 L reactor. The selection of TS content (%) for stripping was the bottleneck TS (i.e., the TS content that led to serious ammonia accumulation and thereby the cessation of methane production) obtained from the previous experiment.

Nitrogen gas was introduced into the liquid phase from the bottom of the reactor via an aquatic air stone. Nitrogen was used as the stripping gas and was also used to stir the mixed liquid in the reactor. The N_2 flow rate was controlled by a flow meter at 1.0 L min^{-1}, 3.0 L min^{-1}, and 5.0 L min^{-1}, respectively. The pH value for all experiments before AD was 8.1 without adjustment. An experiment with no simultaneous stripping was run as a control. The exhaust gas was passed through solutions of 50% (w/w) sulfuric acid (H_2SO_4) and 20% (w/w) sodium hydroxide (NaOH) to prevent the release of ammonia and other volatile compounds into the atmosphere (Zhang et al., 2012) (Figure 4.1). The headspace of the reactors was flushed with N_2 for about 5 minutes to obtain anaerobic conditions, after which they were capped tightly with rubber caps and incubated at 55°C. Experiments were conducted in triplicate for each condition.

4.3.3 Analytical methods

4.3.3.1 Chemical analyses

Total solids, VS, soluble chemical oxygen demand (COD; a measure of soluble substrate), and pH were determined according to standard methods (Clesceri, 1998). Total ammonia nitrogen (TAN) was measured by using a Tecator 2300 Kjeltec Analyzer (Eden Prairie, MN, USA; 4500-NorgB; 4500NH3BC). An elemental analyzer (LECO) was used to measure total carbon (TC), total nitrogen (TN), and total hydrogen (H).

4.3.3.2 Biogas analyses

Biogas production was measured by water displacement every two days, while the total volume of biogas was calculated after AD. Gas samples were collected every two days and stored in 12 mL vacuumed borosilicate vials (Extainer, Labco Limited, Wycombe, England). A gas

chromatograph (GC, CP-3800, Varian Inc., Palo Alto, California, USA) equipped with a thermal conductivity detector was used to analyze the methane content of each biogas sample (Ma et al., 2013).
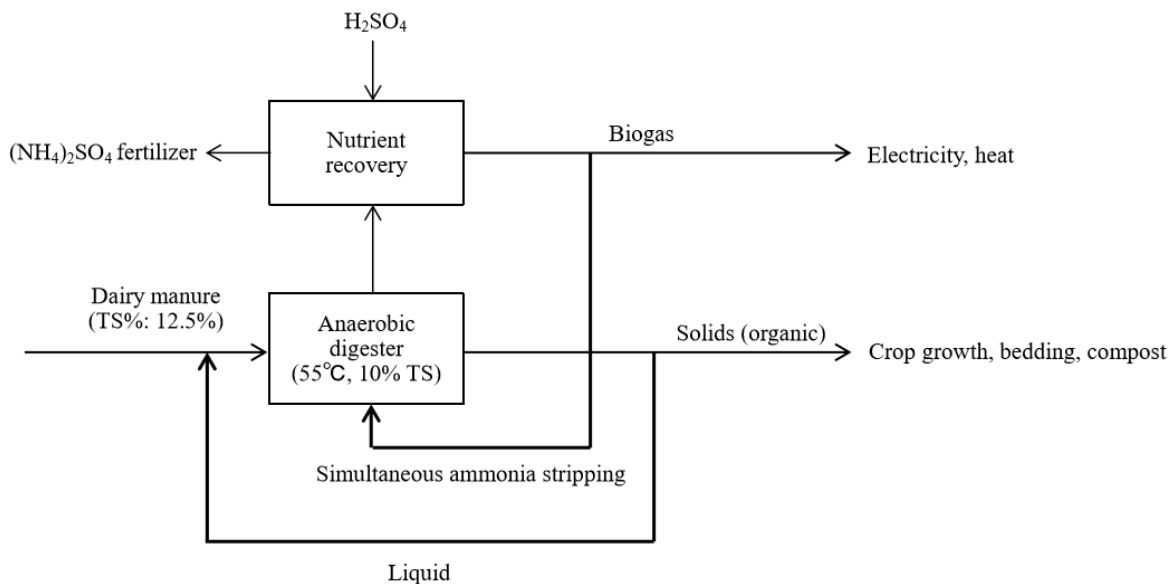
Figure 4.1: Schematic diagram of thermophilic AD coupled with the simultaneous ammonia stripping

4.4 Results and discussion

4.4.1 Part 1 – Review of existing technologies

Anaerobic digestion is used worldwide for the treatment of industrial, agricultural, and municipal wastes. It involves the degradation and stabilization of an organic material under anaerobic conditions by microbial organisms and leads to the formation of methane and inorganic products including carbon dioxide. Bacteria that function without oxygen degrade organic matter inherent in poultry and livestock waste. These microorganisms are not only sensitive to oxygen, but also to temperature, and thus design criteria for systems utilizing anaerobic processes varies regionally (Jones, 2003). It has been reported that the anaerobic treatment of complex organic wastes involves two distinct stages, acid fermentation and methane fermentation (Kelleher et al., 2002).

Importantly, anaerobic treatment process configurations are mainly based on substrate characterization and solid contents. Cattle manure generally has moisture content around 75-80% and dairy cattle manure has an even higher moisture content and is sometimes referred to as "cattle slurry." When solid content is between 25% and 40%, the AD process is described as dry digestion, whereas when solid content is below 15%, it is referred to as wet digestion. Also,

cattle manure has a lower biodegradability than many other types of waste due to the high content of inorganic compounds and fibers not broken down in the cow's digestive system.

Advances in the understanding of anaerobic system functions and reactor design have led to the evolution of a new generation of high-rate anaerobic processes (Ghangrekar and Kahalekar, 2003). Many high-rate anaerobic treatment process configurations have been developed and successfully applied, as described below.

4.4.1.1 Batch digester
Batch reactors are the simplest type of reactor. They are filled with the feedstock and the reaction is carried out with nothing else being introduced or withdrawn until the reaction is completed. Kalia and Singh (2001) tested the anaerobic batch digestion of cattle manure (19.9% TS) under ambient conditions (20-23℃). Their results showed a lower methane yield of $0.103 m^3 kg^{-1}$ VS with 23.9% VS reduction. However, 10% mixing of digested slurry with fresh cattle manure in the batch digester achieved a higher gas production and a shorter hydraulic retention time (HRT) of 14 days with 36.1% VS destruction.

Amon et al. (2007) investigated the influence of feeding intensity on methane yield from dairy cattle manure operated for 60 days in a batch digester at 38℃. They reported the highest methane and biogas yield of 0.166 and 0.245 $m^3 kg^{-1}$ VS from the manure received from dairy cows with medium milk yield that were fed with a well-balanced diet. They concluded that the anaerobic digestibility of dairy cattle manure is clearly influenced by animal diet and performance.

Omar et al. (2008) studied the anaerobic treatment of cattle manure seeded with the palm oil mill effluent-activated sludge in a 10 L batch digester operating under thermophilic conditions for 17 days HRT. A VS reduction of 98% with a substantial methane yield of 0.184 $m^3 kg^{-1}$ VS was reported.

Dubrovskis et al. (2009) investigated the feasibility of digesting cattle manure and mink manure inoculum in a batch digester at 37℃. They obtained a high yield of biogas (0.303 $m^3 kg^{-1}$ VS) and a methane yield of 0.168 $m^3 kg^{-1}$ VS, with a methane content of 56% from the substrate of cattle manure and 25% mink manure inoculum. They concluded that the fresh mink manure has a higher potential for biogas production, but it should be mixed with agricultural waste rich in carbon to increase the carbon nitrogen ratio for optimal AD.

Callaghan et al. (1999) operated two 1 L batch digesters for 14 days HRT for the mesophilic digestion of cattle manure and cattle manure with fish offal and brewery solids. They reported a higher methane yield of 0.37 $m^3 kg^{-1}$ VS with VS reduction of 47.3% from the cattle manure with fish offal, compared to 0.15 $m^3 kg^{-1}$ VS with VS destruction of 31.1% from cattle manure alone. The study concluded that co-digesting the fish offal and brewery solids with cattle slurry produced an increase in methane yield, compared with that of a control digestion using cattle slurry alone. Mladenovska et al. (2003) studied the anaerobic co-digestion of cattle manure and lipids in a continuous stirred tank reactor (CSTR) operated at mesophilic condition for 15 days HRT. A higher methane yield of 0.224 $m^3 kg^{-1}$ VS and higher removal of VS was observed in the reactor co-digesting manure and lipids than in the reactor treating the manure alone. These

results indicate that co-digestion of other waste with cattle manure can increase the methane yield and the stability of the bioreactor.

Thermophilic AD of cattle manure can quickly produce a large amount of biogas. The manure itself provides the appropriate microbial community. The microbial diversity of a thermophilic ecosystem (46-60℃) is lower than that of a mesophilic one (28-38℃) (Mladenovska and Ahring, 2000) and major bacterial and archaeal species are close to thermophilic species. Thermophilic microorganisms are present in cattle manure (a mesophilic ecosystem) at a subdominant level, and quickly become dominant under thermophilic anaerobic conditions.

4.4.1.2 Semi-continuous reactors

Semi-continuous reactors are so named because they are fed semi-continuously through an inlet pipe, and displace an equal amount of slurry through an outlet pipe. The AD of cattle manure in a pilot-scale bioreactor carried out at 35℃ was investigated (Comino et al., 2009). The study reported biogas production of 0.423 $m^3 kg^{-1}$ VS, equivalent to a methane yield of 0.211 $m^3 kg^{-1}$ VS for a period of 56 days with a methane content of 51.4%. The authors concluded that the cattle manure mix had good potential for biogas production when it was co-digested with other types of biomass.

Li et al. (2009) investigated the effect of co-digestion on different mixtures of cattle manure and kitchen waste in both batch and semi-continuous modes under mesophilic conditions. They reported a significant methane yield of 0.233 $m^3 kg^{-1}$ VS with 71.9% VS destruction in the semi-continuous operation. They concluded that the optimal mixing ratio for co-digesting cattle manure and kitchen waste was 3:1. These results indicate that co-digestion of cattle manure can increase methane yield and the stability of the bioreactor.

Alkaya et al. (2010) investigated the effect of temperature and HRT on anaerobic co-digestion of cattle manure and agricultural residues in a semi-continuously fed reactor operated at 20℃ and 35℃ (Alkaya et al., 2010). The reactors were operated at an HRT of 20 and 30 days, with an organic loading rate (OLR) of 3 kg VS $m^{-3} day^{-1}$. The authors observed a clear effect of temperature on reactor performance for both HRTs of 20 and 30 days. They reported a biogas yield in the range 0.299–0.324 $m^3 kg^{-1}$ VS and 0.087–0.138 $m^3 kg^{-1}$ VS for the reactor run at 35℃ and 20℃, respectively. The study concluded that HRT as an operational parameter did not affect AD significantly, as the methane yield with 30 days HRT (0.039–0.182 $m^3 kg^{-1}$ VS) was not significantly different to the one achieved with 20 days HRT (0.042–0.182 $m^3 kg^{-1}$ VS).

4.4.1.3 Continuous reactors

Continuous one-stage
The continuous one-stage system uses mechanical agitation or biogas recirculation to mix the contents of the digester continuously. Ahring et al. (2001) conducted a study to investigate the influence of temperature increase—from 55℃ to 65℃—on the performance of two CSTRs treating cattle manure at 15 days HRT. They obtained a stable biogas output of 0.2 $m^3 kg^{-1}$ VS day^{-1} in 10 days after the start-up at 55℃, with a methane content of 65-71%. However, they observed a fast drop in the biogas production and methane content (less than 45%) after increasing the temperature of the test reactor to 65℃. The study concluded that a temperature

shift from 55℃ to 65℃ led to a lower methane yield and an increased amount of volatile fatty acid (VFA) in the effluent.

In a similar study, the influence of temperature (50℃ and 60℃) on the performance of CSTRs digesting cattle manure was investigated by El-Mashad et al. (2004) at HRTs of 10 and 20 days. Methane production rate at 60℃ was lower than that at 50℃ at all the HRT levels employed, largely due to the effect of temperature on the hydrolysis stage. In another study, Rico et al. (2011) evaluated the influence of bioreactor content recirculation rate on biogas production during the mesophilic (37℃) AD of screened dairy manure in a CSTR digester. They studied the effect of continuous and intermittent operation of the recirculation pump at HRTs of 10 and 20 days. The researchers obtained stable biogas production in the range of 1.26–1.34 $m^3 m^{-3} day^{-1}$ at 10 days HRT. Biogas production at 20 days HRT reached a rather stable value of about 0.7 m^3 $m^{-3} day^{-1}$, with no significant difference between the mixing conditions. They concluded that AD of screened dairy manure at 10 days HRT with 6% TS concentration was affected minimally by recirculation rate. On the other hand, at 20 days HRT recirculation rate did not affect reactor performance at all. Therefore, they found that continuous recirculation was not a feasible means to improve biogas production. Rojas et al. (2010) found that more dilute media in the reactor allowed for a better contact and improved biogas production, hence making stirring not necessary.

Ahring et al. (2001) conducted a laboratory study to investigate the effect of a temperature increase from 55 to 65°C on the performance of an anaerobic reactor treating cattle manure (Ahring et al., 2001). They carried out investigations in two thermophilic, laboratory-scale CSTRs having a working volume of 3 L at an organic loading rate of 3 g VS $L^{-1} day^{-1}$. In the study, the HRT was 15 days. One of the reactors was kept at 55°C for the whole period and the other reactor served as the test reactor. The authors obtained stable biogas production with a methane content of 65-71% (at approximately 200 mL g^{-1} VS day^{-1}) in 10 days after the start-up at 55°C. They observed an instant drop in the volumetric gas production and the methane content of the biogas decreased to 45% with the increase to 65°C of the temperature in the test reactor. The study reported that the temperature increase had a negative effect on the digester's performance and on the microbial activity, and caused changes in the structure of the microbial community of the biogas reactor. The study also emphasized that conversion of propionate, successful at 55°C, was completely inhibited by an increase of the temperature to 65°C. They showed that the temperature shift from 55 to 65°C resulted in a lower methane yield and an increased amount of VFA in the effluent. The study concluded that the VFA-degrading and methanogenic consortia were severely affected by the increase in temperature and that these populations were unable to balance the activity of fermenting populations. Mladenovska et al. (2003) used a laboratory scale CSTR operated at 37°C to investigate biogas reactor performance and the microbial community during AD of cattle manure and a mixture of manure with lipids (Mladenovska et al., 2003). They concluded that the reactor co-digesting manure and lipids exhibited a significantly higher specific methane yield and a higher removal of VS than the reactor treating manure alone.

A phylogenetically and metabolically rich methanogenic community that stably digested cattle manure rapidly adapted to a change in feed to cattle manure plus two-phase olive mill wastes both at 37°C and 55°C, but did not adapt to the digestion of two-phase olive mill wastes alone

(Goberna et al., 2010). The thermophilic co-digestion of the residues was the most productive and had the most diverse methanogenic communities. This is consistent with the theoretical expectation of a more efficient exploitation of resources due to better occupation of available niches. *Methanosarcina* dominated in the starting cattle manure (>96% of total 16S rRNA [ribosomal ribonucleic acid] gene copies; over 45 times more abundant than any other methanogen) at high acetate (0.21 g L^{-1}) and ammonia N (1.3 g L^{-1}) concentrations. Co-digestion at 37°C induced a six-fold increase of *Methanosarcina* numbers, which correlated with methane production. At 55°C, the rise in temperature and H_2 partial pressure induced a burst of *Methanobacterium*, *Methanoculleus*, *Methanothermobacter*, and a group of uncultured archaea. The digestion of cattle manure alone resulted in low but constant biogas production despite certain oscillations in the methanogenic biomass. Higher temperatures (55°C) fostered methanogenic diversity by promoting some H_2 scavengers while yielding the highest methane production.

The effect of a temperature increase from 55 to 65°C on process performance and microbial population dynamics were also investigated in thermophilic, laboratory scale CSTRs (Ahring et al., 2001). The reactors had a working volume of 3 L and were fed with cattle manure at an organic loading rate of 3 g VS L^{-1} day^{-1}. The HRT in the reactors was 15 days. A stable reactor performance was obtained for periods of three retention times, both at 55°C and 65°C. At 65°C methane yield stabilized at approximately 165 mL g^{-1} VS day^{-1}, compared to 200 mL g^{-1} VS day^{-1} at 55°C. Simultaneously, the level of total VFA increased from being below 0.3 g L^{-1} to 1.8-2.4 g acetate L^{-1}. The specific methanogenic activities of biomass from the reactors were measured with acetate, propionate, butyrate, hydrogen, formate, and glucose. At 65°C, decreased activity was found for glucose-, acetate-, butyrate-, and formate-utilizers and no significant activity was measured with propionate. Only the hydrogen-consuming methanogens showed enhanced activity at 65°C. Numbers of cultivable methanogens were significantly lower on glucose, acetate, and butyrate at the increased operational temperature, while the numbers of hydrogenotrophic methanogens remained unchanged.

Continuous two-stage reactors
The continuous one–stage system uses mechanical agitation or biogas recirculation to mix the contents of the digester continuously and processes occur in one reactor. In contrast, the continuous two–stage system employs independent reactors to separate the hydrolysis/acidification and acetogenesis/methanogenesis processes (Wilkie, 2005).

Harikishan and Sung (2003) evaluated the performance of a two-stage continuous reactor treating dairy manure. The first reactor was operated under thermophilic condition at four days HRT and the second reactor was operated under mesophilic condition at 10 days HRT. They reported an overall methane yield of 0.21 m^3 kg^{-1} VS including a high OLR of 5.82 kg VS m^{-3} day^{-1}, achieved for the second reactor with 41% VS reduction.

Nozhevnikova et al. (1999) conducted a study to investigate anaerobic manure treatment under extreme temperature conditions. They carried out laboratory scale experiments on the digestion of cattle and pig manure under psychrophilic conditions (5-20°C) and extreme thermophilic (55-82°C) conditions. In the study, a two-step anaerobic manure treatment process was proposed to achieve acidogenic fermentation at high temperature, with separation for solid and liquid fractions and treatment of the liquid manure fraction under low temperature conditions. The

authors reported that long-term adaptation of active psychrophilic microbial communities was essential to perform sufficient manure treatment at low temperatures.

Comparison between the two-stage system and the conventional one-stage system
Nielsen et al. (2004) operated a laboratory-scale reactor to compare the performance of a two-stage system and the conventional one-stage system for the treatment of cattle manure. In the study, the OLR of 3 kg VS m^{-3} day^{-1} was used for all systems. The two-stage system was connected with a first reactor operated at 68℃ with an HRT of 3 days and the second reactor operating at 55℃ with 12 days HRT, while the conventional one-stage reactor was operated at 55℃ with an HRT of 15 days. They found that the two-stage system has a 6-8% higher methane yield and a 9% more VS removal than the conventional one-stage system. They concluded that increased methane yield and VS removal were achieved when the pretreatment reactor operated at 68℃ with an HRT of 3 days, connected to a 55℃ reactor with an HRT of 12 days (compared with a conventional one-stage reactor operated at 55℃ with an HRT of 15 days). The 68℃ reactor generated 7% to 9% of the total amount of methane of the two-stage system and maintained a VFA concentration of 4.0 to 4.4 g acetate L^{-1}. Population size and activity of aceticlastic methanogens, syntrophic bacteria, and hydrolytic/fermentative bacteria were significantly lower in the 68℃ reactor than in the 55℃ reactors. The density levels of methanogens utilizing H_2/CO_2 or formate were, however, in the same range for all reactors, although the degradation of these substrates was significantly lower in the 68℃ reactor than in the 55℃ reactors. Temporal temperature gradient electrophoresis profiles (TTGE) of the 68℃ reactor demonstrated a stable bacterial community along with a less divergent community of archaeal species.

Demirer and Chen (2005) compared the performance of the one-phase conventional configuration with the two–phase continuous reactors treating unscreened dairy manure under mesophilic conditions. In the study, the two-phase configuration was operated at an HRT of two days for the first acidogenic reactor and an HRT of eight days for the second methanogenic reactor, while the one-phase conventional reactor was operated at an HRT of 20 days. The results indicated that the OLRs between 1 and 6.3 kg VS m^{-3} day^{-1} were found to be sufficient to represent the conventional one-phase anaerobic digester treating the dairy manure. On the other hand, the two-phase configuration made an elevated OLR of 12.6 kg VS m^{-3} day^{-1} that was not possible with the one-phase reactor. In addition, the two-phase reactor resulted in a 50% and 67% higher biogas production at an OLR of 5 and 6 kg VS m^{-3} day^{-1}, respectively. They concluded that the use of the two-phase configuration lead to significant cost savings due to both high performance and reduced volume.

4.4.1.4 Plug flow reactor (PFR)
The PFR is an unmixed system where waste flows semi-continuously as a plug through a horizontal reactor (Wilkie, 2005). The mixed plug-flow loop reactor (MPFLR) is an AD system designed and implemented by DVO (Chilton, Wisconsin, U.S.). According to a 2013 survey by the U.S. Environmental Protection Agency, nearly 40% of the AD systems operated on U.S. dairy farms used the MPFLR design. The popularity of MPFLR digesters stems from their simplicity in construction and maintenance, along with their operational reliability. A MPFLR system is essentially a horizontally oriented U-shaped tank. The waste influent enters an MPFLR digester at one end, flows forward and loops back as a 'plug', and finally exits from the other

end. The digester content is continuously mixed by biogas in the direction perpendicular to the plug-flow of the reactor. A portion of the effluent is typically recycled to inoculate the influent and improve AD.

In Li et al. (2014), the microbiome in a MPFLR digester operated on a mega-dairy farm was examined three times over a 2-month period. Within the 23 days of retention time, 55-70% of total manure solid was digested. Metagenomic analysis showed that, with some temporal variations, the bacterial community was rather stable spatially in the digester. The methanogenic community was also stable both spatially and temporally in the digester. Among methanogens, the genus *Methanosaeta* dominated in the digester. Quantitative polymerase chain reaction (qPCR) analysis and metagenomic analysis yielded different relative abundance values for individual genera of methanogens, especially for *Methanobacterium*, which was predominant based on qPCR analysis.

Hills and Mechlschau (1984) studied the biogas yield from cattle manure in a PFR, obtaining a high yield of 1.31 $m^3 m^{-3} day^{-1}$ with conditions of 15 days of HRT, 35℃ and 15.2% TS. Performance data showed that various reactor configurations—such as fixed-film reactor, anaerobic filter, fixed dome plant, up-flow anaerobic sequencing batch (UASB), CSTR, upflow anaerobic filter (UAF), temperature-phased anaerobic digestion (TPAD), anaerobic hybrid reactor, and two-stage anaerobic systems—are used in anaerobic processing of cattle manure. Most of the studies were conducted in laboratory-scale reactors varying between 0.1 and 9.0 L attached-film bioreactor, anaerobic rotating biological reactor, batch reactors, and down flow reactor (Sakar et al., 2009). In those studies, different temperature ranges—varying from psychrophilic conditions (5-20°C) to extreme thermophilic (55-82°C)—were evaluated. Retention or operation times used in those studies ranged from 0.5 days to 140 days. Organic loading rates (influent VS introduced in a unit volume of the reactor) ranged from 0.117 to 7.3 g VS $L^{-1} day^{-1}$.

In recent years, a number of novel reactors designs have been adapted and developed, allowing a significantly higher rate of reaction per unit volume of reactor (Bouallagui et al., 2005). Among such novel reactors are the up-flow anaerobic sequencing batch (UASB) reactor, the film reactor, and the leaching bed reactor. The UASB and anaerobic fluidized bed reactors can accumulate high biomass concentration and can permit long solid retention time even with low HRT, allowing for wider application in treating different types of waste (Ghazi et al., 2008).

4.4.1.5 Up-flow anaerobic sequencing batch (UASB) reactor
Among the high-rate reactors, the UASB process is the most commercially successful. Hundreds of full-scale treatment plants have been installed over the past decade for the treatment of various types of wastewater (Fang and Chung, 1999). Marañón et al. (2001) conducted a study to investigate the effect of HRT on the treatment of cattle manure in a UASB reactor. They found that the maximum COD removal of 75.5% for an HRT of 22.5 days at an OLR of 2.35 kg COD $m^{-3} day^{-1}$. In that study, the biogas production values varied between 0.20 and 0.39 $m^3 kg^{-1}$ COD.

Castrillon et al. (2002) conducted a study on the anaerobic thermophilic treatment of cattle manure in a UASB reactor with an HRT range of 7.3-22.5 days. They observed the highest COD removal of 79.7% for an HRT of 22.5 days. The authors found that the COD removal was

somewhat greater under the mesophilic condition obtained in a previous study (Marañón et al., 2006). Another study investigated the influence of HRT on the treatment of cattle manure in UASB reactors (Marañón et al., 2001). The authors found that the highest percentage of COD removal was 75.5% for a HRT of 22.5 days. In that study, gas production varied between values of 0.20 and 0.39 $m^3 kg^{-1}$ COD, with a methane content of up to 64%.

Castrillon et al. (2002) also carried out studies on anaerobic thermophilic (55°C) treatment of cattle manure in 9 L UASB reactors made of transparent PVC (polyvinyl chloride). In that study, a HRT range of 7.3-22.5 days was employed. They found that the highest percentage of COD removal obtained was 79.7% for an HRT of 22.5 days. The authors compared the results obtained at a thermophilic temperature with those obtained at a mesophilic temperature obtained in a previous study. Although the main advantage of thermophilic anaerobic treatment was reported to be the faster inactivation of viruses and bacteria, the reduction in COD was found to be slightly greater under mesophilic conditions maintained in the study. In the case of cattle manure, the authors of this article had previously studied its anaerobic treatment in the mesophilic range using UASB-type reactors at the laboratory scale with good results, obtaining high organic matter removal rates (Marañón et al., 2001). Different HRTs (22.5, 16, 10.6, 8.9, and 7.3 days) were employed and organic matter, total solids, metals, and biogas production were determined for anaerobic thermophilic treatment of cattle manure in UASB reactors. The level of COD removal achieved in the thermophilic range is generally slightly lower than those obtained in the mesophilic range. The highest percentage of COD removal obtained on thermophilic condition was 79.7% for an HRT of 22.5 days.

4.4.1.6 Fixed film reactor and suspending contact reactor
High-rate anaerobic systems can be divided into suspended growth and attached-growth processes (including expanded/fluidized bed reactors and fixed-film processes) (van Lier, 2006). In suspended growth systems, bacterial sludge is present as flocs or granules, whereas in attached growth systems microorganisms are adhered to a moving or fixed media. In an expanded/fluidized bed reactor, suspended carrier media (such as sand or porous inorganic particles) are used to develop an attached film. Fixed-film processes rely on the bacteria attaching to a fixed media, such as rocks, plastic rings, or modular cross-flow media. Some systems, such as the anaerobic hybrid process, combine suspended and attached-growth processes in a single reactor to utilize the advantages of both types of biomass.

Fixed-film reactor
The microbial consortium in AD reactors consist of three types: fermentative bacteria, acetogenic bacteria, and methanogenic bacteria. Methanogenic bacteria grow at a slower rate than fermentative and acetogenic bacteria, which makes them sensitive to changes in environmental conditions. Since the species composition and density of methanogenic bacteria most significantly affects the performance of an AD reactor, it is important to develop and maintain a large density, stable, and viable population of methane-producing bacteria in an AD reactor (Vartak et al., 1997).

The selection of a high-efficiency biofilm carrier is important for the enrichment of high-density methanogens and to prevent the biomass being washed out in the effluent. Previously reported studies have shown the retention of microorganisms in AD reactors with a biofilm carrier can potentially increase their productivity by increasing the amount of methanogens in AD reactors

(Hill and Bolte, 1992). Biofilms have improved in their packing material properties and several biofilm carriers have been evaluated for microbial immobilization (Gong et al., 2011). Based on the characteristics of the materials used, traditional carriers can be mainly classified into two categories: granular and fibrous carriers. One noticeable drawback of using a granular carrier is that the pores of granule media are susceptible to clogging resulting from biofilm growth. In comparison, the fibrous carriers are capable of overcoming this disadvantage when the space between carriers is kept smooth enough. It is still not well understood what the most suitable biofilm carrier is for AD systems. The use of a biofilm carrier requires the materials to be biocompatible with the predominant microorganism in its application.

To maintain a high methanogen concentration in a digester, the immobilization of microbial cells on various biofilm carriers has been studied. Picanco et al. (2001) investigated the performance of AD reactors using three types of fibrous biofilm carriers, including activated carbon fiber, polyvinyl alcohol fiber, and glass fiber. The activated carbon fiber carrier performed better than the other two types of carriers in achieving higher amount of biogas and methane production and pollutants' removal. The experimental results also demonstrated that the activated carbon fiber carrier could sustain greater biogas and methane production than the control during the duration of the experiment.

Lo et al. (1984) performed a laboratory-scale comparison of two anaerobic digesters (conventional and fixed-film) for methane production from screened dairy manure. They carried out studies in a 4 L reactor at 30°C with intermittent mixing. In the study, they obtained a maximum methane productivity of 6.33 L day^{-1} from the fixed-film reactor at a loading rate of 672 g VS L^{-1} day^{-1} for one-hour HRT. The authors concluded that a high gas production rate was not sustained in the conventional digester because of bacterial biomass washout at a HRT of less than 6 days.

Vartak et al. (1997) conducted a study to investigate the effectiveness of attached-film bioreactors on psychrophilic AD of dairy manure. They operated eight digesters having a nominal working volume of 5 L (internal diameter, 15.2 cm; height, 30.5 cm) with the temperature varying between 10 and 37°C. In the study, they compared the average performance of bioreactors having different support medium types such as polyester and limestone material. The study concluded that the polyester medium with its high porosity and surface-to volume ratio had the best performance in terms of biogas production, methane production, methane yield, and specific methane productivity at 37°C.

Lo et al. (1986b) studied AD of screened dairy manure using one- and two-phase laboratory-scale AD systems. They reported that separation of acidogenic and methanogenic phases of digestion resulted in a significant increase in methane production rate. Maximum methane production rate of 2.32 L L^{-1} day^{-1} for a fixed-film methane phase reactor was obtained at 1-day HRT.

Suspending contact reactor
Lo et al. (1986a) carried out studies on mesophilic (35°C) digestion of screened dairy manure using an anaerobic rotating biological contact reactor. In the study, 5.5 L reactors were fed with 3.0% VS at a HRT range of 1-11 days. The maximum biogas productivity was found to be 1.89 L day^{-1} at 1-day HRT and the maximum methane yield obtained was 0.093 L g^{-1} VS at 11 days

HRT. The authors reported that the anaerobic rotating biological contact reactor performed better than the fixed-film reactor for methane production.

Comparison of the fixed-film reactor and the suspending contact reactor
The efficiency of the removal of organic matter in fixed-bed reactors is directly related to the characteristics of the support material used for the immobilization of anaerobes, and is greater in a fixed-film reactor than in a fluidized bed reactor (Picanco et al., 2001). After immobilization of anaerobes on polyurethane foam in a thermophilic, fixed-bed, anaerobic digester supplied with acetate, the results of real-time polymerase chain reaction (PCR) analysis indicated that the major immobilized methanogenic archaea were *Methanosarcina* spp., and that the major free-living methanogenic archaea were *Methanosarcina* spp. and *Methanobacterium* spp. Immobilized methanogenic archaea could be present in concentrations 1000 times greater than those in the original anaerobically digested sludge from a completely mixed thermophilic digester supplied with cattle manure. On the other hand, immobilized bacteria could be present at a concentration only 10 times greater. The cell densities of the immobilized methanogenic archaea and bacteria were higher than those of the free-living methanogenic archaea and bacteria in the reactor.

4.4.1.7 Leaching bed reactors (LBR)
Leaching bed reactors (LBR) constitute a promising option for the dry anaerobic biogasification of animal manure. The concept of a leaching bed reactor (also known as percolating anaerobic or dry anaerobic digestion) was developed to convert various organic waste materials containing high levels of particulate matter into methane to serve as a readily usable energy source. This is basically a one-stage column reactor operated in batch mode, in which leachate (or liquor) collected at the bottom of the reactor is continuously recirculated to the top.

Promising results obtained in the AD of the organic fraction of municipal solid waste led to development of different modifications of this reactor, including a two-phase hydrolysis/acidification and methanogenesis process (Chugh et al., 1999; Vieitez et al., 2000). In this process, leachate that is passed over the waste extracts organic acids formed from the initial steps of hydrolysis and fermentation. The organic acids are then passed to a methanogenic reactor that is optimized for methane production. By separating acid formation from methanogenesis, the more sensitive methanogenic system can be operated as a high-rate reactor. The concept of a two-stage leaching bed in a methanogenic reactor has been used effectively for AD of the organic fraction of municipal solid waste, fruit and vegetable wastes (Viturtia et al., 1995), sorghum, food wastes (Ghanem et al., 2001; Han et al., 2002), and poultry mortalities, as well as for biohydrogen production by anaerobic fermentation of food waste (Han and Shin, 2004).

One of the controlling parameters in the anaerobic fermentation process is pH (Myint and Nirmalakhandan, 2009) because the acid production and consumption phases demand different pH levels for optimal performance. As such, the two-phase configuration has been proposed, where the acid production and consumption phases are physically separated from each other to maintain appropriate pH levels in each phase. For example, improvement of cellulose hydrolysis and conversion efficiency by separating the two phases has been demonstrated (Myint and Nirmalakhandan, 2009). Utilizing a leached reactor with leachate recirculation in the first phase

enables the pH in the leached reactor to be controlled by adjusting the recirculation rate as it increases the acidification rate and subsequently decreases the pH (Wang et al., 2002).

Another parameter that can enhance the performance of the leached reactor is the porosity of the bed. In anaerobic hydrolysis of organic solid wastes that have high solid content and fibrous material, mass transfer limitations may hinder the transport of enzymes and leachates within the leached reactor (Chynoweth and Pullammanappallil, 1996). Mass transfer limitations may be reduced by improving the hydraulic conductivity of the bed, which in turn can be controlled by the particle size and volume fraction of solids in the bed. Following the above, it is hypothesized in this study that mixing the organic solid wastes with inert fillers such as pistachios-half-shell can increase the porosity of the leach bed and minimize mass transfer limitations.

Various digester configurations are employed using different approaches such as one-stage or two-stage digesters, wet or dry/semi-dry digesters, batch or continuous digesters (Chowdhury and Fulford, 1992), attached or non-attached biomass digesters, high-rate digesters, and digesters with combination of different approaches (De Baere and Mattheeuws, 2008). Gas production varies considerably with time, and several units must be operated simultaneously to maintain a constant gas supply (Rao and Baral, 2011). The fermentation that takes place with TS content of 6-10% is known as wet fermentation, and with a TS content of more than 20% is known as dry fermentation. It is reported that the fermentation can proceed at TS contents up to 32% (Jewell et al., 1981). Choice of reactor type is driven by waste characteristics, particularly TS content. High TS substrates are mainly treated in CSTRs, while soluble organic waste substrates are treated using high rate biofilm systems such as UASB reactors (Kaparaju et al., 2009).

After comparing the most common types of anaerobic digesters based on technical performance, biological considerations, and reliability, it's clear that the batch digester is the simplest and least expensive type of anaerobic digester (Lissens et al., 2001; Steadman, 1975). Therefore, the batch digester is the most feasible approach for small and medium sized dairy farms, in order to reduce operating costs, and costs for equipment purchasing and maintenance.

4.4.2 Part 2 – Technological and economic challenges limiting success

Temperature is an important factor affecting AD. Several previous studies have demonstrated that the growth rate of microbes is higher—and the AD process becomes faster and more efficient—at thermophilic temperatures, when compared with mesophilic temperatures (Krishania et al., 2013a). For cattle manure, which has a high content of coarse fibers, thermophilic AD can facilitate decomposition (Nasir et al., 2012). However, AD at thermophilic temperatures can lead to high risk of ammonia inhibition, as the temperature increase can result in an increase in ammonia toxicity (Weiland, 2010). Total solids content is also a significant factor affecting AD. While a high TS content can enhance the treatment capacity of AD, it can also lead to ammonia inhibition of the AD process.

High TS AD is popular for optimizing the treatment capacity of livestock waste and economizing water resources. From the perspective of liquid effluent recycling, high TS can lead to little or no effluent emission, which is environmentally beneficial. Previous work has mainly focused on the

effects of TS or temperature on AD of cattle manure. Anaerobic digestion of cattle manure at thermophilic temperatures and high TS content is significant and practical from the perspective of environmental protection and energy recovery. Several previous studies have focused on chicken manure for methane recovery through AD. However, few studies exist on AD of cattle manure at thermophilic temperature with high TS due to the ammonia inhibition that occurs under these conditions. Overcoming this issue will be important for the widespread adoption of AD technology.

There have been several attempts to avoid the accumulation of ammonia during methane fermentation. Cattle manure diluted with water can be treated anaerobically either in a semi-solid form containing 10-11.5% TS (Bujoczek et al., 2000), or in a wet form containing 0.5-3% TS (Rao et al., 2008; Yetilmezsoy and Sakar, 2008). A few studies have demonstrated that the acclimation of the methanogenic consortia of microorganisms to high ammonia levels was effective in raising ammonia tolerance for methane production. Although the spontaneous acclimation of methanogenic consortia to high levels of ammonia is well demonstrated, the process of acclimation takes quite a long time and the methane yield of 31 mL g^{-1} VS in the treatment of cattle manure was low (Abouelenien et al., 2009a).

Physicochemical or biological removal of ammonia from methanogenic sludge is another approach to reducing ammonia inhibition. Physicochemical methods include chemical precipitation processes such as the magnesium ammonium phosphate process, and the zeolite and clay process (Tada et al., 2005). Nitrification/denitrification processes and anaerobic ammonia oxidization (ANAMMOX) process constitute the biological methods for ammonia removal (Dong and Tollner, 2003). Effectiveness of all the methods mentioned above, however, relies on dilution of the manure to a TS level of 0.5-3.0% (Chen et al., 2008). Stripping of ammonia from wastes in a liquid form has been useful for the removal of ammonia from AD effluent as well as from poultry litter leachate. A few other studies have also reported the application of ammonia stripping from dehydrated waste-activated sludge and cattle manure that had a high TS content. However, previous studies used ammonia stripping only for the removal of the ammonia produced, as a separate step. Further improvements were necessary to reduce the cost and the time consumed by the multi-step process of ammonia stripping during dry fermentation of cattle manure.

4.4.3 Part 3 – Evaluation of strategies to overcome challenges

The above challenge can be addressed through ammonia stripping coupled with AD for methane production. To the best of our knowledge, at present there are no reports of previous studies on ammonia fermentation coupled to ammonia stripping in a single reactor system.

4.4.3.1. Thermophilic AD of dairy manure at different high solids concentrations

Process performance
The daily methane production for 6%, 8%, and 10% TS are shown in Figure 4.2. Each of the three treatments had clear peaks appear during AD. In addition, for 6%, 8%, and 10% TS, the peaks in daily methane production appeared on day 14, day 16, and day 18, respectively, and were 13.8 L kg^{-1} VS, 24.3 L kg^{-1} VS, and 14.7 L kg^{-1} VS, respectively. For 8% TS, the process of

methane production was not stable from days 16 to 20. The level of daily methane production was higher for 8% TS than for 10% TS. In addition, AD for 8% TS continued until day 40. Low organic matter content for 6% TS is a major reason that methane production dropped off. As a result, daily methane production was greater for 8% TS than for 6% or 10% TS.

In terms of methane content, the values for the three treatments were generally similar (Figure 4.3). For 6%, 8%, and 10% TS, the methane content was greater than 50% on day 10, day 10 and day 14, respectively (Brown and Li, 2013; Zhu et al., 2010). Values for 8% and 10% TS were 51.8-63.5%, 55.7-69.2%, and 57.4-64.8%, respectively. Therefore, for 10% TS, stable status was reached 4 days later than for 6% and 8% TS.
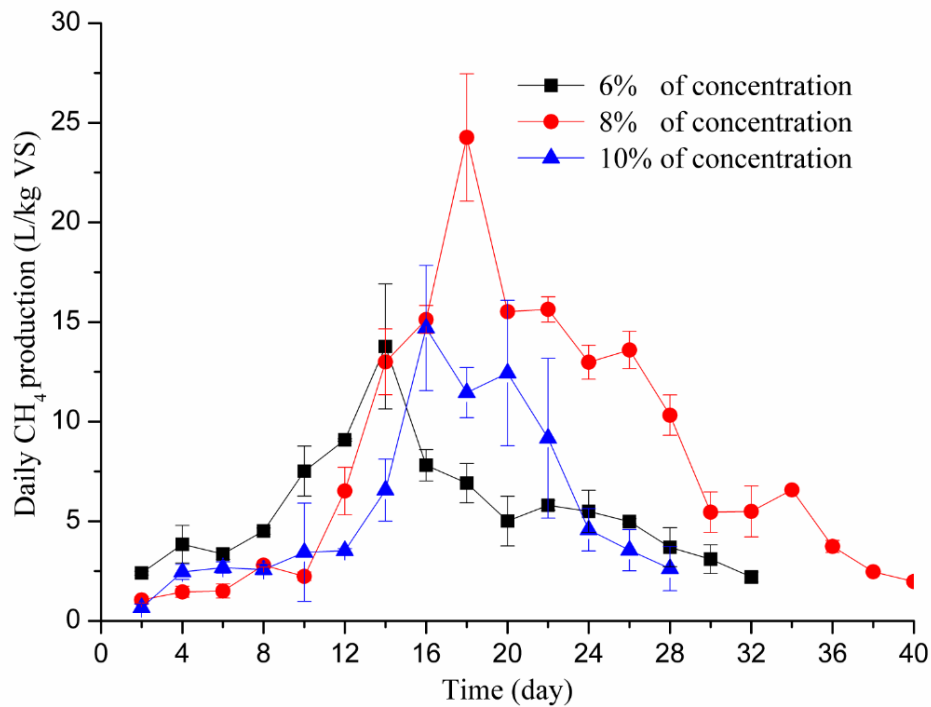
Figure 4.2: Daily methane production from anaerobic digestion of dairy manure at different levels of total solids
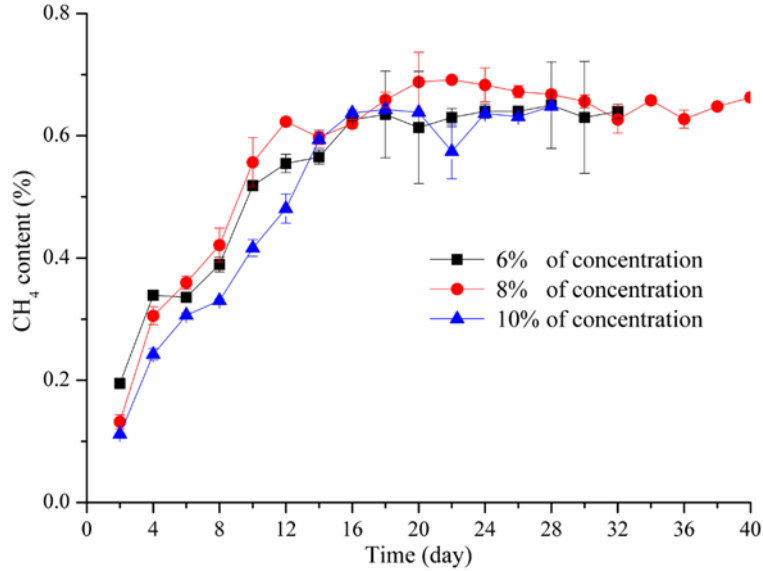
Figure 4.3: Methane content from anaerobic digestion of dairy manure at different levels of total solids

Soluble chemical oxygen demand (COD), total ammonia nitrogen (TAN), and pH changes
Levels of soluble COD were associated with TS (Figure 4.4). Levels of soluble COD for 6% TS were the lowest, while the highest level of soluble COD was for 10% TS. This can be attributed to the variation of organic matter content with the TS content. Soluble chemical oxygen demand for 10% TS fluctuated during the AD process due to high organic matter content. The soluble COD for 6% and 8% TS followed a similar trend, increasing during the initial period of AD and followed by a slow decrease. This was generally in accordance with their corresponding trends of daily methane production. The initial slow increase in methane production was due to the limited number of microbes and the rate-limiting hydrolysis step (Yao et al., 2013). As particulate matter or complex particulate compounds were converted into soluble substrate (soluble COD) and the population of hydrolytic bacteria increased and acclimated, there was in increase in daily methane production. After most of the soluble COD was utilized by microbes, daily methane productions decreased.

Like soluble COD, TAN levels were associated with TS (Figure 4.5). As reported previously, the acidification and methanogenesis stages can be negatively affected by the concentration of free ammonia (Nasir et al., 2012). It was reported that ammonia inhibition occurred at an ammonia content of 1.2 g L^{-1} at the pilot scale (Mata-Alvarez et al., 2000). For 6% TS, the level of TAN was the lowest, and remained below 1.2 g L^{-1} through the whole AD process, meaning that no ammonia inhibition occurred. When TS was increased from 6% to 8% and 10%, TAN content increased substantially and was much higher than 1.2 g L^{-1}.

71
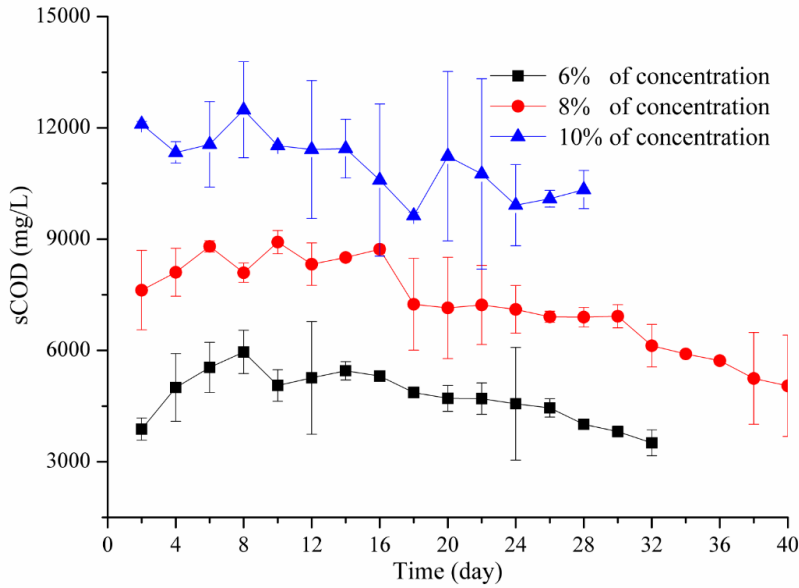
Figure 4.4: Soluble chemical oxygen demand (COD) concentrations at different levels of total solids
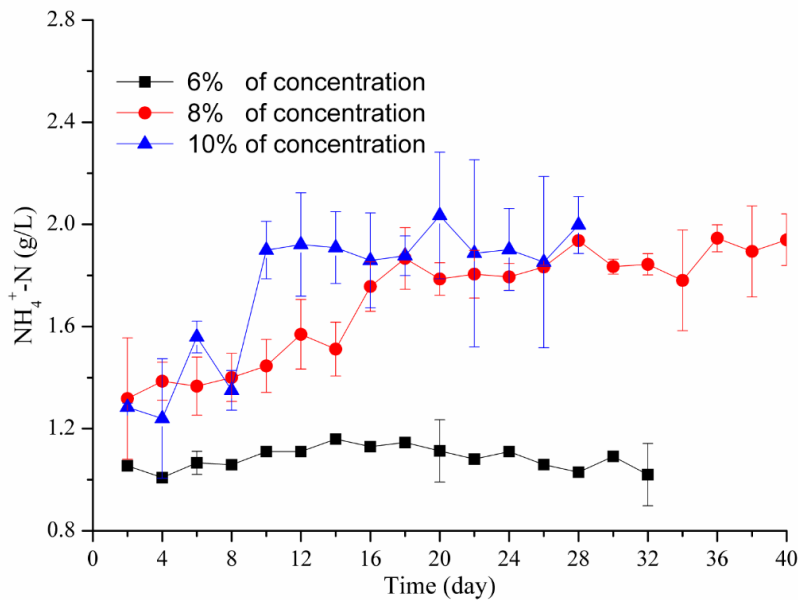
Figure 4.5: Total ammonia nitrogen (TAN) concentrations during anaerobic digestion of dairy manure for different levels of total solids

Some studies have found that ammonia inhibition occurred in the range of 1.5-3.0 g L^{-1}. Ammonia is claimed to be toxic irrespective of pH when present at concentrations greater than 3 g L^{-1} (Calli et al., 2005). Total ammonia nitrogen levels for 8% and 10% TS increased during the AD process and stabilized at about 1.8 g L^{-1}, indicating that ammonia inhibition was occurring. This is likely the reason that the peaks of daily methane production for 8% and 10% TS appeared

later than for 6% TS. Levels for 10% TS were the highest (1.8-2.0 g L^{-1} after 8 days of AD), so the AD process was inhibited much more seriously than with 8% TS and ceased on day 28. In the pH range of 6.5-8.5, methanogenic activity decreased with the increase in TAN content, and dropped to 0 at 5.88-6.6 g L^{-1} of TAN. In other studies, the effect of ammonia inhibition can be intensified with a TS content higher than 5%, when chicken manure was used as the substrate for AD (Bujoczek et al., 2000). The inhibitory effect occurred at a lower TS content with chicken manure because it has a higher initial TAN content than cattle manure.

Trends for pH were similar for different TS values (Figure 4.6). First, they decreased, then increased slowly and maintained a stable level. pH ranged from 6.45-8.55, similar to the wide pH range of 6.5-8.5 for AD noted by Mata-Alvarez et al. (2000). For the initial period of AD, the decrease in pH could be attributed to the acidification of the coarse fibers not digested by the cow. After that, pH increased along with an increase in the release of ammonia. This phenomenon also occurred with chicken manure as substrate for methane production at thermophilic temperature and high TS (Abouelenien et al., 2010).
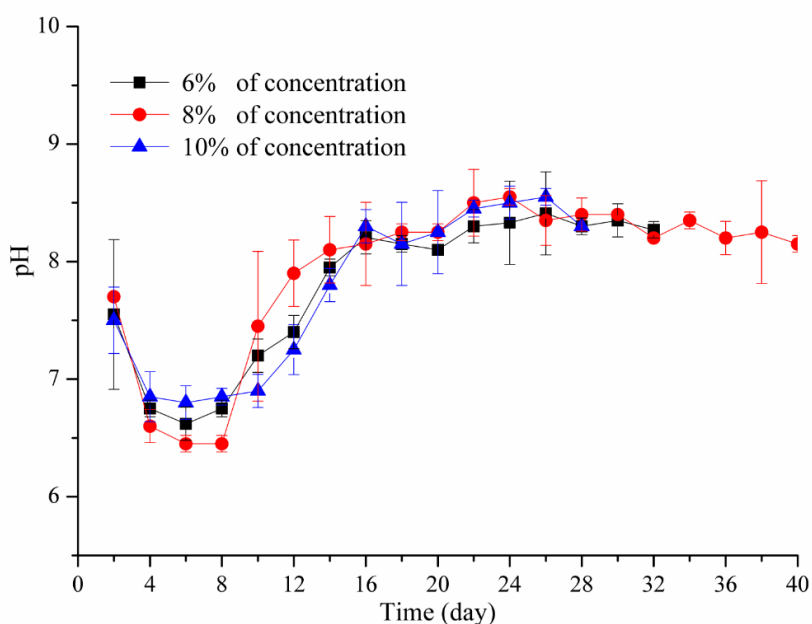
Figure 4.6: pH values during anaerobic digestion of dairy manure at different levels of total solids

Cumulative methane production
Cumulative methane production for 6%, 8%, and 10% TS was 89.5 L kg^{-1} VS, 161.7 L kg^{-1} VS, and 80.4 L kg^{-1} VS, respectively (Figure 4.7). The cumulative methane production obtained with 8% TS was 1.8-fold and 2.0-fold more, respectively, than methane production with 6% and 10% TS. Despite the fact that there was no ammonia inhibition at 6% TS, the cumulative methane production was the lowest. Although there was some ammonia inhibition that occurred for 8% TS, the inhibitory effect was not severe.

73
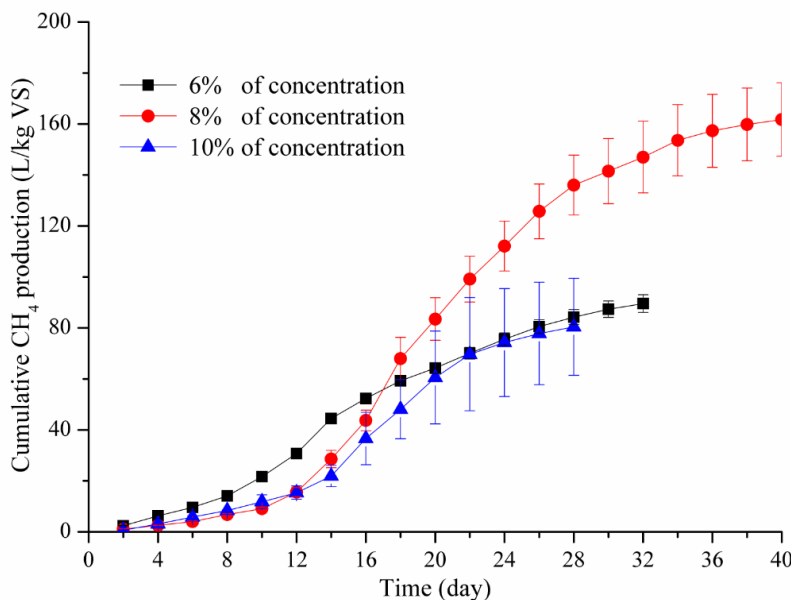
Figure 4.7: Cumulative methane production during anaerobic digestion of dairy manure at different levels of total solids

In summary, severe ammonia inhibition during thermophilic AD of dairy manure occurred at 10% TS, and the total methane production was the lowest for this treatment. For this reason, 10% TS was established as the threshold for conducting simultaneous ammonia stripping experiments.

4.4.3.2. Simultaneous ammonia stripping for thermophilic AD of dairy manure at the threshold solids concentration (10% TS)

Process performance

Simultaneous ammonia stripping at 1 L min^{-1} was beneficial for the enhancement of process performance (Figure 4.8). Levels of daily methane production at different gas flow rates can be ranked as 1 L min^{-1} > 3 L min^{-1} > control > 5 L min^{-1}, with the highest level of daily methane production obtained at 1 L min^{-1}. Distinct peaks were observed at 1 L min^{-1} and 3 L min^{-1}, with methane production of 23.6 L kg^{-1} VS day^{-1} and 21.3 L kg^{-1} VS day^{-1}, respectively. For the control, the highest peak (5.0 L kg^{-1} VS day^{-1}) appeared on day 14, which was 2 days later than those at 1 L min^{-1} and 3 L min^{-1}. These results indicate that simultaneous ammonia stripping was beneficial for thermophilic AD at the threshold TS content. However, at 5 L min^{-1}, the level of daily methane production was even lower than the control and the lowest of any of the treatments. The stripping rate is important for thermophilic AD of substrates with a high nitrogen content. High stripping rate has some disadvantages such as water evaporation, foaming, and cooling of the substrate (De la Rubia et al., 2010; Liao et al., 1995). Loss of organic materials due to high stripping rate may also contribute to a reduction in daily methane production (Zhang et al., 2012). Another possible cause of the low level of daily methane production resulting from the high stripping rate may be that the high stripping rate disturbs energy and nutrient transfer between microbes in the AD system.
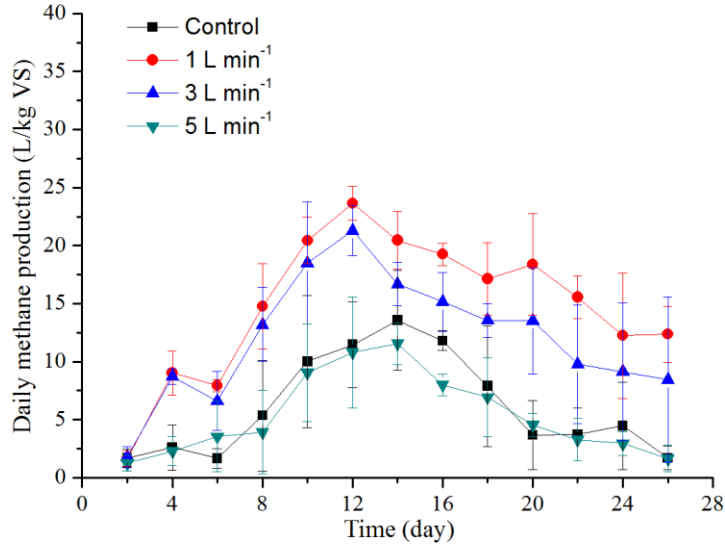
Figure 4.8: Daily methane production at different stripping rates

Trends for methane content of the treatments were similar except for that of 5 L min^{-1} (Figure 4.9). For the control, 1 L min^{-1}, 3 L min^{-1}, and 5 L min^{-1} treatments, methane content reached 50% on day 10, day 6, day 6, and day 8, respectively. Therefore, at 1 L min^{-1} and 3 L min^{-1}, duration of the start-up period and time required to reach stable status were the shortest, which was four days shorter than for the control. Similar to results for daily methane production, methane content at 1 L min^{-1} was the highest (56.5-75.5%). The methane content of the 5 L min^{-1} treatment had great fluctuation throughout the AD process and the lowest level (44.5-61%). Methane content for the control and the 3 L min^{-1} were 55.9-65.1% and 61.9-68.5%, respectively. As with the daily methane production results, methane content ranked as: 1 L min^{-1} > 3 L min^{-1} > control > 5 L min^{-1}. These results indicate that stripping rate at 1 L min^{-1} reduced the time used for reaching stable status and improved methane content.
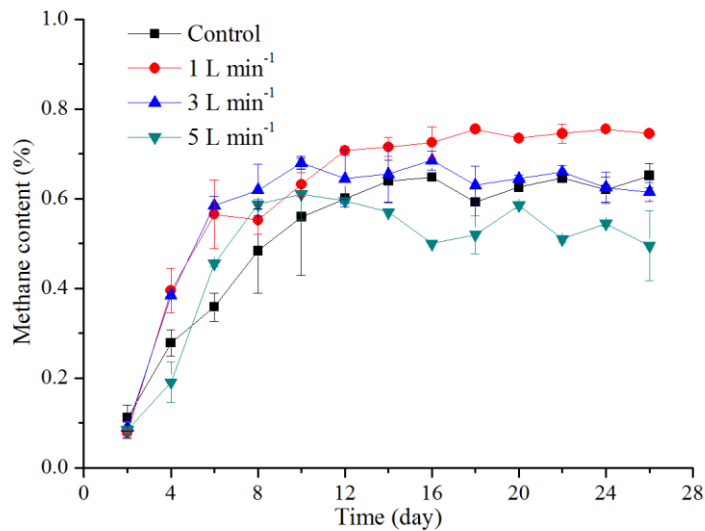
Figure 4.9: Methane contents at different stripping rates

Soluble chemical oxygen demand, total ammonia nitrogen, and pH changes

Simultaneous ammonia stripping at 1 L min⁻¹ was beneficial for the utilization of available substrate and for the removal of ammonia inhibition. Soluble chemical oxygen demand was used as an indicator of the level of available substrate for microorganisms in the AD system. For all the experiments, soluble COD content experienced initial increases followed by a slow decrease (Figure 4.10). This was in general accordance with their corresponding trends for daily methane production. The reason was similar to that mentioned previously. This result is in agreement with previous studies (Zheng et al., 2009). When most of the soluble COD was utilized by microbes, daily methane production decreased accordingly. For control, the peak appeared on day 8, which was later than the peaks in the other treatments. According to Abouelenien et al. (2009b), the microbes responsible for decomposing substrate were inhibited by high levels of ammonia (Figure 4.11) (Abouelenien et al., 2009b). However, after a long period of microbial acclimation to the high levels of ammonia, an increase of soluble COD release occurred. The level of soluble COD for the control was the highest. This can be attributed to ammonia inhibition of methanogenesis, which led to the accumulation of available substrate that could not be utilized by methanogens for methane production. However, the level of soluble COD at 1 L min⁻¹ was the lowest among all treatments, suggesting that microbial activity at 1 L min⁻¹ consumed the available substrate throughout the AD process.

76
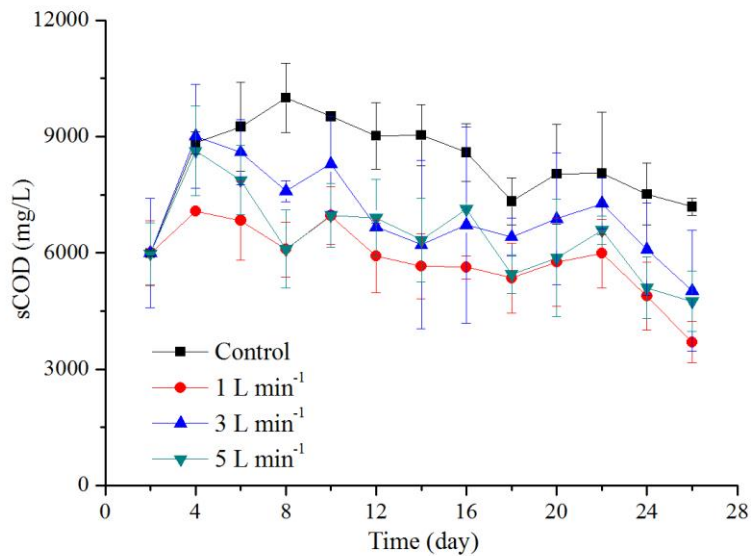
Figure 4.10: Soluble chemical oxygen demand (soluble COD) at different stripping rates
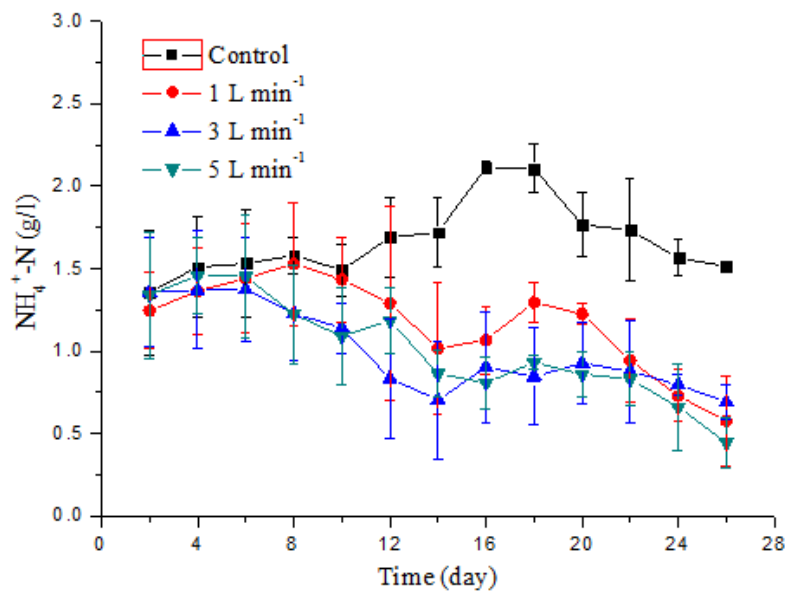
Figure 4.11: Total ammonia nitrogen concentrations at different stripping rates

Changes in pH for the control, 1 L min^{-1}, 3 L min^{-1}, and 5 L min^{-1} treatments were similar: an initial decrease in pH followed by a slow increase (concurrent with an increase in ammonia release), and then maintenance of a stable level (Figure 4.12). The pH values were 6.45-8.55, which were within the suitable pH range of 6.5-8.5 for AD (Mata-Alvarez et al., 2000). In

general, animal manure has higher moisture, nutrient, salt contents, and higher pH buffer capacity than food scraps, which is beneficial for maintaining pH stability in an anaerobic digester (Angelidaki and Ellegaard, 2003). The pH of a digester is usually used to indicate the process stability. pH level is associated with the level of VFAs in an AD system (Liew et al., 2011; Yao et al., 2013; Yao et al., 2014a; Yao et al., 2014b). An acidic environment is caused by the accumulation of VFA, which leads to a decrease in pH (Liew et al., 2011). Therefore, low pH is associated with a high level of VFAs and vice versa. For the initial period of AD, the decrease in pH can be attributed to the acid from the cow's digestive system (Nasir et al., 2012). The accumulation of acidic products, such as acetate, usually occurs in the initial period of AD (Abouelenien et al., 2010). The accumulation of acidic products leads to a decrease in pH, which reduces the efficiency of ammonia fermentation (Abouelenien et al., 2010).
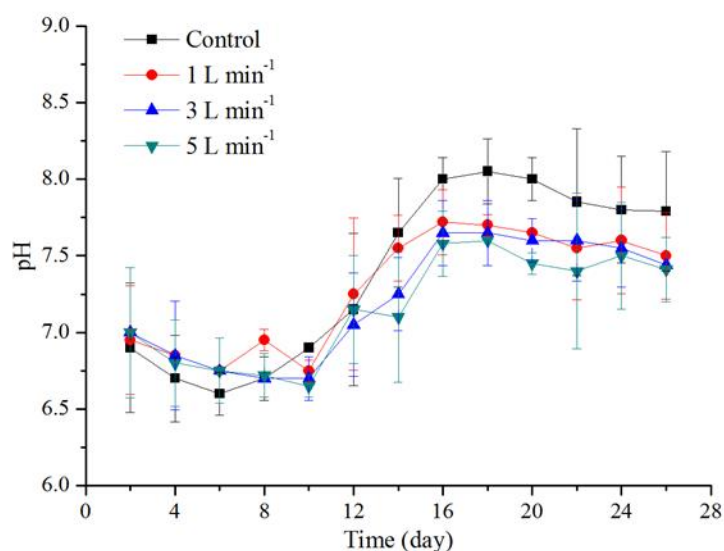
Figure 4.12: pH at different stripping rates

The level of TAN in the initial period of AD increased slowly and maintained a relatively stable state (Figure 4.11). After a period of adaptation and breeding of methanogens, acidic products were further utilized for methane production, in agreement with a previous study (Yao et al., 2013), and the pH increased accordingly (Figure 4.12). The increase in pH leads to an increase in TAN (Chen et al., 2008). As a result, TAN level for the control increased relatively quickly starting around day 10 (Figure 4.6B). During days 14-20, the level of TAN was the highest, which was in line with low pH values (Figure 4.6C). Values of TAN for the control were 1.5-2.1 g L^{-1} within the range of 1.5-3.0 g L^{-1} that leads to ammonia inhibition in AD (McCarty, 1964). However, for the experiments with simultaneous ammonia stripping, the levels of TAN were lower than the 1.5-3.0 g L^{-1} (McCarty, 1964), thus it was unlikely that ammonia inhibition occurred.

Efficiency of ammonia removal was 53.5%, 44.4% and 64.4% at 1 L min^{-1}, 3 L min^{-1}, and 5 L min^{-1}, respectively. Efficiency of methane production at 1 L min^{-1} was the highest among all

treatments. The highest stripping rate (5 L min^{-1}) led to the loss of organic matter as mentioned above, and the resulting mass loss may explain the high efficiency of ammonia removal. Ammonia removal at 3 L min^{-1} was the lowest. Comparatively, efficiency of ammonia removal for previous studies was higher, where 88.2% ammonia removal was obtained at fermentation conditions involving ammonia stripping for 12 hours and higher pH (9.0, 9.5, 10.0, and 11.0) (Zhang et al., 2012). Ammonia removal of 78% was obtained at pH 12 by Lei et al. (2007), who applied ammonia stripping to AD effluent (Lei et al., 2007). Ammonia removal of 75-95% was reached by applying ammonia stripping for poultry leachate at 1-2% TS.

There are some reasons for the lower efficiency of ammonia removal obtained in this study (44.4-64.4%), compared to traditional ammonia stripping. First, higher pH is usually used for a separate ammonia stripping process in systems without AD (Abouelenien et al., 2009a; Zhang et al., 2012). Second, TS is usually lower during ammonia stripping, which allows better gas-liquid association to trap more ammonia due to higher fluidity (Abouelenien et al., 2010; Rao et al., 2008). And third, a higher temperature is used for the separate ammonia stripping process (Nakashimada et al., 2008). However, these parameters (high pH ($\geq$9.0), high temperature ($>$ 60°C), and low TS are not economically feasible in this situation because of energy requirements and cost. For these reasons, creating these parameters to improve ammonia stripping is only feasible when the ammonia stripping is a separate stage, not when AD is coupled with ammonia stripping.

Cumulative methane production
The maximum cumulative methane production was obtained at 1 L min^{-1}. Cumulative methane production for the control, 1 L min^{-1}, 3 L min^{-1}, and 5 L min^{-1} treatments were 84.7 L kg^{-1} VS, 192.3 L kg^{-1} VS, 168.8 L kg^{-1} VS, and 68.5 L kg^{-1} VS, respectively. The maximum cumulative methane production was obtained at 1 L min^{-1}, which was 127.0%, 13.9%, and 180.7% higher than those of the control, 3 L min^{-1}, and 5 L min^{-1} treatments, respectively (Figure 4.13), so the improvement in cumulative methane production was significant ($p < 0.05$). The value obtained at 5 L min^{-1} was the lowest. The reasons behind the highest stripping rate affecting the methane yield include mass transfer and the loss of organic matter. The low methane production of the control can be attributed to ammonia inhibition, as mentioned above. Decreases in methane production in thermophilic AD of nitrogen-rich manure has been attributed to the accumulation of ammonia (Lei et al., 2007; Nakashimada et al., 2008; Rao et al., 2008). Simultaneous ammonia stripping at 55°C, instead of a higher temperature and without pH adjustment, is cost efficient. Therefore, dairy manure could be used for efficient methane production via simultaneous ammonia stripping at a flow rate equivalent to 1 L min^{-1}.

Substrate degradation
The highest level of substrate utilization was obtained at 1 L min^{-1}. Reductions in TS and VS were calculated to evaluate the biodegradability of the substrate and the efficiency of methane production at different stripping rates. The greatest reductions of TS and VS (34.4% and 41.3%, respectively) were obtained at 1 L min^{-1} (Table 4.2), and were in line with the maximum cumulative methane production. Compared to the control, TS and VS reduction were enhanced by the ammonia stripping process by 251% and 250%, respectively. In general, greater degradation is associated with greater methane production. However, in this study, cumulative methane production of the control was greater than that of the 5 L min^{-1} treatment, while TS and VS reductions for the control were lower than those of the 5 L min^{-1} treatment. This may be due to the high stripping rate of 5 L min^{-1} leading to the loss of organic substrate (Zhang et al., 2012).

The optimal stripping rate of 1 L min^{-1} was beneficial for the prevention of ammonia inhibition and led to a high efficiency of feedstock degradation. Higher stripping rates led to the loss of volatile matter, while the control with no ammonia stripping led to the accumulation of ammonia and subsequent ammonia inhibition. These results indicate that a stripping rate of 1 L min^{-1} was the most effective for the utilization of dairy manure for methane production.
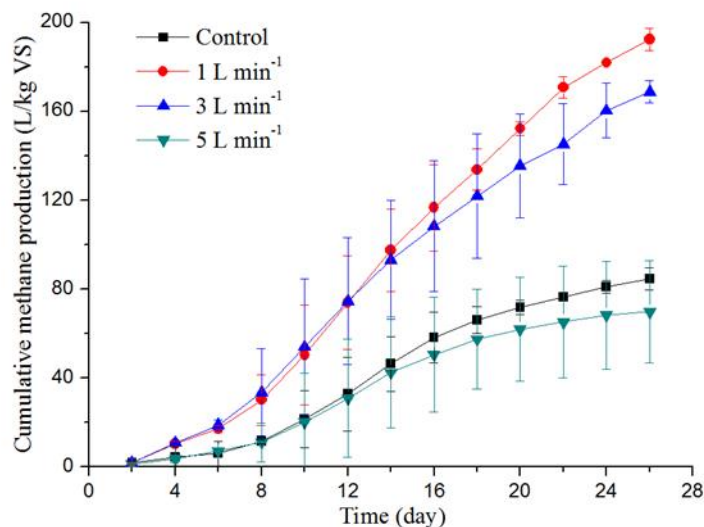
Figure 4.13: Cumulative methane production at different stripping rates

Table 4.2: Total solids and volatile solids before and after anaerobic digestion and degradation

		Control	1 L min^{-1}	3 L min^{-1}	5 L min^{-1}
Before AD (g)	Total solids	52.1	52.1	52.1	52.1
	Volatile solids	42.0	42.0	42.0	42.0
After AD (g)	Total solids	46.9 ± 1.1	34.1 ± 2.5	36.9 ± 1.1	46.2 ± 6.3
	Volatile solids	37.0 ± 5.7	24.7 ± 1.8	27.3 ± 4.7	36.5 ± 4.2
Degradation (%)	Total solids	9.8 ± 4.2	34.4 ± 1.1	29.1 ± 3.9	11.1 ± 7.3
	Volatile solids	11.8 ± 2.8	41.3 ± 1.9	34.9 ± 4.7	13.1 ± 2.4

4.5 Conclusions and recommendations

It is necessary to improve the efficiency of energy recovery in digesters for small- and medium-sized dairies in order to make adoption of this technology economically feasible. Inhibition of thermophilic AD due to ammonia accumulation is a major challenge in these systems. Inhibition

of AD occurred at a threshold level of 10% TS. The results show that simultaneous ammonia stripping helped to overcome ammonia inhibition that occurred in thermophilic AD at the threshold TS. The optimum stripping rate was identified as 1 min L^{-1} and a maximum methane production of 192.3 L kg^{-1} VS was achieved. Overall, this approach has the potential to improve the efficiency of dairy manure utilization for methane production. The proposed approach simplifies the system by combining ammonia stripping and thermophilic AD within the same digester, making this process simpler and more cost effective than other reported options. Therefore, for small- and medium-scale dairy digesters that are not economically sustainable, this approach makes it possible to digest manure with a high TS content, improve methane production, and reduce wastewater discharge while recovering nutrients. Because this approach demonstrated satisfactory efficiency in this study, the simultaneous ammonium stripping technique coupled with small- and medium-scale thermophilic AD and high TS can be applied to other nitrogen-rich materials like food scraps, though the technique needs to be optimized for those specific conditions. As a result, the developed technique has the potential to enhance the availability of small- and medium-scale AD in Washington State.

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5. Engineered Industrial Biochar Adsorbents as Alternatives for Nutrient Management and Removal of Escherichia coli from Anaerobic Digester Effluent

Michael Ayiania, Matthew Smith, Alex Dunsmoor, Waled Suliman, and Manuel García-Pérez

5.1 Background

The use of biochar for the recovery of nitrogen (N) and phosphorus (P) from anaerobic digestion (AD) processes has the potential to mitigate environmental issues such as greenhouse gas emissions and nutrient runoff leaching in soils, a serious and persistent problem in agriculture. Washington State University's (WSU) biochar program is advancing the design and use of biochar to adsorb and retain nutrients from AD effluent. This research shows promise for addressing environmental concerns due to nutrient runoff and creating a valuable market for engineered biochar, as well as for improving the hydrological and microbial transport properties of marginal soils.

Spectroscopic methods currently used to characterize the biochar adsorbents were originally developed for the characterization of graphitic carbons and, hence, are inaccurate for the analysis of the amorphous biochar produced from AD fibers. To better understand the chemical make-up of biochar and to better understand the adsorption mechanism of pollutants towards biochar, there is a need to compared spectroscopic data collected from Raman, nuclear magnetic resonance (NMR) and X-ray photoelectron spectroscopy (XPS) with simulated spectra and examine for a wide variety of ordered and defective polyaromatic structures.

This biennium, the research team aimed to develop biochar adsorbents as one of several alternative technologies that are currently proposed for large-scale AD biorefineries and small-scale anaerobic digesters. Specifically, researchers targeted the development of carbonaceous adsorbents for the removal of hydrogen sulfide (H_2S) in mixture with methane (CH_4) for biogas purification, ammonia, phosphate, and *Escherichia coli* from aqueous effluents; as well as the development of new analytical techniques for biochar characterization using density functional theory (DFT) calculation.

5.2 Objectives

The objective of this project was to develop an engineered biochar from AD fiber and Douglas fir to perform the following tasks: (1) adsorption of H_2S from biogas, (2) removal of phosphorus from the liquid effluent of an anaerobic digester, (3) adsorption of ammonia gas, and (4) retention of *E. coli* using biochar with nitrogen functional groups.

To accomplish these tasks, the research team developed the biochar surface through physical (carbon dioxide) and chemical (phosphoric acid) activation. They also produced biochar with nitrogen functional groups, which they hypothesized would be suitable for *E. coli* retention. The team also developed new analytical techniques for the analysis of biochar using density functional theory calculation, and thereby characterized the resulting activated biochar.

5.3 Methods

5.3.1 Development of novel spectroscopic methods for biochar characterization

Spectroscopic methods currently used to characterize biochar adsorbents include Raman, nuclear magnetic resonance, and X-ray photoelectron spectroscopy. These methods, however, were developed for the characterization of graphitic carbons and are inaccurate when applied to the amorphous biochar produced from AD fiber. To better understand the spectroscopic data collected from Raman, NMR, and XPS, simulated spectra were generated and examined for a wide variety of ordered and defective polyaromatic structures. These structures were simulated using density functional theory level calculations on the high-performance cluster at WSU. A Becke 3 Lee Yang Parr exchange correlation function was used with an augmented double (Raman) or triple (NMR and XPS) zeta basis set, 6-31G(d) and 6-311G(d,p), respectively. Raman and NMR spectra were determined directly from the calculated polarizability and shielding tensors, respectively. The Raman response was corrected by an empirical linear factor proposed by Halls et al. (2001), while the NMR data shifted relative to tetramethyl silane. The XPS signals were determined by calculating the binding energy of 1s electrons for each atom and assuming the initial state approximation first proposed by Tian et al. (2015).
To ensure that accurate reproduction of real spectra was achieved, several reference compounds were examined for each method, including: naphthalene (Raman), pyrene (XPS and Raman), benzo[a]pyrene (Raman), benzo[g,h,i]perylene (Raman), perylene (Raman), coronene (Raman), tetracarboxylic perylene (Raman, XPS, and NMR), and cellobiose (XPS). The theoretical carbon structures examined were formed from various regular polyaromatic structures such as coronene, circumpyrene, and circumcoronene. These structures were modified by the inclusion of single and double point defects (one or two carbons removed), with hydrogen or oxygen added, and the structure rearranged to maintain a neutral charge. These defects resulted in several non-hexagonal ring systems, including pentagonal and heptagonal rings, as well as large irregular gaps within the aromatic structure. To examine the isolated effect of pentagonal and heptagonal rings in larger systems, these rings were placed in the center of a theoretical molecule and surrounded by hexagonal rings. In addition to these defects the edge of coronene structures was modified with various oxygenated groups, including: hydroxyls, carbonyls, lactones, and carboxyls. Each of the theoretical spectra were examined for trends, which were used to construct a series of modified deconvolution parameters and interpretations for peak fitting of real biochar spectra.

The new methods developed were tested by examining the Raman, XPS, and NMR spectra of a thermoseries of cellulose biochars produced at 300°C to 700°C using a spoon reactor at WSU. Treatment times were 30 minutes with a nitrogen sweep gas used to minimize vapor-biochar interaction. Following treatment, samples were allowed to cool to 25°C under nitrogen, to

minimize reaction with atmospheric oxygen. Biochar samples were mixed with spectroscopic grade potassium bromide (KBr) at a 1:20 w/w (weight to weight) ratio for Raman analysis. Raman spectra were collected using a Horiba LabRAM Raman microscope equipped with a 50 mW 532 nm laser. Signals were collected using a 99% filter to minimize potential heating. Final spectra are the average of three to five spots each based on four scans and smoothed using a five-point boxcar average. The XPS spectra were collected using a Kratos Axis 165 XPS system with a MgKα source operated at a pass energy of 40eV. The system was calibrated using gold. Present charging was corrected using a flood gun. The NMR spectra were collected using a Bruker Avance 400 NMR. A multiple-pass, cross-polarization sequence adapted from Johnson and Schmidt Rohr (2014) was used for polarization with signals recorded on a Hahn Echo. Dephasing was achieved using either standard gated dephasing for times less than one rotor period, or a long range REDOR-type method (Mao and Schmidt-Rohr, 2003) for longer periods.

5.3.2 Production of carbonaceous adsorbents for H_2S adsorption

The adsorption tests were carried out in vertically-oriented glass tubes. In each experiment, 300 mg of activated biochar were packed in the tube. The tests were conducted at atmospheric pressure and at room temperature. The simulated gas used contained 2000 ppm of H_2S, 65% CH_4, and the balance was carbon dioxide (CO_2). Hydrogen sulfide was then passed through the column of adsorbent at a rate of 10 mL per minute. The flow rate of gas was controlled by a volumetric flow meter. A 0.01 N hydrochloric acid (HCl) solution (500 mL, using tap water) was employed to moisturize the biogas before reaching the column. The concentration of H_2S was monitored using a gas chromatography analyzer (GC; Varian GC3800, equipped with a CP-Silica PLOT 50m x 0.53mm x 4µm column) with a computer data acquisition program. The breakthrough concentration was set to be 10% of the initial concentration of H_2S. The test was allowed to reach the saturation point of the activated biochar before it was stopped (Figure 5.1 is a schematic representation and Figure 5.2 is a photo of the H_2S adsorption setup).

5.3.3 Integration of fiber pretreatment with existing AD technologies

Anaerobic digestion fiber was received from Regenis and Andgar Company. Preliminary studies indicated that deposition of calcium on the fiber surface prior to pyrolysis can strongly improve phosphate retention of the resulting biochars. This deposition can be achieved through pH elevation in a calcium-rich solution. In this study, the biochars resulting from the fibers activated with CO_2 were investigated. A sodium phosphate solution buffered to pH 7 was used to test these biochars for phosphate removal.
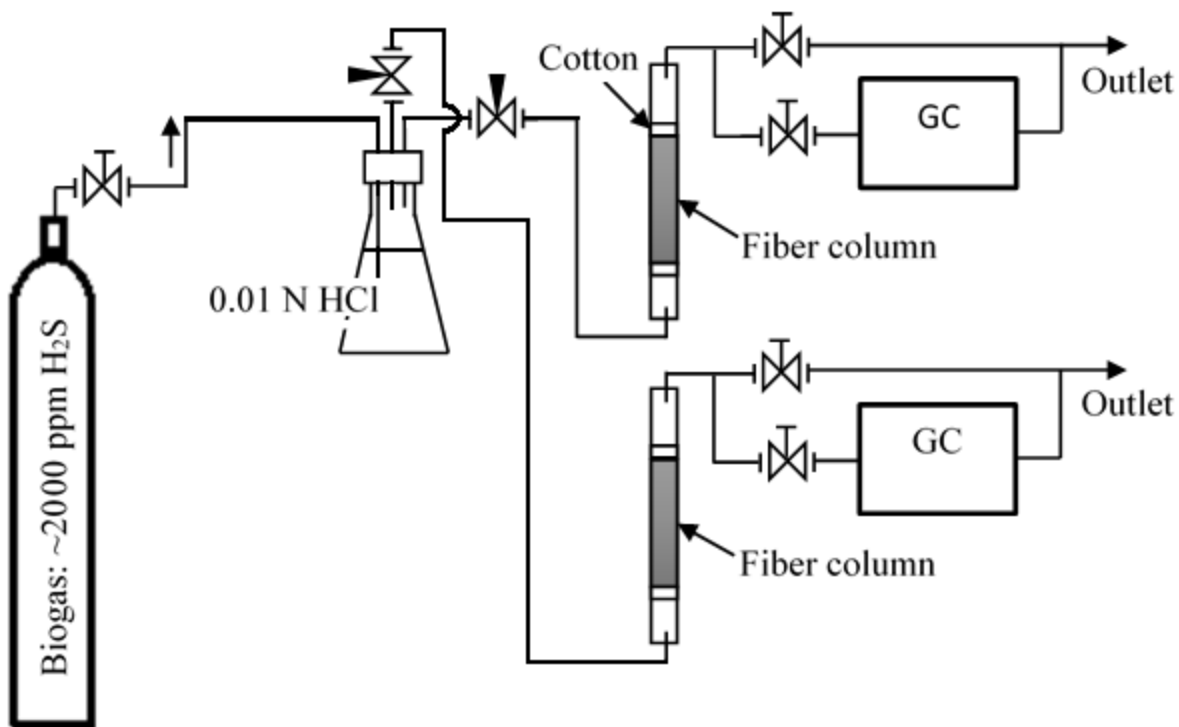
Figure 5.1: Schematic of the experimental setup designed to carry out the hydrogen sulfide breakthrough experiments

5.3.4 Biochar surface area development (Physical Activation)

A series of biochars, activated physically with CO_2, were produced at temperatures between 350°C and 800°C from AD fiber in a tube furnace reactor. Briefly, the sample was introduced into the furnace under nitrogen flow for a period of 30 minutes at 25°C. Later, the temperature began to increase from 25°C to the expected final temperature at a heating rate of 10°C per minute. After the final temperature was reached, the sample underwent a two-stage process (carbonization and activation) for a total residence time of two hours. The first stage involved carbonization of the sample to biochar under nitrogen flow, while the second stage consisted of switching from nitrogen gas to CO_2 for activation of the biochar for one hour. A flow rate of 500 mL per minute was employed in the reactor zone to minimize vapor-biochar interactions. Samples were then cooled to temperatures below 25°C under nitrogen gas, before exposure to air. The activated carbon obtained was characterized and used for the adsorption studies.
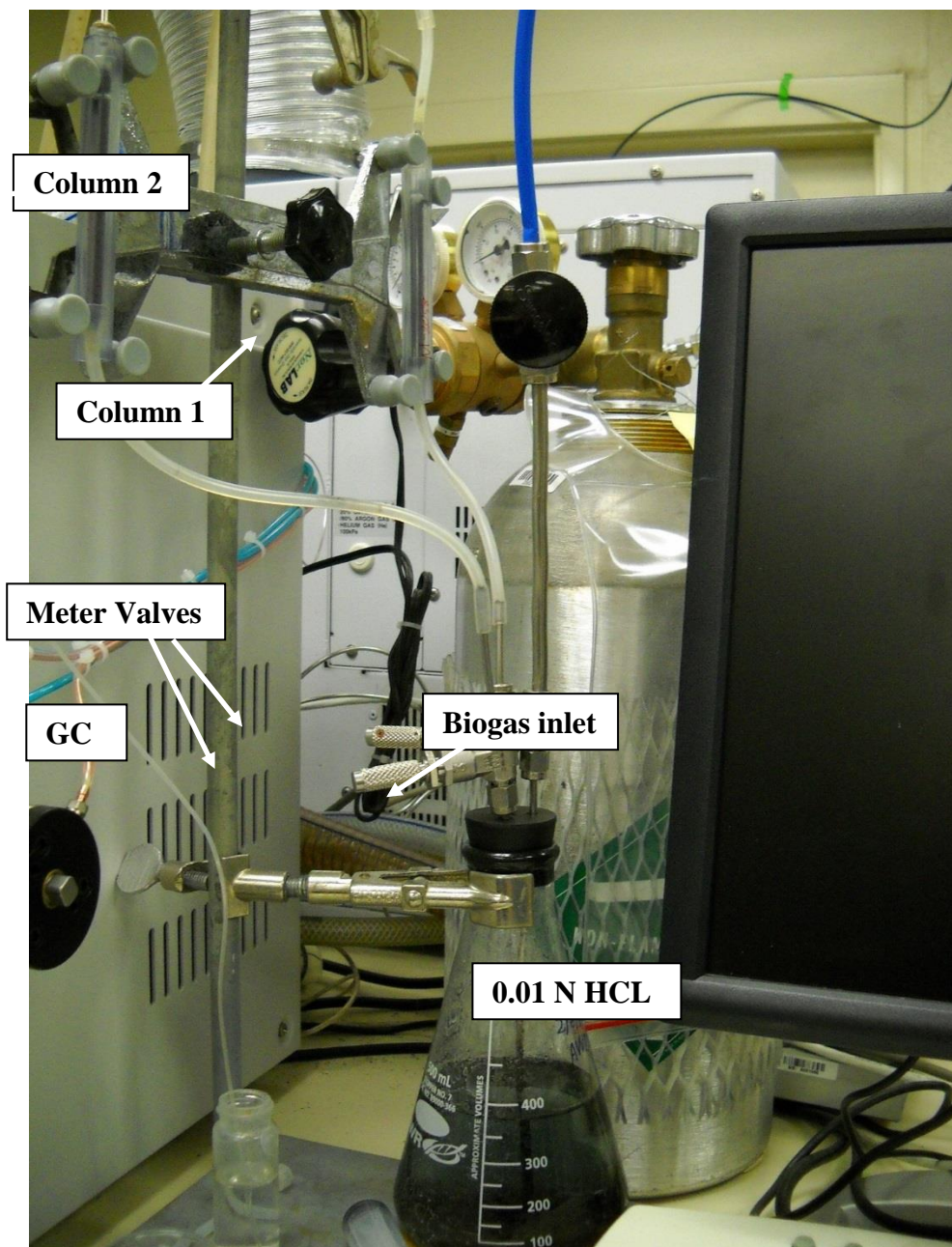
Figure 5.2: Experimental apparatus used for the hydrogen sulfide breakthrough experiments

5.3.5 Biochar surface area development (Chemical Activation)

Anaerobic digestion fiber was passed through 30- and 32-mesh screens to obtain a uniform particle size distribution. The AD fiber was next impregnated with phosphoric acid (H_3PO_4) solution in a 2:1 ratio of H_3PO_4 to AD fiber. The impregnated samples were allowed to stand under room conditions for one hour. Next, the samples were dried for 24 hours in an oven set to 105°C. Prior to carbonization, 4.0 g of impregnated samples were placed in a stainless-steel boat, and then later introduced into a tube furnace equipped with automated time and temperature control. Samples were placed in the middle of the tube to ensure a uniform distribution of heat.

The carbonization was carried out at temperatures between 350°C and 600°C. The heating rate was 10°C per minute with a nitrogen flow of 500 mL per minute. The isothermal carbonization time was one hour. Finally, the resulting biochar was characterized and used for the adsorption studies.

5.3.6 Phosphate adsorption studies

To evaluate the effectiveness of activated biochar produced from AD fiber in removing nutrients from effluents, we conducted adsorption experiments using phosphate (PO_4^{3-}). A solution of phosphate was prepared with sodium phosphate monobasic (NaH_2PO_4) and sodium phosphate dibasic (Na_2HPO_4), to guarantee a buffer solution of pH 7. The concentration range (0, 10, 30, 50, and 100 mg L^{-1}) was chosen to represent the range of phosphate concentrations commonly observed in dairy manure AD effluent.

The activated carbon produced was added to the phosphate solution at the rate of 200 mg per 25 mL solution (8 g L^{-1}). The mixture was then placed on a horizontal mechanical shaker and left for 24 hours with two replicates for each concentration. After shaking, the suspension was allowed to stand for at least two hours for the particles to settle at the bottom of the tube, and was later filtered through 0.45 µm filters.

The equilibrium concentrations of filtrates from the adsorption studies were measured by the molybdovanadate with acid persulfate digestion method (1.0 to 100 mg L^{-1}). The experimental results obtained were later fitted to the Freundlich and Langmuir models. The amount of PO_4^{3-} uptake at equilibrium, q_e (mg g^{-1}), was calculated using the mass balance equation, as follows:

$$q_e = \frac{(C_o - C_e)V}{m} \qquad\qquad 1$$

where C_o (mg L^{-1}) is the initial PO_4^{3-} concentration, C_e (mg L^{-1}) is the concentration of PO_4^{3-} at equilibrium, m (g) is the mass of biochar used, and V (L) is the volume of PO_4^{3-} solution. All chemicals used in this study were analytical reagent grade.

5.3.7 Adsorption of ammonia from the gas phase

In this study, highly acidic biochars activated with H_3PO_4 were produced. Douglas fir was the feedstock and it was impregnated with phosphoric acid at a 2:1 ratio of phosphoric acid:Douglas fir. The activation was done using 85% H_3PO_4 at a temperature range of 350-600°C. The resulting biochar was characterized by elemental analysis, proximate analysis, and surface area analysis.

Ammonia adsorption was tested in a packed bed filter set-up, and the breakthrough curves were obtained. This experiment entailed passing ammonia in a nitrogen gas stream (200 ppm) through the sample of activated biochar, and measuring the ammonia in the off-gas over time. Ammonia was measured using a tin oxide-based, solid-state gas sensor (MQ-135). The analog output was registered by a 10-bit resolution analog-to-digital

converter (ATmega 328) with a 1.1 voltage reference input. The MQ-135 is designed to operate in air, requiring the presence of oxygen at its atmospheric concentration (21% by volume); pure oxygen was introduced to the gas stream after the ammonia gas passed through the biochar where it flowed through a static mixture to ensure homogeneity by the time it reached the sensor. The output from the sensor was recorded every 10 seconds and saved to a text file. The complete experimental setup is illustrated in Figure 5.3.
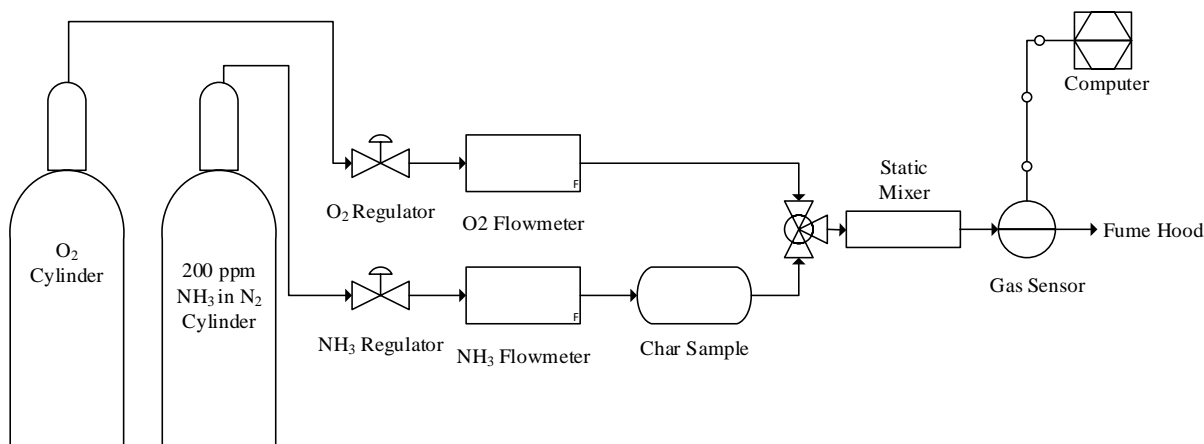
Figure 5.3: Experimental setup for gas phase ammonia adsorption

5.3.8 Production of biochar with surface amine groups for *E. coli* retention

In this study, high protein material (white mushrooms from Walmart) was mixed with a non-protein feedstock (Douglas fir) at a ratio of 1:1. This biochar was produced at the same conditions described elsewhere in the previous sections. The aim was to introduce nitrogen functional groups onto the resulting biochar. Previous studies demonstrate that biochar has negative charges due to oxygen functional groups. Introducing nitrogen functional groups to biochar may result in a positively charged surface which the researchers hypothesized would be effective for *E. coli* retention by electrostatic forces. The materials were physically activated with CO_2 to enhance the surface area.

The *E. coli* bacterial strains were prepared by growing for twelve hours at 37°C in a media of tryptic soy broth on a shaker rotating at 150 rpm. Following incubation, 1% of the volume of the activated culture was transferred into 10 mL of tryptic soy broth medium and grown at the conditions described above until the late stationary phase of growth (usually four to five hours of growth). After growth, bacterial suspensions were centrifuged at 5,100 rpm for 10 minutes, and were then collected and washed three times with deionized water. Bacterial pellets were then suspended in deionized water to a concentration of 0.1 optical density at 600 nm (equivalent to

2.4 x10^8 colony forming units (cfu) mL^{-1}, the initial concentration for analysis) (Suliman et al., 2017).

5.4 Results and discussion

5.4.1 Development of novel spectroscopic methods for biochar characterization

Analysis of theoretical Raman, XPS, and NMR spectra yielded new information regarding the deconvolution and assignment of various spectral features. The interpretation of Raman signals (

Table 5.1) for deconvolution parameters and assignments showed strong separation of pentagonal, hexagonal, and heptagonal (and octagonal) breathing modes that can be used in peak assignments. For this, pentagonal rings showed breathing modes near 1,400-1,460 cm^{-1}, hexagonal rings near 1,350 cm^{-1} and heptagonal rings showed a doublet structure near 1,000 and 1,200 cm^{-1}. In addition, distributions within the 1,550-1,600 cm^{-1} were also strongly influenced by the degeneracy of the structure, allowing interpretation of system ordering. Out of plane deformations have been observed to shift intensity within this region toward 1,500 cm^{-1}.

Table 5.1: Summary of peak assignments

Position (cm^{-1})	Peak	Shape	Assignment
975-1,075	S_L	Gaussian	Breathing modes for small aromatic regions, secondary breathing mode for 7+ membered ring
1,150-1,200	S	Gaussian	Breathing mode for rings containing 7+ carbons with Kekulé modes in adjacent benzene rings, benzene ring breathing modes adjacent to heteroatom defects
1,250-1,300	D_S	Gaussian	Assorted breathing modes for most PAHs[a]
1,340-1,380	D	Gaussian	Combined breathing/Kekulé vibrations for PAHs. Larger more symmetric systems show peaks near 1,350 cm^{-1}, while peaks for smaller systems move towards 1,400 cm^{-1}
1,400-1,460	A_1	Gaussian	Breathing mode for 5-membered rings with Kekulé vibrations in adjacent 6-membered rings and near pure Kekulé in small ring systems and moieties
1,480-1,550	A_2	Gaussian	Mixed breathing and asymmetric stretch vibrational modes for sp^2 carbons near defects causing out of plane deformation. Heteroatom defects tend to cause greater red shift
1,570-1,600	G_G	Gaussian	Distributed asymmetric vibrations for distribution of small PAHs
1,570-1,600	G_L	Lorentzian	Standard E$_{2g}$ mode for large PAHs
1,605-1,650	D'	Gaussian	Double resonance activated breathing mode *weak: E$_{2g}$ mode near ether inclusions

| 1,750-1,800 | C | Gaussian | Carbonyl stretching mode, very weak |

* New peak observed in this study, interpretation from figures presented previously.
[a] PAH = polycyclic aromatic hydrocarbon

These deconvolution strategies were applied to a thermoseries of cellulose biochars. The development of spectral intensity associated with pentagonal structures as temperature increased and a sharp increase in out of plane deformations at 700°C were observed (Figure 5.4). The thermoseries showed an increasingly ordered structure based on analysis of the G-band region (1,570-1,600 cm^{-1}). The increased intensity near 1,350 cm^{-1} and the slight shift in maximum peak intensity indicated increasing aromatic condensation as temperature increased.
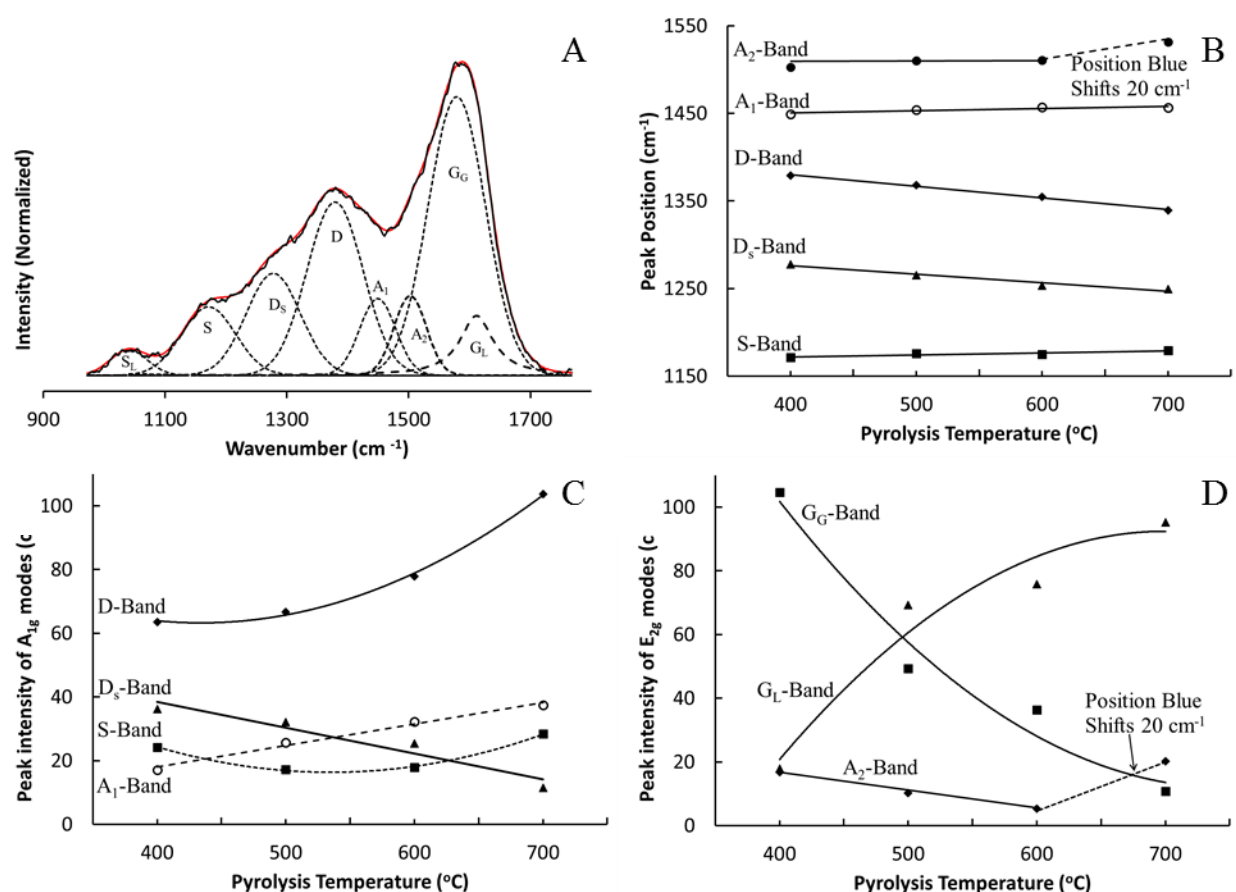
Figure 5.4: (A) Deconvolution of Raman spectrum of a cellulose biochar produced at 400°C using the newly proposed method, (B) Peak position, (C) Peak intensity of breathing modes, and (D) peak intensity of asymmetric stretch modes.

Analysis of the XPS signals provided strong evidence of the existence of 3 C-C peaks near 283.6, 284.4, and 285 eV, respectively. These peaks and interpretations have been added to the deconvolution parameters given in

Table 5.2. Carbons contained within pentagonal rings have been linked to spectral intensity near 283.6 eV, while those in heptagonal and octagonal rings show intensity near 285 eV. Simulations also suggested that the presence of carbonyl groups can cause weak shifts in non-adjacent carbons, resulting in additional intensity near 285 eV. To more accurately fit these regions, a new deconvolution algorithm has also been proposed (Figure 5.5) which utilizes the deconvolved O1s spectrum as a constraint in the C1s deconvolution. This prevents over or under expression of carbon-oxygen groups during analysis.

Table 5.2: Peak assignments and parameters for the interpretation of O1s and C1s spectra of biochars

Peak	Assignment	BE (eV)	FWHM (eV)	G:L (0-1)
O-C(1)	Ether and hydroxyl groups bonded to aromatics	533.0-533.4	1.8-2.2	0-0.1
O-C(2)	Ether and hydroxyl groups bonded to aliphatics and carbonyl shake-up	532.5-532.9	1.8-2.2	0-0.1
O=C	In carbonyl, lactone, and carboxylic groups	531.2-531.6	1.8-2.2	0-0.1
(H$_2$O)	Absorbed water/oxygen, sub monolayer	534.8-535.2	1.8-2.2	0-0.1
C-C low	Cyclopentane ring atoms within cluster	283.4-284	1.2-2	0-0.3
C-C Primary	Primary C-C/C-H peak	284.2-284.6	1.2-2	0-0.3
C-C High	C in cycloheptane or larger rings within clusters, C in small clusters containing C=O bonds, sp3 bonded carbons	284.8-285.4	1.2-2	0-0.3
C-O	Ether and hydroxyl bonded C, C associated with ether bond in lactone/esters	285.9-286.6	1.8-2.2	0-0.1
C=O	Carbonyl groups and carbons attached to two ether/hydroxyl groups	286.7-287.5	1.8-2.2	0-0.1
COO	Carboxyl, lactone, and ester groups	288.3-288.9	1.8-2.2	0-0.1
Pi-Pi*	HOMO-LUMO transition for primary C-C peak	291-292	2-3	0-0.1

Survey scan, O1s and C1s high resolution scans,
additional high resolution scans if needed
Desired Peaks
Bounds for all free parameters
See Table 8 for recommended conditions

Evaluate survey scan for all elements present

Did the Carbon show Charging?

Yes → Lock the asymmetry parameter (TS) at 0 for the primary C-C peak

No → Specify the maximum allowable asymmetry (0.20 is recommended for chars)

Select desired peak and correct peak position
Binding Energy likely shifted 1-2 eV

Correct peak position to 284.4 eV for C-C bonds
Binding Energy likely <0.5 eV

Apply baseline correction to O1s Peak

Deconvolute O1s peak using 4 symmetric GLsum peaks, repeat until fit reaches minimum

Calculate maximum and minimum apparent C-O/C=O/O-C=O, total C-O, total C=O and total C:O bonds as shown in Equations S2-S12

Correct O-C and O=C values as needed if additional compounds (eg CaO) are present

Deconvolute C1s peak using input bonds and bonds from the O1s Deconvolution, repeat until fit reaches minimum

Reasonable fit achieved?

No → Adjust initial guess parameters and re-optimize

Yes → Optimize DHE, DCL and C:O ratio by comparison of C1s and O1s deconvolution results using equations S13-S15

reasonable agreement (<10% error)?

Multiple No → Additional adjustment of fitting parameters required for material

No → Evaluate survey scan for additional elements, reevaluate assignments for non C/O deconvolutions, adjust initial guess for O1s and C1s Peaks

Yes → O1s and C1s fit results and parameters

End

Figure 5.5: Basic algorithm for the combined O1s/C1s deconvolution of XPS spectra of biochars and amorphous carbons

97

Analysis of the cellulose thermoseries (**Error! Not a valid bookmark self-reference.**) indicated that pentagonal ring systems likely decreased with temperature between 400°C and 700°C, as did the heptagonal and octagonal ring systems and all other defect types. While this result contrasted with the intensity increase observed near 1,400 cm^{-1} in the Raman spectra, such changes may be the result of resonant enhancement within larger structures. The XPS results also indicated preferential stability of ether and lactone type groups over hydroxyl and carboxyl groups as temperature increased, consistent with results reported for temperature programmed desorption from activated carbons. The XPS results also indicated increasing aromatic condensation at 600°C and 700°C due to lack of charging effects (increased electron conduction within the sample) and the development of an asymmetric peak shape associated with the primary aromatic C-C peak. This peak shape is highly prevalent in activated carbons and graphitic materials, and has been previously linked to polyaromatic clusters larger than 1 nm.

Table 5.3: Peak distribution and C:O ratios determined using proposed deconvolution scheme

Deconvolution peaks	Cellulose feedstock	Pyrolysis Temperature (°C)				
		300	400	500	600	700
C-C low (%)	5	4	11	6	5	4
C-C Primary (%)	6	14	36	52	67	75
TS (asym. factor)	0	0	0	0	.11	.16
C-C High (%)	1	5	27	16	14	9
C-O (%)	66	60	18	16	5	4
O-C-O/C=O (%)	20	15	0	2	0	0
O-C-O estimated	20	15	0	1	0	0
COO (%)	1	2	5	4	3	3
Pi-Pi[a] (%)	1	0	3	4	4	4
C:O[a]	1.26	1.54	4.63	5.11	11.40	10.86
O-C	98	98	80	69	61	62
C=O	2	2	20	31	39	38
D_{HE}[b]	0.57	0.70	0.64	0.84	0.91	0.52
D_{CL}[c]	0.55	0.64	0.64	0.58	0.66	0.42

[a] Results from comparison of C1s and O1s fine scans
[b] D_{HE} = distribution of hydroxyl and ether groups
[c] D_{CL} = distribution of carboxyl and lactonic groups

Examining the NMR shielding tensors associated with various defects indicated that both pentagonal and heptagonal and octagonal type defects shifted signal intensity from approximately 130 to 140 ppm. Oxygenated inclusions were also found to shift second nearest neighbor carbons to between 110 and 120 ppm. All other results were found to be consistent with previously reported chemical shifts. The assigned deconvolution regions are given in Table 5.4.

Table 5.4: Peak assignment table

Chemical Shift	Assignment
5-50 ppm	Aliphatic CH_x
20-60 ppm (slow dephasing)	Aliphatic CR_4
45-90 ppm (110 for double)	Aliphatic ether
100-120 ppm	Aromatic C near oxygen defects
120-140 ppm	Aromatic 5 and 6-member rings and olefins
140-150 ppm	Contribution for ether bonded carbon in furans and
	~ ½ of carbons in larger non-oxygenated rings
150-160 ppm	Aromatic linked ethers
160-180 ppm	Carboxylic/lactone/ester
185-210 ppm	Carbonyl

Analysis of the NMR spectra for the cellulose thermoseries provided quantifiable data regarding the regular aromatic structure, defective structures, and oxygenated structures. The dephasing behavior of these spectra provided information on the overall size and special distribution of these structures. Based on these results, a series of representative structures have been proposed (Figure 5.6). It must be noted that these are not absolute structures but only one of many possible forms that provide a reasonable fit to the NMR data obtained. These results detailed the increasing aromatic condensation of the biochars. As pyrolysis temperature increased, the fraction of defective structures and oxygenated decreased, in agreement with previous XPS data.

Cellulose ($C_{12}H_{20}O_{10}$)   C300 ($C_{12}H_{18}O_9$)   C400 ($C_{22}H_{10}O_5$)

C500 ($C_{24}H_9O_4$)   C600 ($C_{32}H_{12}O_3$)   C700 ($C_{37}H_{12}O_3$)
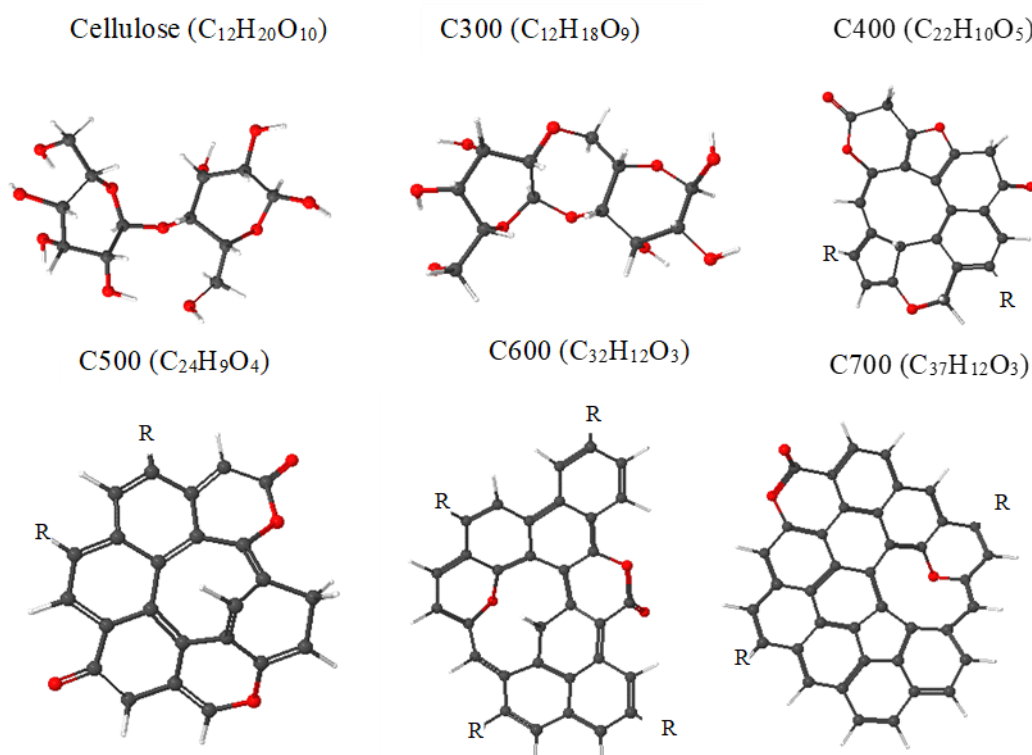
Figure 5.6: Example biochar structures based on composition shown in Analysis of the cellulose thermoseries (Error! Not a valid bookmark self-reference.**) indicated that pentagonal ring systems likely decreased with temperature between 400°C and 700°C, as did the heptagonal and octagonal ring systems and all other defect types. While this result contrasted with the intensity increase observed near 1,400 cm-1 in the Raman spectra, such changes may be the result of resonant enhancement within larger structures. The XPS results also indicated preferential stability of ether and lactone type groups over hydroxyl and carboxyl groups as temperature increased, consistent with results reported for temperature programmed desorption from activated carbons. The XPS results also indicated increasing aromatic condensation at 600°C and 700°C due to lack of charging effects (increased electron conduction within the sample) and the development of an asymmetric peak shape associated with the primary aromatic C-C peak. This peak shape is highly prevalent in activated carbons and graphitic materials, and has been previously linked to polyaromatic clusters larger than 1 nm.**

Table 5.3, and C-H distance and quantity from Table 5.4 for C300-C700

5.4.2 Integration of fiber pretreatment with existing AD technologies and testing for phosphorus removal

5.4.2.1 Surface area development

The bulk properties of the biochar activated at different temperatures are shown in Table 5.5 and Table 5.6.

100

Table 5.5: Bulk properties associated with activated biochar (CO_2 activation)

Temperature (°C)	500	550	600	650
Moisture (wt. %)	1.97 (0.38)[b]	2.53 (0.13)	2.90 (0.25)	3.23 (0.25)
$SA^c_{(CO2)}$ (m^2 g^{-1})	209.96	240.74	246.46	285.02
V_{micro}^d (cm^3 g^{-1})	0.0842	0.0965	0.0988	0.1142
V_{meso}^e (cm^3 g^{-1})	-	-	-	0.0294
C (wt. %)	52.82 (0.027)	54.19 (0.33)	53.21 (0.12)	49.53 (0.00)
H (wt. %)	1.68 (0.058)	1.34 (0.001)	0.95 (0.003)	0.62 (0.00)
N (wt. %)	2.49 (0.007)	2.41 (0.008)	2.38 (0.018	2.26 (0.00)
O^a (wt. %)	10.42	7.61	7.41	5.99
Ash (wt. %)	32.59 (0.17)	34.45 (1.97)	35.82 (1.57)	41.60 (1.42)

[a] calculated by difference
[b] standard deviation
[c] SA = surface area
[d] V_{micro} = volume of micropores
[e] V_{meso} = volume of mesopores

Table 5.6: Bulk properties associated with activated biochar from AD fiber (CO_2 activation)

Temperature (°C)	700	750	800	Raw AD Fiber
Moisture (wt. %)	2.18 (0.15)	3.01 (0.30)	0.46 (0.14)	7.02 (0.66)
$SA^c_{(CO2)}$ (m^2 g^{-1})	304.97	305.31	31.07	-
V_{micro}^d (cm^3 g^{-1})	0.1222	0.1224	0.01245	-
V_{meso}^e (cm^3 g^{-1})	0.0592	0.117	-	-
C (wt. %)	45.21 (0.00)	36.08 (0.00)	9.48 (0.039)	43.37 (0.039)
H (wt. %)	0.52 (0.011)	0.38 (0.00)	0.13 (0.006)	4.98 (0.006
N (wt. %)	2.26 (0.028)	1.96 (0.00)	0.21 (0.029)	2.07 (0.03)
O^a (wt. %)	8.6	5.26	0.4	34.08
Ash (wt. %)	43.32 (1.84)	56.31 (2.32)	90.47 (0.14)	15.49 (0.33)

[a] calculated by difference
[b] standard deviation
[c] SA = surface area
[d] V_{micro} = volume of micropores
[e] V_{meso} = volume of mesopores

5.4.2.2 Phosphate adsorption studies

Adsorption isotherms were used to describe the relationship between PO_4^{3-} concentration and the activated biochar at a constant temperature (Figure 5.7).

The results obtained were fitted to Langmuir and Freundlich models. The parameters of these models were estimated using equations reported elsewhere (Hai et al., 2016)
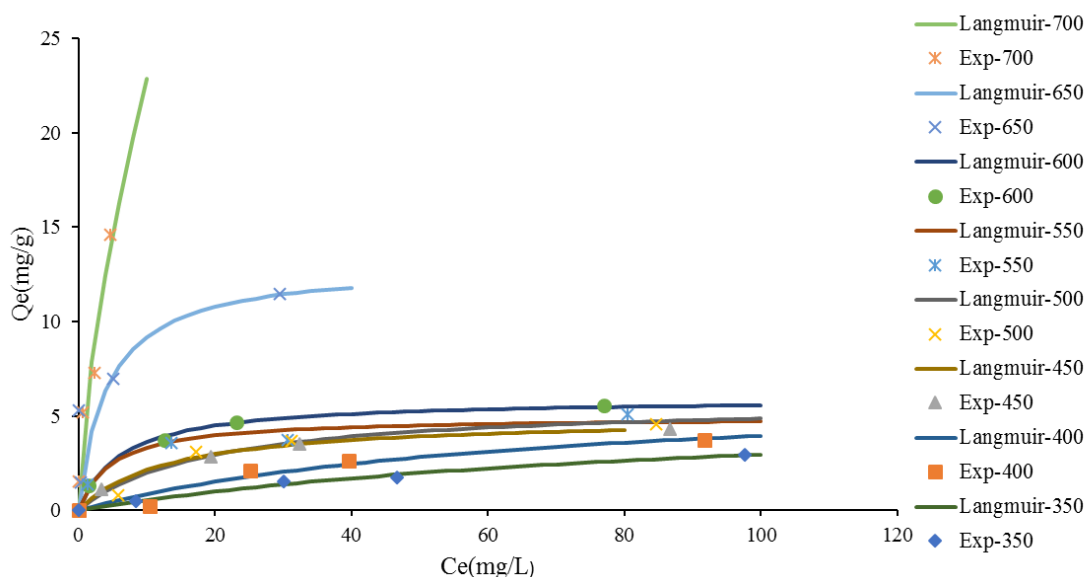
Figure 5.7: Adsorption isotherms of PO_4^{3-} on activated biochar produced at different temperatures

Table 5.7: Isotherm parameter of PO_4^{3-} adsorption and deduced parameter from Langmuir and Freundlich models

BIOCHAR	Langmuir Parameters					Freundlich Parameters			
	Q_{max} (mg g^{-1})	K_L (L mg^{-1})	R^2	χ^2	K_F	$1/n$	R^2	χ^2	R_L
BC-700-60min	37.5	0.13	0.918	1.86	4.96	0.66	0.945	0.79	0.06—0.4
BC-650-60min	13.1	0.24	0.993	0.38	3.45	0.36	0.976	0.36	0.03—0.3
BC-600-60min	5.9	0.16	0.988	0.07	1.68	0.29	0.929	0.25	0.05—0.3
BC-550-60min	4.9	0.20	0.924	0.18	1.03	0.37	0.887	0.34	0.04—0.3
BC-500-60min	5.8	0.05	0.940	0.28	0.81	0.41	0.834	0.63	0.14—0.6
BC-450-60min	4.9	0.08	0.996	0.02	0.95	0.35	0.948	0.13	0.10—0.5
BC-400-60min	6.6	0.02	0.906	0.58	0.22	0.64	0.858	0.75	0.4—0.9
BC-350-60min	5.8	0.01	0.987	0.03	0.14	0.67	0.986	0.05	0.5—0.9

102

The constant, correlation coefficient (R^2), and chi-square (χ^2) values of both Langmuir and Freundlich models are presented in Table 5.7. The R^2 values of the Langmuir model (0.918 to 0.996) were higher than those of the Freundlich model (0.834 to 0.986). In contrast, the chi-square values of the Langmuir model were significantly lower than those of the Freundlich model. Therefore, considering these adsorption parameters (R^2 and χ^2), researchers focused on describing the data with the Langmuir model.

Hall et al. (1966) identified that the adsorption characteristics of the Langmuir model can also be interpreted in terms of a dimensionless factor called the separation factor (R_L). This factor is used to determine whether the adsorption is favorable ($0<R_L>1$) or unfavorable ($R_L>1$). An $R_L=0$ means adsorption is irreversible, whereas $R_L=1$ means there is a linear relationship (Tran et al., 2016).

The separation factor (R_L) ranged from 0.034 to 0.888 (Table 5.7), indicating that the adsorption of PO_4^{3-} was favorable. As can be seen in Table 5.7, as the carbonization temperature increased, the adsorption capacity of the biochar also increased. Statistically, the maximum adsorption capacity of the activated biochar varied significantly ($p < 0.05$) from 650°C upward, and the maximum adsorption was reported at 700°C (37.5 mg g^{-1}). Thus, we demonstrated that activated biochar produced from AD fiber is effective for phosphate removal.

5.4.3 Testing adsorption of H_2S from the gas phase

The breakthrough results obtained with a thermoseries of activated carbon from AD fiber (Figure 5.8) indicated that the biochar's capacity for H_2S adsorption was highly influenced by the carbonization temperature. The studies demonstrated that physically activated carbon produced at lower temperatures (350°C, 400°C, and 450°C) had very poor adsorption of H_2S (not shown). Another key point to notice is that the adsorption of H_2S was influenced not only by the surface area and pore size, but also by the surface chemistry of the activated biochar. Based on the curve, researchers noticed that two mechanisms of adsorption may be occurring: (1) filling of the micropores and (2) oxidation of the H_2S to elemental sulfur due to the high ash content present in the activated carbon (see ash content in Table 5.5 and Table 5.6). Activated carbon produced at 500°C and above performed as well as commercial activated carbon.
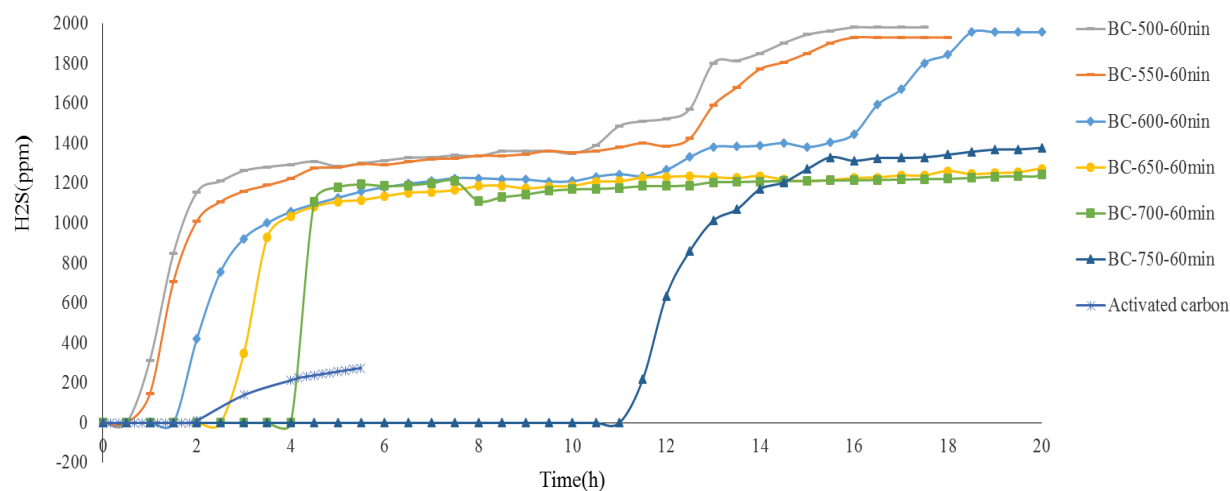
Figure 5.8: Adsorption isotherms of H₂S on activated biochar produced at different temperatures

Biochar treated chemically performed poorly in terms of H_2S adsorption, with practically no adsorption occurring (Figure 5.9). This was attributed to the high ash content of the raw feedstock. The phosphoric acid was insufficient to neutralize the mineral content, which led to poor surface area development. The sulfur content before and after adsorption of H_2S on produced activated biochar is presented in Table 5.8.
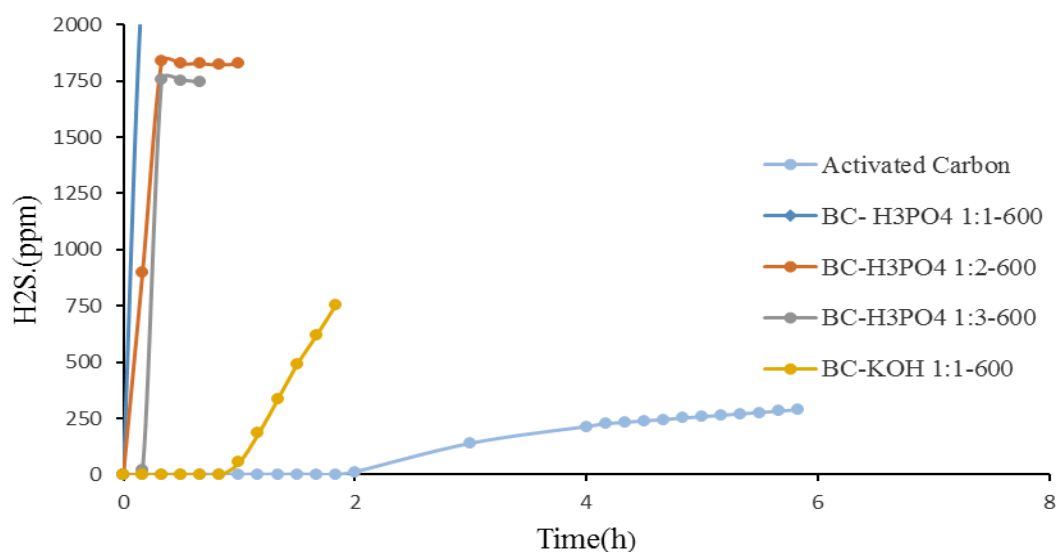
Figure 5.9: Adsorption isotherms of H₂S on chemically activated biochar produced at different temperatures

Table 5.8: Sulfur content in activated biochar before and after adsorption of H₂S

	% Sulfur	
Samples	**Before H₂S adsorption**	**After H₂S adsorption**
BC-800-60min	0.46	9.24
BC-750-60min	0.72	10.95
BC-700-60min	0.59	6.44
BC-650-60min	0.4	5.58
BC-600-60min	0.24	4.77
BC-550-60min	0.23	5.755
BC-500-60min	0.013	4.395

From a technological perspective, focusing on the production and physical activation (with CO_2) of biochar from AD fiber is a viable approach.

5.4.4 Testing adsorption of ammonia from the gas phase

5.4.4.1 Surface development of biochar for ammonia gas adsorption

The characteristics of the biochar used for ammonia gas adsorption are presented in Table 5.9. These biochar samples were chemically activated with 85% phosphoric acid. The resulting biochar was used for the adsorption study without washing off phosphoric acid prior to carbonization (Table 5.9).

Table 5.9: Bulk properties associated with activated biochar from Douglas fir (phosphoric acid activation)

Temperature (°C)	600	550	500	450	400	350
C (wt. %)	33.91	25.95	21.06	21.61	20.65	20.12
H (wt. %)	1.26	1.35	1.9	1.58	1.97	2.08
N (wt. %)	0.08	0.03	0.02	0.03	0.01	0.05
O^a (wt. %)	50.43	59.43	67.64	67.61	70.67	71.6
Ash (wt. %)	14.32	13.24	9.38	9.17	6.69	6.15

^{a} calculated by difference

5.4.4.2 Ammonia adsorption studies

Activating biochar with phosphoric acid greatly increased ammonia adsorption capacity. This increase in adsorption capacity may be attributed to changes in biochar surface functionalities and to the decrease in pH resulting from activation. The breakthrough curves (Figure 5.10) indicate the time required for the concentration of ammonia at the outlet of the biochar to reach 10% of the initial concentration (200 ppm). Biochar produced at 550°C took eight hours to break through, which meant that it was more effective than commercial activated carbon, which only took 30 minutes to break through (Figure 5.10).
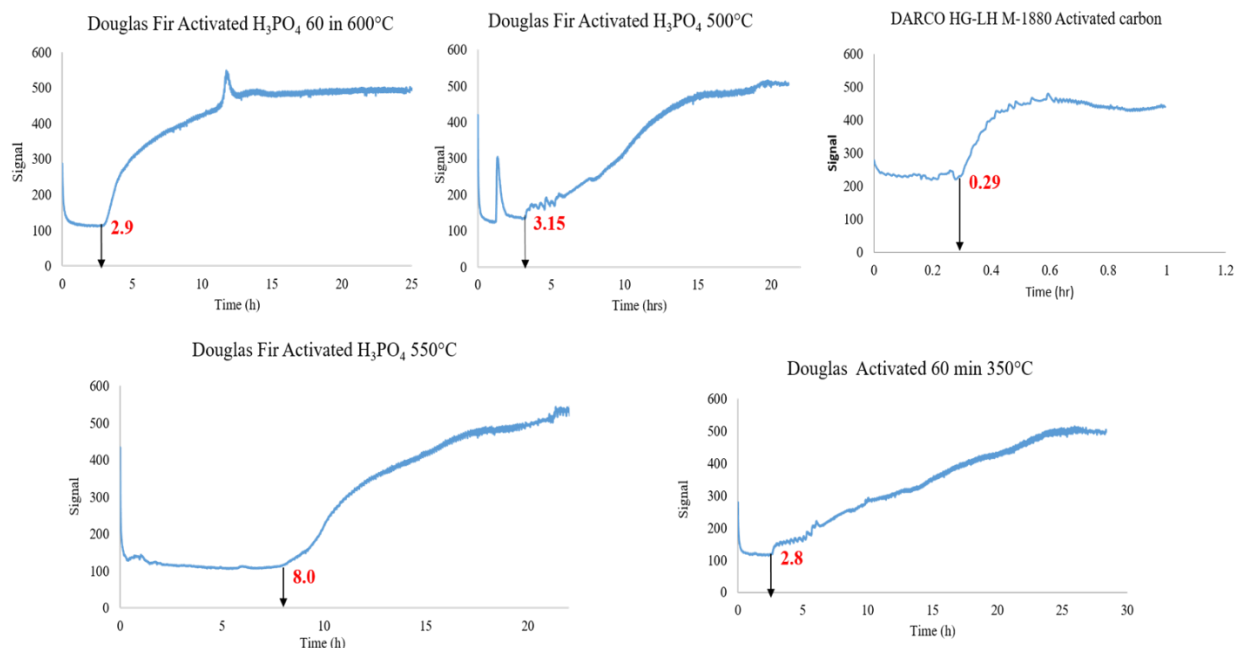
Figure 5.10: Ammonia adsorption breakthrough curves for biochar produced at different temperatures and for DARCO commercial activated carbon

5.4.5 Production of biochar for the retention of *E. coli*

5.4.5.1 Surface development of biochar for *E. coli* retention

Characteristics of the biochar used for *E. coli* retention are presented in Table 5.10. The high nitrogen feedstock was carbonized and activated with carbon dioxide. The resulting biochar was tested for retention of *E. coli*.

Table 5.10: Bulk properties associated with CO_2 activated biochar

Temperature (°C)	400	500	600	700	750
$SA^a_{(CO2)}$ (m^2 g^{-1})	55.55	223.33	223.34	296.21	343.45
V_{micro}^b (cm^3 g^{-1})	0.0223	0.0895	0.0895	0.118	0.138
C (wt. %)	65.95	63.5135	66.98	67.37	64.12
H (wt. %)	3.12	2.01	1.14	0.54	0.17
N (wt. %)	4.41	4.08	4.11	4.29	4.21
O^a (wt. %)	10.32	12.05	8.83	9.23	2.89
Ash (wt. %)	15.48	16.37	18.94	18.57	28.6

[c] SA = surface area
[b] V_{micro} = volume of micropores

106

5.4.5.2 *E. coli* adsorption studies

The breakthrough curves (Figure 5.11) represent the adsorption of *E. coli* on the biochar produced with high nitrogen content (Table 5.10). The capacity of biochar to adsorb *E. coli* was dependent on its surface functionalities and the temperature at which it was produced. Biochar produced at 750°C demonstrated an excellent adsorption of *E. coli*, followed by biochar produced at 700°C. There was an increase in bacterial attachment when biochar was used instead of sand, which may be due to the overall increase in active attachment sites in the biochar. Another possible explanation for the effectiveness of biochar for retention of *E. coli* is the enhanced positive charge on the surface of high nitrogen biochar. This enhanced charge may result in an overall increase in electrostatic forces between biochar with high nitrogen content and the negatively charged surface of *E. coli* (Hermansson, 1999).
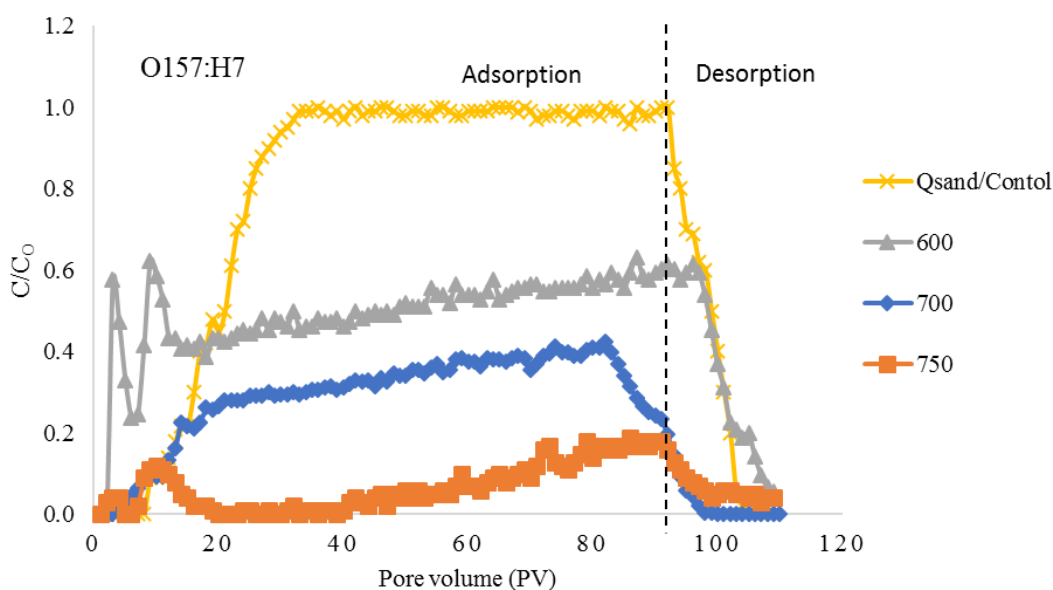
Figure 5.11: *E. coli* **(strain O157:H7) adsorption breakthrough curves for biochar produced at different temperatures**

5.5 Conclusions and recommendations

New spectroscopic methods (Raman, XPS, and NMR) for biochar characterization were developed as a result of this study. These methods allowed the researchers to quantify the presence of defects in the biochar structure which could have a major impact on its capacity to adsorb pollutants.

Several samples of biochar derived from AD fibers were prepared and tested for their capacity to retain H_2S. Some of the biochars produced had a capacity to adsorb H_2S comparable to that of commercial activated carbon. The AD biochar engineered for phosphate retention achieved 97.62% removal, demonstrating a very high capacity for phosphate retention due to the high ash content of this biochar. Also, biochar activated with phosphoric acid effectively retained

ammonia, producing 170.1 mg of NH_3 per g of biochar, compared to the commercial activated carbon, which produced 16.2 mg of NH_3 per g of activated carbon. The researchers suspect that the phosphoric acid bound to the acid-activated biochar contributed to the exceptionally high ammonia adsorption capacity. Surface area is a key factor in adsorption, but in this case surface acidic functional groups were the most important factor for ammonia adsorption. Engineered biochar with nitrogen functional groups demonstrated a high capacity for the removal of *E. coli*, removing up to 82.2% of *E. coli* from effluent and reducing the transport of a pathogenic strain of *E. coli* (O157:H7) due the electrostatic interaction between the positive surface charge of the biochar and the negative surface charge of *E. coli*.

The researchers' recommendations for further work include:

- To continue investigating the value of the biochar that has adsorbed nutrients as a soil amendment.
- To focus on how to develop general design rules that can be applied for the selection of feedstock and carbonization conditions leading to the formation of biochar with desired pollutant removal characteristics and for land application.
- To continue the research on *E. coli* retention by using engineered biochar with high ash content.

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SECTION 3

TESTING OF BIOPRODUCTS FROM ANAEROBIC DIGESTION SYSTEMS

6. Producing Biofertilizer as a Co-Product of Anaerobic Digestion

Rishikesh J. Ghogare, Allan H. Gao, Jose Martinez, and Shulin Chen

6.1 Background

Anaerobic digestion (AD) is a rapidly emerging technology, mainly due to its ability to process large amounts of waste, including agricultural waste, sustainably. In the United States, 250 million tons of animal manure are generated annually by concentrated animal feeding operations alone (Jiang et al., 2009). Animal manure has a high nutrient content, but transporting it is cost-prohibitive. The waste is therefore generally applied to nearby land as fertilizer (Salminen et al., 2001). However, over-application of manure can result in eutrophication of waterways, localized nutrient accumulation leading to degradation of soil quality, and methane release that contributes to air pollution and greenhouse gases emissions (USDA, 2004). These issues have driven the development of AD technology for handling large quantities of dairy waste. More than 200 dairy-based anaerobic digesters are in operation in the U.S. (EPA, 2016), and this number is expected to increase over the coming years, mainly due to governmental regulations and policies related to waste management. The U.S. dairy industry has set a goal to reduce greenhouse gas emissions by 25% by 2020. Meeting this goal will require more widespread adoption of AD technology (USDA, 2014).

Adoption of AD technology reduces air pollution by the entrapment of methane, prevents soil and water quality issues caused by direct land application of manure, and reduces pathogen count in manure (Abbasi et al., 2012; Power et al., 2001). Although AD improves the management of large quantities of waste, it also results in large volumes of wastewater, since the influent requires dilution before treatment. The wastewater produced is rich in nitrogen, phosphorus, volatile acids, and solids (Lei et al., 2007), and requires treatment using processes such as nutrient recovery to meet environmental regulations. National Pollutant Discharge Elimination System licenses are required for any feeding operation that discharges or proposes to discharge waste to reduce its environmental impact, maintain appropriate nutrient levels, and show compliance with the Clean Water Act (Zeb et al., 2017).

Recently, several physical and chemical processes have been developed for the treatment of AD effluent, to remove solids and phosphorus (Bowers and Westerman, 2005; Braguglia et al., 2006; Chen et al., 2008; Le Corre et al., 2007; Zhang et al., 2010). Along with phosphorus, AD effluent is also rich in nitrogen, most of which is in the form of ammonia or inorganic nitrogen. Ammonia from AD effluent is commonly recovered using the process of ammonia stripping (Lei et al., 2007). However, ammonia stripping poses several challenges such as pH adjustment, clogging of stripping towers, high gas flow rate, and the requirement of external heating for recovery of free ammonia (Bonmatí and Flotats, 2003; Saracco and Genon, 1994). Recent

developments and improvements in ammonia stripping, such as direct aeration-ammonia stripping, have been shown to be feasible for recovering ammonia with high efficiency (Dvorak et al., 2013). With the application of sequential screening or solid separation in combination with ammonia stripping methods, 75% of phosphorus and 70-80% of nitrogen can be recovered from the AD effluent (Jiang, 2010).

Despite these technological developments, the resulting waste stream still requires wastewater treatment and regulation by government agencies to address environmental concerns resulting from disposal of the large volume of waste generated. The AD effluent is both a potential pollutant and an underutilized resource. Application of the effluent to fields has few advantages due to its low nutrient content, and heavy application may result in contamination of drinking water, eutrophication of waterways, or salting of cropland. The cost of transportation of liquid waste is also cost-prohibitive. One potential method to alleviate this concern is to utilize the nutrient-recovered stream for production of a co-product, such as biofertilizer, which would have an economic benefit on the overall AD process.

Biofertilizers (also called microbial fertilizers) are an array of organisms that fix nitrogen or solubilize phosphate. Some microbial strains provide additional benefits, such as suppressing the growth of harmful microorganisms or releasing micronutrients (Singh et al., 2007). There are many organisms which can act as biofertilizers, including bacteria in the genera *Azospirillum, Azotobacter,* and *Rhizobia* (Hayat et al., 2010). In the past, the production of microbial biofertilizers has used sugars (derived from sources such as sugar cane) or from starchy wastes, including cassava and potato (Hayat et al., 2010; Ogbo, 2010).

Such biofertilizers have the capacity to release nutrients over time (Wu et al., 2005), reduce leaching from the soil, and suppress the growth of harmful microorganisms (Chen, 2006). On the other hand, some drawbacks of biofertilizers are that they have a lower nutrient content than conventional, chemically-derived fertilizers, and require ideal soil conditions to achieve maximum effectiveness (Mohammadi and Sohrabi, 2012). Despite these disadvantages, the supplementation of chemical fertilizers with small amounts of microbial biofertilizers has the potential to reduce the amount of fertilizer that farmers must purchase and apply to their fields.

In this report *Azotobacter vinelandii* was used as the biofertilizer species. *Azotobacter vinelandii* and *Azospirillum brasilense* are free-living soil bacteria which are capable of fixing nitrogen. Both of these species of nitrogen-fixing bacteria have been extensively studied for their nitrogen-fixing ability and improvement of crop yield (Mohammadi and Sohrabi, 2012). However, a previous study this research team conducted showed that *A. vinelandii* had higher specific growth rate than *A. brasilense,* resulting in greater amount of biomass (Gao et al., 2015). Also, *A. vinelandii* demonstrated appreciable growth on cellulosic sugars without affecting its nitrogen-fixing ability (Gao et al., 2015). Hence, *A. vinelandii* was chosen as a potential biofertilizer candidate to be tested for growth using AD effluent.

6.2 Objectives

This project was focused on developing biofertilizer as a co-product of AD technology. The main goal was to develop a technology which complemented the nutrient recovery process

developed at Washington State University (WSU), which involves the production of organic fertilizer. Development of biofertilizers from the waste stream after nutrient recovery (that is, from the nutrient-recovered AD effluent) would add more fertilizer options to the existing suite of products. The further reduction of nutrients in nutrient-recovered AD effluent through biofertilizer production would minimize the adverse effects to soil and water quality caused by over-application of this effluent to fields.

The major objectives of this project were: (1) to optimize the growth of the microbial biofertilizer *Azotobacter vinelandii* in a laboratory setting using nutrient-recovered AD effluent, and (2) to conduct a techno-economic assessment for a modified a biorefinery that includes the production of biofertilizer. The optimization of growth objective was achieved by testing growth in different dilutions of AD effluent and measuring utilization of glucose and nitrogen. As the high nitrogen content of the nutrient-recovered effluent may inhibit the optimal growth of biofertilizer, the effluent was diluted. The measurement of residual nitrogen and glucose utilization was conducted to determine the nutrient level in the effluent that remained after the production of the biofertilizer. The major performance parameters were biomass productivity and titer of *A. vinelandii.* The techno-economic assessment produced an estimate of the cost of biofertilizer production at a local scale, providing an evaluation of its economic feasibility for use in organic farms in Washington State.

6.3 Methods

6.3.1 Optimizing biofertilizer growth

In this study, we developed a methodology for the production of biofertilizers using an underutilized resource: nutrient-recovered AD effluent.

6.3.1.1 *Azotobacter* culture

Azotobacter vinelandii (ATCC 478 type strain) was obtained from the American Type Culture Collection and established on ATCC medium 12—*Azotobacter* supplement agar plates. Cultures were then replated onto Burk's N-free medium and Ashby's N-free medium, as described by Stella and Suhaimi (2010), and maintained at 30°C (Ashby's and Burk's media are standard nitrogen-free media for culturing *Azotobacter* species).

6.3.1.2 *Azotobacter* culture on non-nutrient-recovered AD effluent

Solid-separated AD effluent, obtained from the step prior to nutrient recovery, was collected from Dr. Pius Ndegwa's laboratory at WSU. Residual solids were separated from the effluent by centrifugation at 14,000 rpm for 10 minutes. *A. vinelandii* was grown on AD effluent supplemented with 20 g L^{-1} of glucose and other nutrients of Ashby's media (Table 6.1), for testing effect on growth. Ashby's media and AD effluent without any sugars were used as controls. Here, Ashby's media was selected because in a previous study (Gao et al., 2015), Ashby's media supported better growth than Burk's media, resulting in greater biomass accumulation of *A. vinelandii.*

6.3.1.3 *Azotobacter* culture on nutrient-recovered AD effluent

Nutrient-recovered AD effluent was obtained from a commercial dairy digester managed by Regenis in Ferndale, Washington. Culture of *A. vinelandii* for sample measurements occurred in 250 mL shake flasks with 50 mL of culture media. "Control 1" contained Ashby's media, the standard nitrogen-free medium for the growth of *Azotobacter*, and "Control 2" was composed of Ashby's media components, 20 g L^{-1} glucose, and 0.5 g L^{-1} ammonium acetate. "Control 2" was used to mimic conditions in the AD effluent, as nutrient-recovered AD effluent contains inorganic nitrogen and is supplemented with glucose. Growth of *A. vinelandii* was tested on different concentrations of nutrient-recovered AD effluent (10%, 20%, 50%, and 100%), each supplemented with 20 g L^{-1} glucose and Ashby's media components (Table 6.1).

Table 6.1: Components of media and effluent supplement used

Control 1 (Ashby's media)		Control 2		Effluent supplement	
Components	(g L^{-1})	Components	(g L^{-1})	Components	(g L^{-1})
Mannitol	20	Glucose	20	Glucose	20
Potassium phosphate	0.20	Potassium phosphate	0.20	Potassium phosphate	0.20
Magnesium sulfate	0.20	Magnesium sulfate	0.20	Magnesium sulfate	0.20
Sodium chloride	0.20	Sodium chloride	0.20	Sodium chloride	0.20
Potassium sulfate	0.10	Potassium sulfate	0.10	Potassium sulfate	0.10
Calcium carbonate	5.0	Calcium carbonate	5.0	Calcium carbonate	5.0
		Ammonium acetate	0.5		

Starter cultures were inoculated from single colonies on streaked plates and allowed to grow for 48 hours (180 rpm, 30°C). Triplicate flasks were then inoculated to an optical density of 0.1 and allowed to incubate for 120 hours (180 rpm, 30°C). Samples were collected at different time intervals for analysis.

6.3.1.4 Measurement of glucose

Initial glucose concentration in all cultures was maintained at 20 g L^{-1} and the final concentration of residual sugars was measured using a Dionex ICS–3000 with a Dionex Pac PA20 column and a CarboPac PA20 guard column. Glucose concentration was quantified using an external standard method (Gao et al., 2012).

6.3.1.5 Sampling, total nitrogen, and dry cell weight

Samples of 2 mL were taken at different time intervals. Due to the formation of alginate (salt of alginic acid, mainly comprised of polysaccharides) in the culture, centrifugation did not serve to adequately separate cell mass from the solution media. Samples were boiled at 100°C for 5 minutes to facilitate separation, and were then centrifuged for 10 minutes at 13.8 x 103 g, washed with deionized water, and centrifuged again. Dry cell mass was measured after 24 hours of drying in a 105°C oven. Total nitrogen content was analyzed by a total nitrogen reagent kit (Hatch method 10071, Product # 2672245/2672145), using 2 mL samples of cell culture taken directly from the flask. In the case of *A. vinelandii* grown on effluent, the culture was centrifuged and a 2-mL sample of supernatant was used for the nitrogen measurement.

6.3.2 Techno-economic analysis and assumptions for a modified biorefinery for biofertilizer production

6.3.2.1 Software used for the model

To analyze the impact of a biofertilizer co-product on large scale production, a model was developed using Aspen Plus software (Aspen Technology Inc., Bedford, Massachusetts). The analysis was carried out using the Aspen ONE technology suite 8.6. The software module Aspen Plus 8.6 was used to design the unit operations and the Aspen Economic Analyzer was used to predict capital costs. Operating costs were derived from the literature and market values for chemicals and energy. Three "departments" of a biorefinery were designed and compared to the base case: ozone and aqueous ammonia soaking (OSAA) pretreatment, biofertilizer culture and separation, and lignin precipitation. The biofertilizer production was carried out using cellulosic sugars from the OSAA process, the feasibility of which was previously demonstrated at a laboratory scale (Gao et al., 2015).

6.3.2.2 Base case

The base case used for comparison was from a National Renewable Energy Laboratory technical report titled "Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol" (Humbird et al., 2011). This report details the economics of a lignocellulose biorefinery comprising the following operations:

1. Dilute acid pretreatment of corn stover
2. Neutralization of pretreated biomass for enzymatic saccharification
3. Enzymatic saccharification and fermentation
4. On-site enzyme production
5. Distillation of ethanol and solids recovery
6. Wastewater treatment
7. Storage
8. Boiler/turbogenerator
9. Utilities

This analysis examined the replacement of three unit operations, as follows:

1. Dilute acid pretreatment of corn stover was replaced with OSAA pretreatment of wheat straw
2. Enzymatic saccharification and fermentation was modified such that one quarter of the available sugar after enzymatic saccharification will instead proceed to a biofertilizer fermentation process
3. Boiler/turbogenerator where, rather than having the boiler burn lignin for energy, the base case will recover the lignin for sale as a resin precursor. Thus, the turbogenerator will be removed and the electricity will be purchased from the local grid.

6.3.2.3 Assumptions underlying the techno-economic analysis

The following assumptions were made:

1. The cost per ton of wheat straw was assumed based on the corn stover estimated cost. The costs of transportation, handling, and milling of wheat straw were assumed to be the same as those for corn stover.
2. Wastewater pretreatment cost was assumed to be the same as for the base case and modified pretreatment processes.
3. The enzyme production and saccharification operations were assumed to be the same as in the National Renewable Energy Laboratory (NREL) base case, due to similar enzyme loading for OSAA pretreated and dilute acid pretreated wheat straw (Bule et al., 2013b; Humbird et al., 2011).
4. The base case was downsized using an engineering factor of 0.6 for operations which were smaller than needed for the modified biorefinery. For example, the base case fermentation operation would be 25% smaller, since 25% of available sugar is utilized for biofertilizer production. The calculated engineering factor was thus $0.75^{0.6}$, or 0.841. Operations that were scaled down were:
   - Fermentation to ethanol (part of the enzymatic saccharification and fermentation operation)
   - Distillation of ethanol and solids recovery
5. The turbogenerator was removed from the capital cost estimate. The modified biorefinery will be purchasing electricity solely from the grid, and using residual material in a boiler to meet steam requirements.

6.3.2.4 Modeling of the OSAA pretreatment process

The OSAA pretreatment process was modeled based on a mass balance established from previous work on the pretreatment process (Bule et al., 2013a). Wheat straw is initially ozonated for 10 minutes, followed by a three-hour treatment with soaking aqueous ammonia. The aqueous ammonia solution is produced by bubbling gaseous ammonia through water to form saturated ammonium hydroxide. The ozonated biomass is then treated with the ammonium hydroxide solution in a stirred tank reactor for 3 hours. Following the soaking in aqueous ammonia pretreatment, ammonia is recovered through a flash tank at 90°C, and residual ammonia in the aqueous phase is steam-stripped and re-utilized for the generation of ammonium hydroxide (Zeng et al., 2006). The biomass solids are run through a belt press, and are then subjected to enzymatic saccharification. The soluble solids, which contain a significant amount of lignin (Table 6.2), are utilized for ammonia recovery.

Table 6.2: Initial mass components entering pretreatment and mass components entering lignin recovery after pretreatment. The remaining biomass is routed to enzymatic hydrolysis and used for production of sugar for ethanol fermentation and biofertilizer production

Into Pretreatment		Into Lignin Recovery	
Biomass Component	BDST* hr^{-1}	Biomass Component	BDST* hr^{-1}
Glucan	33.76	Glucose	1.12
Galactan	0.40	Galactose	0.15
Xylan/Mannan	14.57	Xylose/Mannose	2.67
Arabinan	1.99	Arabinose	0.54
Lignin (Insoluble)	22.05	Lignin (Insoluble)	n/a
Lignin (Soluble)	1.90	Lignin (Soluble)	13.23
Protein	1.43	Protein	n/a
Ash	5.41	Ash	n/a
Water	1.21	Water	132.88
Extractives	8.87	Extractives	4.23
Total Solids	91.60	Total Solids	14.26
BDST yr^{-1}	769,414.8	BDST yr^{-1}	119,784

* BDST: Bone Dry Short Ton

6.3.2.5 Modeling of microbial biofertilizer production on lignocellulose sugar

Microbial biofertilizer production was modeled based on the methodology for cultivation of biofertilizer strains reported in the literature (Mohammadi and Sohrabi, 2012; Pena et al., 2008). Briefly, microbes are brought up through a seed train and then inoculated into larger, aerated fermenters. The media components used for culturing are shown in Table 6.3, and consist of Ashby's growth media for *A. vinelandii*.

Table 6.3: Growth components of *A. vinelandii* based on initial wheat straw sugar input

Component	Loading	Unit (lb lb^{-1})	lb per ton straw	$ ton^{-1}	lb per ton straw
Dipotassium phosphate	0.01	per lb sugar	6.48	1840	5.9616
Magnesium sulfate	0.01	per lb sugar	6.48	65	0.2106
Sodium chloride	0.01	per lb sugar	6.48	42	0.13608
Potassium sulfate	0.005	per lb sugar	3.24	209	0.33858
Calcium carbonate	0.25	per lb sugar	162	50	4.05
pH adjustment (NaOH)	0.005	per lb sugar	3.24	250	0.405
Water	10	per lb sugar	20,000	0.40	4
Electricity	1.2	MWhr	1.2	77.5	0.0465

Twenty five percent of the available glucan from the initial biomass was used for microbial biofertilizer culture. Based on 33.76 tons per hour glucan input, 8.44 tons per hour of glucan were routed to biofertilizer production. With a 90% sugar yield after enzymatic hydrolysis, this was equivalent to 8.35 tons per hour of glucose. Prior work in culturing of *A. vinelandii* showed that 1 ton of glucose could result in the production of 0.275 tons of biofertilizer (Gao et al., 2015). As a result, the production of biofertilizer was calculated at 2.29 tons per hour.

The price of biofertilizer was set based on the potential chemical fertilizer it could displace. Traditional ammonia fertilizer costs $714 per ton, or $0.357 per lb. Prior work has found that 13.2 lbs of biofertilizer can replace up to 40 kg of ammonia fertilizer per hectare (Biari et al., 2008; Ozturk et al., 2003), giving biofertilizer an expected value of $2.38 per lb, or $4,760 per ton. Based on the fact that microbial biofertilizer is an untested technology and may not perform as expected, a lower price of $2,000 per ton was chosen. This price could enable entry into the fertilizer market while maintaining the revenue required for the process.

6.3.2.6 Modeling of lignin recovery through acid precipitation and centrifugation

Lignin can be precipitated from ammonia effluent by adjusting the pH (Bouxin et al., 2014). Lignin is soluble in ammonia effluent due to the formation of a phenoxide anion in basic media. Neutralization of the effluent results in lignin returning to a neutral charge and becoming an insoluble solid. Other soluble biomass components, such as the monomer sugars seen in Table 6.2, remain soluble under neutral conditions. Extractives also remain soluble for the most part (Sluiter et al., 2005), allowing separation of a relatively clean lignin fraction.

6.4 Results and discussion

6.4.1 Optimizing production of biofertilizer

6.4.1.1 Culturing *A. vinelandii* on AD effluent

The ability of *A. vinelandii* to grow on AD effluent (prior to nutrient recovery) was tested. The growth of *A. vinelandii* was significantly better on the AD effluent supplemented with glucose compared to Ashby's media. The concentration of biomass production in Ashby's media on day five was 1.5 g L^{-1}, as compared to 7 g L^{-1} in AD effluent with glucose supplementation (Figure 6.1). This resulted in a yield of 0.35 g of biomass per g of glucose. The rapid increase in biomass compared to Ashby's media could be due to assimilation of nitrogen present in the AD effluent (Strandberg and Wilson, 1968). There was no biomass production detected when *A. vinelandii* was grown on AD effluent alone.
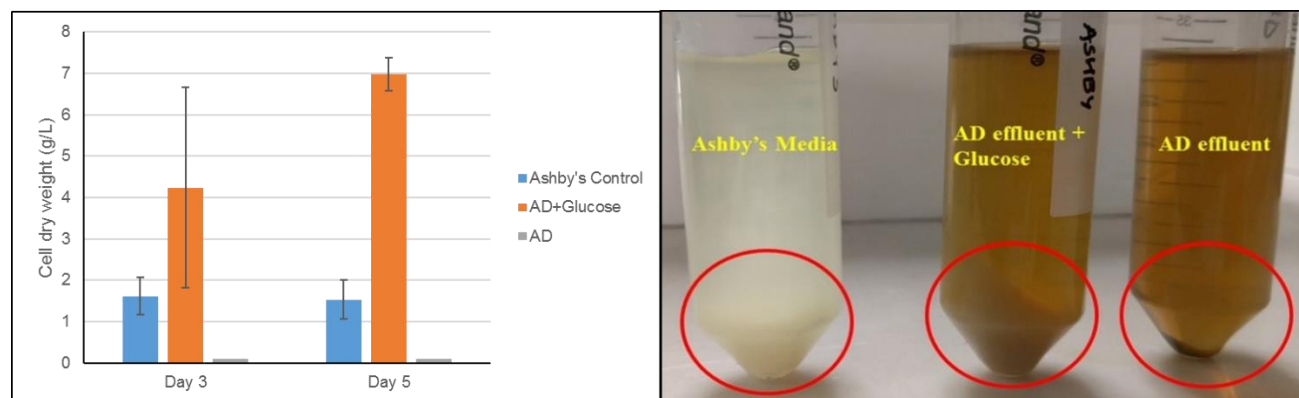
Figure 6.1: Comparison of *A. vinelandii* biomass produced on nitrogen-free Ashby's media, AD effluent supplemented with glucose, and AD effluent alone

6.4.1.2 Analysis of total nitrogen of *A. vinelandii* grown on non-nutrient-recovered AD effluent

Total nitrogen analysis showed that, when *A. vinelandii* was grown in nitrogen-free Ashby's media, the fixation of atmospheric nitrogen resulted in 17 mg L^{-1} of nitrogen in the media. The total nitrogen concentration in the AD effluent prior to fermentation was 121.6 mg L^{-1}, which was reduced when *A. vinelandii* was grown on AD effluent supplemented with glucose, resulting in 58.6 mg L^{-1} nitrogen in the media. This suggests that nitrogen from the media was utilized by *A. vinelandii* for biomass production (Figure 6.2). The nitrogen-fixing ability of this bacterium is suppressed when grown on media containing nitrogen, due to the lack of nitrogenase activity (Strandberg and Wilson, 1968). This result is also indicated by the reduced alginate formation when *A. vinelandii* was grown on AD effluent supplemented with sugar (Figure 6.1), as alginate formation is positively correlated with nitrogen fixation (Nosrati et al., 2012).
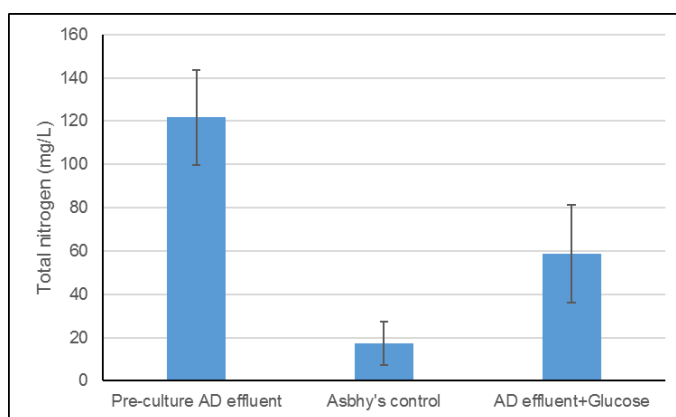
Figure 6.2: Comparison of total nitrogen content in solutions prior to culture: initial (or pre-culture) AD effluent, Ashby's media (as a control), and AD effluent supplemented with glucose

6.4.1.3 Growth and nitrogen assimilation by *A. vinelandii* on nutrient-recovered AD effluent

To test the feasibility of biofertilizer production using a combination of lignocellulosic sugars and nutrient-recovered AD effluent, we assessed the ability of nutrient-recovered AD effluent to support the growth of *A. vinelandii*. The growth of *A. vinelandii* was tested in different effluent concentrations supplemented with glucose. It was observed that diluted effluent promoted a significantly greater accumulation of *A. vinelandii* biomass compared to Control 1 (Ashby's media) and Control 2 (Ashby's media supplemented with glucose and ammonium acetate).

The cultures containing 10% and 25% effluent showed no lag phase in growth and had rapid increases in biomass in the first 48 hours, reaching 4.43 g L^{-1} and 6 g L^{-1}, respectively, on the sixth day (Figure 6.3). In a culture with 50% nutrient-recovered AD effluent, a prolonged lag phase was observed for the first 48 hours, followed by a rapid increase in growth and biomass accumulation, with the final dry cell weight reaching 6.23 g L^{-1} on day 6. Complete inhibition of growth was observed when *A. vinelandii* was grown on 100% AD effluent supplemented with sugars (Figure 6.3). These results indicate the presence of potential growth inhibitors such as excess ammonia, which affects *A. vinelandii* growth when AD effluent is used in high concentration. The prolonged lag phase followed by rapid growth and biomass accumulation are

119

a result of *A. vinelandii*'s ability to acclimatize or overcome the effect of inhibitors present in the AD effluent. Control 1 and Control 2 consistently accumulated less biomass compared to AD effluent cultures, accumulating 2.68 g L^{-1} and 2.82 g L^{-1}, respectively. These results are promising for biofertilizer production using nutrient-recovered AD effluent and lignocellulosic sugars, as eventually the effluent would be diluted to some degree, due to addition of the lignocellulosic sugars.
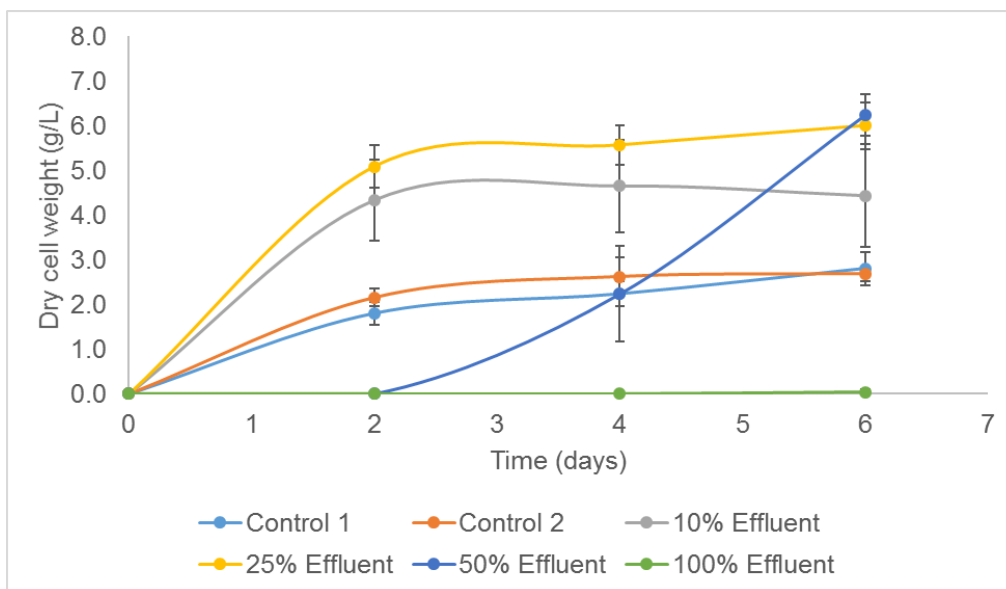
Figure 6.3: Cell mass of *A. vinelandii* grown on different concentrations of nutrient-recovered AD effluent supplemented with glucose and Ashby's media component, Control 1 (Ashby's media), and Control 2 (Ashby's media supplemented with glucose and ammonium acetate)

Analysis of total nitrogen content prior to and after growth of *A. vinelandii* showed a significant reduction in total nitrogen content in nutrient-recovered AD effluent, resulting in a final effluent stream with reduced nutrient content. The total nitrogen content in cultures with 10%, 25%, and 50% effluent prior to *A. vinelandii* inoculation was 120.33 ppm, 288 ppm, and 479 ppm, respectively (Figure 6.4). The final nitrogen content (after biofertilizer production) in cultures with 10%, 25%, and 50% effluent concentration was 26.33 ppm, 66.67 ppm, and 142 ppm, respectively (i.e., decreases of 78%, 76.8%, and 70%, respectively). Similarly, over 90% reduction in total nitrogen was observed in Control 2 (containing glucose and ammonium acetate) after six days of *A. vinelandii* growth (Figure 6.4).

These results indicate that the growth of biofertilizers on nutrient-recovered AD effluent acts as a second nutrient recovery process, resulting in a final effluent with a very low nitrogen content while producing a valuable biofertilizer product that can be marketed along with the organic fertilizers produced from the AD process.

6.4.1.4 Sugar utilization by *A. vinelandii* grown in nutrient-recovered AD effluent
Upon analysis of residual glucose, it was observed that most supplemented glucose was utilized during biofertilizer growth. The initial glucose concentration for all cultures was maintained at

20 g L^{-1}. After six days of *A. vinelandii* growth, cultures containing 10% and 25% effluent contained 6.8 g L^{-1}and 2 g L^{-1} of residual glucose. The glucose was completely utilized in the culture containing 50% effluent (Figure 6.5). These results indicate that *A. vinelandii* was able to utilize the majority of the supplemented sugars.
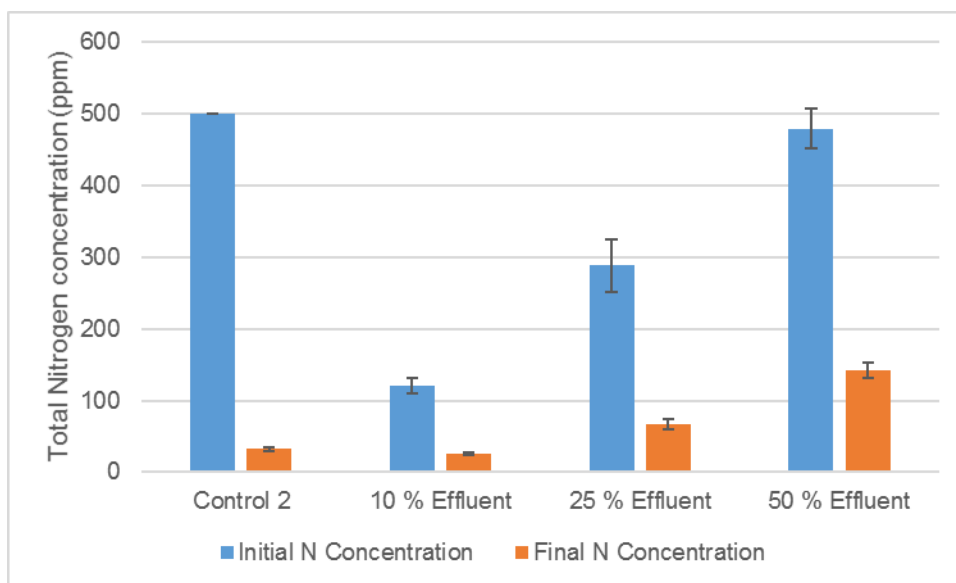
Figure 6.4: Total nitrogen concentration in nutrient-recovered AD effluent and Control 2 (Ashby's media supplemented with glucose and ammonium acetate) before and after growth of A. vinelandii. Final samples were collected on day 6. Error bars represent standard deviation of triplicate samples
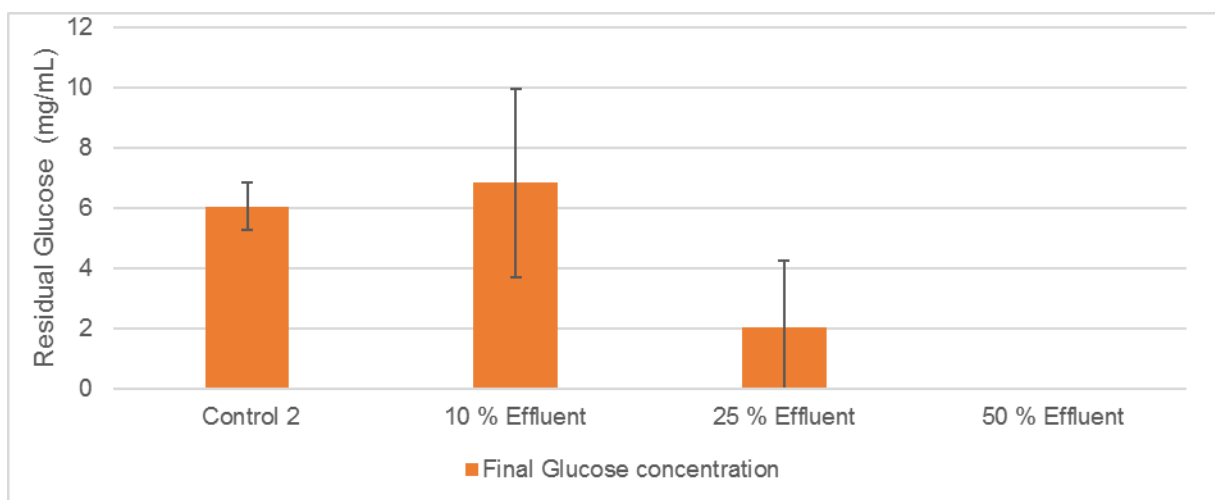
Figure 6.5: Concentration of residual glucose in different concentrations of nutrient-recovered AD effluent and Control 2 (Ashby's media supplemented with glucose and ammonium acetate) after biofertilizer production. Final samples were collected on day 6. Error bars represent standard deviation of triplicate samples

121

6.4.1.5 Developing manure fiber as a microbe carrier

In preliminary experiments, researchers observed that the manure fiber contained high levels of lignin and the pretreatment and hydrolysis yielded a much lower sugar content than what would be needed to support biofertilizer growth. The high lignin content would require an expensive pretreatment process, resulting in an increase in the cost of biofertilizer production. Given these results, the development of manure fiber as a carrier for biofertilizer was not carried out.

Researchers assumed that direct application of biofertilizer using traditional methods (such as spraying) would help save additional cost required for separation and mobilization of the biofertilizer. The immobilization of biofertilizers would require additional steps—such as obtaining appropriate carrier material, neutralization, sterilization, packing and transport—which would result in additional costs. Direct application of liquid fertilizers also has advantages, such as lower chances of contamination, longer shelf life, ease of quality control checks, and ease of use by farmers.

6.4.2 Techno-economic assessment of microbial biofertilizer production

The pretreatment capital cost was significantly lower in the modified biorefinery than in the NREL base case, with a predicted capital cost of $8.61 million compared to $29.9 million (Table 6.4). This decrease in cost was due to the ability to use mild steel construction in the OSAA pretreatment, and the lack of a need for a high-pressure screw extrusion system (which would be required for the dilute acid treatment) to feed the pretreatment reactor.

The addition of two unit operations in the form of lignin recovery and biofertilizer culturing had an added capital cost of $3.70 million and $11.50 million, respectively. A large proportion of the cost for the biofertilizer system was due to the need for a set of two stirred tank reactors with volumes large enough to accommodate a 120-hour fermentation period.

Table 6.4: Capital cost for base case and modified case

	Base Case	Modified Case
	Capital Cost (million USD*)	Capital Cost (million USD*)
Pretreatment	29.90	8.61
Neutralization and Conditioning	3.00	3.00
Saccharification & Fermentation	31.20	26.25
On-site Enzyme Production	18.30	18.30
Distillation and Solids Recovery	22.30	18.76
Wastewater Treatment	49.40	49.40
Storage	5.00	5.00
Boiler/Turbogenerator	66.00	29.01
Utilities	6.90	6.90
Biofertilizer production	-	11.50
Lignin precipitation	-	3.70
Total	**232.00**	**180.43**
45% Indirect cost added	**421.82**	**328.05**

* USD = U.S. dollars ($)

Finally, capital cost in the modified biorefinery was lowered due to the removal of the turbogenerator. This resulted in a required purchase of electricity in the operating expenditure, but represented a significant savings of $37 million in capital expenditure. The reduction in installed capital costs due to process improvements totaled $51.57 million. Accounting for indirect costs, which the National Renewable Energy Laboratory estimated at 45% of total invested capital, a reduction in installed cost of $51.57 million translates to $93.76 million in total capital expenditure savings.

There was an increase in operating cost in the modified biorefinery over that of the base case of $15.14 million (Table 6.5). Three areas contributed to this increase in cost: biofertilizer production, lignin precipitation, and the electricity cost.

Table 6.5: Operating cost for base case and modified case

	Base Case	Modified Case
	(million USD*)	(million USD*)
Feedstock	45.20	45.20
Pretreatment	5.46	12.06
Neutralization and Conditioning	2.83	2.83
Saccharification and Fermentation	2.15	1.81
On-site Enzyme Production	12.82	12.82
Distillation and Solids Recovery	n/a	n/a
Wastewater treatment	n/a	n/a
Storage	n/a	n/a
Boiler/Turbogenerator	1.51	n/a
Utilities	1.91	1.91
Fixed costs (labor, etc.)	10.70	10.70
Electricity	-	4.56
Lignin recovery	-	4.95
Biofertilizer production	-	2.31
Capital Depreciation (5.7% per year)	13.22	10.28
Total	**95.80**	**110.94**

* USD = U.S. dollars ($)

Biorefinery revenue was improved significantly through the addition of biofertilizer and lignin co-products, which added $38.58 million and $50.01 million per year in revenue, respectively (Table 6.6). A loss of electrical sales revenue of $6.60 million per year occurred due to the removal of the turbogenerator from the unit operations.

Table 6.6: Revenue and profit for base case and modified case

Base Case	USD* per unit		million USD* per year
Ethanol	$2.15 per gallon		131.15
Electricity	n/a		6.60
Total			137.75
Revenue - operating costs			41.95
Internal rate of return			9.94
Modified Case	**USD* per unit**	**Units per year**	**million USD* per year**
Ethanol	$2.15 per gallon	45.74 million	98.34
Lignin	$500 per ton	100,018	50.01
Microbial biofertilizer	$2,000 per ton	19,288	38.58
Total			186.93
Revenue - operating costs			75.99
Internal rate of return			23.16

* USD = U.S. dollars ($)

6.5 Conclusions and recommendations

Results from the growth of *A. vinelandii* using AD effluent prior to nutrient recovery and glucose showed much higher biomass production than nitrogen-free media. Growth of *A. vinelandii* was also tested on different concentrations of nutrient-recovered AD effluent. *A. vinelandii* could accumulate the greatest biomass (6.23 g L^{-1}) with complete utilization of supplemented sugars. *A. vinelandii* could overcome the growth inhibition and was able to utilize more than 70% of residual nitrogen present in the effluent, resulting in a cleaner waste stream. These results suggest that AD effluent, when supplemented with cellulosic sugars, has potential for producing biomass of *A. vinelandii.* Based on the laboratory-scale experiments in this and a previous study (Gao et al., 2015), 1 ton of wheat straw could potentially yield 648 lbs of C6 sugar, which could then be converted into about 201 lbs of biofertilizer using nutrient-recovered AD effluent. Prior research on the use of biofertilizer has shown that 13.2 lbs of dry cell mass was required for effective inoculation of 1 hectare (Biari et al., 2008). Ozturk et al. (2003) found that wheat and barley that had received an application of microbial biofertilizer supplemented with 40 kg of chemical nitrogen fertilizer exceeded the growth of wheat and barley that were grown with 80 kg of chemical nitrogen fertilizer alone. This suggest that use of relatively small quantities of agricultural residue to generate biofertilizer has the potential to solve problems caused by the direct application of nutrient-recovered AD effluent without requiring large amounts of residue to be removed from fields.

For cost effectiveness, researchers recommend that application of biofertilizers should occur through traditional methods, such as spraying. Because manure fiber has a high lignin content,

pretreatment of fiber for sugars is expensive and would offset the benefits of producing biofertilizers. Immobilization of the biofertilizer on a carrier would also require additional steps (with associated costs) in the commercial process. This research suggests that another source of lignocellulose (such as wheat straw) should be used to provide the sugar substrate for biofertilizer production.

Modification of the base case by changing the pretreatment and adding a unit operation for production of a biofertilizer co-product decreased capital cost by $93.77 million and increased the annual operating cost by $15.14 million per year. The reduction in capital cost combined with revenue generated from the co-products ($88.59 million per year), resulted in an increased internal rate of return (10% to 23.16%) (Table 6.6). This model indicates that production of biofertilizer from cellulosic sugars has a positive effect on the overall economics of the biorefinery. However, the biofertilizer is an untested technology. Further efforts to develop and optimize a low-cost process to produce sugar as an energy source for growing the biofertilizer using AD effluent would help in the commercialization of this technology.

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7. Production of Composite Materials from Anaerobic Digestion Fiber

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7.1 Background

Dairy manure is rich in recalcitrant fibrous matter that survives the animal digestive process, representing around 52.6% of total dry matter (Chen et al., 2003). Traditional anaerobic digestion (AD) does little to reduce this fibrous mass, resulting in a large by-product of fibrous solids. Traditionally, AD fiber is used directly as animal bedding or treated to produce compost for soil amendment. Composted AD fiber can be used for topsoil bedding, nursery or greenhouse bulk soil, turf top dressing, or peat replacement (Winandy and Cai, 2008; MacConnell et al., 2010).

There is also interest in using AD fiber for producing other types of products, such as engineered biocomposite products (Winandy and Cai, 2008). Some characteristics of AD fiber (e.g., its particle size and geometry) make it a suitable substitute fiber for engineered wood products (Winandy and Cai, 2008) such as wood plastic composites (WPCs). WPCs are engineered wood products widely used for decking. The global market for WPCs has been growing rapidly (Eder and Carus, 2013) because they provide better outdoor durability than wood if fungicides and insecticides are added to the formulations for producing the composite (Morrell et al., 2010) and because they do not contribute to deforestation (Markets and Markets, 2014).

One problem that limits the use of WPCs in environments with high moisture content is water affinity. Tests carried out under laboratory (Cameron, 2009) and outdoor (Kiguchi et al., 2007) conditions show that weathering deteriorates WPC surfaces, and water penetration reduces WPC strength and stiffness (Pilarski and Matuana, 2005; Panthapulakkal et al., 2006). Moisture sorption causes WPC swelling, which stresses the interfacial bond between fibers and plastic and compromises the composite's strength (Stark, 2001). Because the properties of WPCs are affected by the chemical composition of the fiber used in the composite, modifying the

composition of raw materials is a strategy for improving critical properties of WPCs, such as water resistance (Pelaez-Samaniego et al., 2013a, b; Chen et al., 2014).

Hot water extraction (HWE) can potentially be used to pretreat AD fiber, as no previous steps (e.g., drying) are required. Previous studies with softwood have shown that HWE at 160°C for 90 minutes removes approximately 20% of the original mass, in the form of an aqueous solution that is rich in sugars, acetic acid, and other compounds, mostly derived from hemicellulose (Chaffee, 2011; Pelaez-Samaniego et al., 2015). Based on these previous studies, HWE may be an effective strategy for improving the properties of WPCs manufactured from AD fiber.

7.2 Objectives

The objectives of this work were to evaluate the impact of HWE on the composition of AD dairy fiber and to assess how changes in AD fiber composition and structure affect the properties of WPCs.

7.3 Methods

7.3.1 Production of composite material

Anaerobic digestion dairy fiber was obtained from George DeRuyter and Sons Dairy Farm in Outlook, Washington, which is a 3000 wet-cow equivalent dairy that uses a typical high-energy feed. The manure was collected using a flush manure handling system, where the dilute manure wastewater is sent to a clarifier before entering a mesophilic, 20-day mixed plug-flow anaerobic digester. After digestion, the effluent is sent to a slope screen attached to dewatering roller presses for mechanical recovery of digested fibrous solids with a moisture content of approximately 72%. A representative portion of the digested fiber (approximately 30 kg) was transferred to Washington State University (WSU), where it was partially dried at room conditions for one week. The AD fiber was subsequently dried at 103°C for 24 hours and stored prior to its characterization and the production of WPCs. Untreated AD fiber (i.e., not thermochemically treated and not ground) is herein referred to as fiber 'as received' (or UUF). Other materials used for WPC production were (a) Eastern white pine (*Pinus strobus*) wood flour (American Wood Fibers, passed through a 60-mesh sieve), (b) ground (passed through a 60-mesh sieve), not thermochemically treated AD fiber, and (c) ground (passed through a 60-mesh sieve), thermochemically treated AD fiber, herein referred to as (a) pine, (b) GUF, and (c) GTF, respectively.

Other additives used for WPC production were virgin high-density polyethylene (HDPE) (Lyondell/Equistar LB010000) (Equistar Chemicals, LP, Mansfield, Texas, U.S.), zinc stearate (former Crompton Corp, Middlebury, Connecticut, U.S.) as lubricant, zinc borate (U.S. Borax/RioTinto, http://www.borax.com) as a biocide, and talc (Nicron 403 from RioTinto, http://www.imerystalc.com). These formulations were chosen to obtain products comparable to previously reported composites (Pelaez-Samaniego et al., 2013a).

7.3.2 Hot water extraction

Anaerobic digestion fiber 'as received' was subjected to hot water extraction using a 1-L Parr 4521 bench top reactor (Moline, Illinois, U.S.) controlled by a 4842 Parr controller and coupled with a water circulating cooling system (Pelaez-Samaniego et al., 2015). Five different temperatures (120°C, 140°C, 160°C, 180°C, and 200°C) were tested for 60 minutes. The proportion of E-pure-water to fiber was 5:1 (in mass). This relatively high water to fiber ratio was used to keep the fiber moist because small particles absorbed water very quickly.

In HWE, some sugars are degraded during the heating-up phase, especially when this step is long (Borrega et al., 2011). For this reason, conversion of the heating-up time into an equivalent isothermal reaction time by adding a temperature time correction factor is recommended (Borrega et al., 2011; Pelaez-Samaniego et al., 2015). The activation energy of the fiber required for this correction was determined using thermogravimetric analysis (TGA) (TGA/SDTA 851e Mettler Toledo, Columbus, Ohio, U.S.) of the 'as received' fiber at different heating rates (1, 2, 5, and 10°C per minute), following ASTM E1641-07 (2007). The computed activation energy of the AD fiber was 190 kJ mol^{-1}, which was used to calculate the time-temperature correction, resulting in a total treatment time of 62 minutes (instead of 60 minutes).

After each treatment, a sample of the liquid phase was collected by wrapping the HWE material with cotton tissue and squeezing it to filter the liquid and retain the solid particles. The collected liquid sample was then stored at 4°C for further analysis of sugars. The pH of each liquid fraction was measured.

7.3.3 Characterization of the liquid products

Approximately 5 mL of the liquid fraction of each HWE sample was filtered using a Milex®-HV 0.45 µm sterile filter with Durapore® PVDF membrane. The filtrate was then diluted with E-pure water and used for sugars analysis using a Dionex ICS-3000 ion chromatograph (Waltham, Massachusetts, U.S.), using the method described by Pelaez-Samaniego et al. (2015).

7.3.4 Characterization of the solid products

Immediately after HWE, the solid phase of each sample was washed with tap water several times, using a 200-mesh sieve to avoid loss of the product. The material was then dried at 103°C until the mass change was negligible, and stored in glass containers. The mass loss during HWE was calculated from the difference between the initial dry mass (i.e., before HWE) and the final dry mass (after HWE). The dried materials were used for chemical composition, proximate, and elemental analyses. The material treated at 160°C was chosen for WPC production, and was analyzed for particle size distribution and bulk density, both before and after grinding it to pass through a 60-mesh sieve. Pine and AD fiber 'as received' were also analyzed for particle size distribution and bulk density. Both untreated and treated fiber were additionally analyzed using a FEI Quanta 200F scanning electron microscope (SEM, Hillsboro, Oregon, U.S.) to visualize the structure of the fibers. Prior to microscopy, the fibers were coated with gold using Hummer V sputtering equipment.

7.3.4.1 Proximate analysis
The ash content of the fibers was determined by burning the material at 550°C for 2 hours (ASTM D1102-84, 2007). Volatiles were indirectly determined using TGA by heating the fiber in a nitrogen environment from 25°C to 600°C at a heating rate of 10°C per minute (ASTM E1868-04, 2004). Fixed carbon was calculated by difference.

7.3.4.2 Elemental analysis
Carbon, hydrogen, and nitrogen content were determined using a LECO® TruSpec CHN instrument (St. Joseph, Michigan, U.S.) coupled with a LECO® 628S S module, in duplicates, as described in Pelaez-Samaniego et al. (2015).

7.3.4.3 Chemical composition
Cellulose, hemicellulose, and lignin in AD fiber were determined following the National Renewable Energy Laboratory (NREL) method (Sluiter et al., 2010). Insoluble lignin was considered as the mass lost after burning the hydrolysis solid fraction in a furnace at 550°C. Soluble lignin was determined by analyzing the hydrolysis liquid fraction with a Shimadzu UV-2550PC UV/Vis spectrophotometer (Kyoto, Japan). The total lignin is the sum of both soluble and insoluble lignin. A Dionex ICS-3000 ion chromatograph (Sunnyvale, California, U.S.) was used to determine sugars in the hydrolysis liquid.

7.3.4.4 Particle size distribution and bulk density
The particle size distribution of the pine wood floor and of the fibers were analyzed according to ANSI/ASAE S319.4 (2013). The sieves used for pine, ground untreated AD fiber (GUF), and ground treated AD fiber (GTF) ranged from 70 to 140 mesh, and those used for fiber 'as received' (UUF) ranged from 14 to 45 mesh. Bulk density of the fibers was tested following ASTM E873-82 (2006).

7.3.5 Wood plastic composites production and testing

7.3.5.1 Wood plastic composites production
Prior to WPC manufacture, torque rheology was conducted for formulations of HDPE with pine, UUF, GUF, and GTF. A mixture of 60% of each fiber and 40% HDPE (50 g total, to fill more than 80% of the rheometer chamber) was used in a Haake Rheomix 600 torque rheometer (Waltham, Massachusetts, U.S.) equipped with roller-blade rotors for 10 minutes at 20 rpm and 160°C. Composites were produced via extrusion, in which 2.5 kg of a mixture containing 58% dry filler, 32% HDPE, 3% lubricant (zinc stearate), 2% zinc borate, and 5% talc were blended for 10 minutes in a Ross ribbon blender. This formulation was chosen following the one used by Pelaez-Samaniego et al. (2013a), for comparison of results. Prior to extrusion, the blends were left in the oven at 103°C for 24 hours. The extrusion process was carried out in a 35-mm intermeshing twin-screw extruder (Cincinnati Milacron Inc., Cincinnati, Ohio, U.S.) equipped with a 37×10 mm cross-section die. The extruder operating conditions were set with a 160°C barrel temperature, screen and die temperatures of 155°C, and a screw rotation of 5 rpm.

7.3.5.2 Wood plastic composites evaluation

Evaluation of the properties of WPCs consisted of flexural (modulus of rupture [MOR] and modulus of elasticity [MOE]) and water sorption tests. For flexural, six specimens of each formulation, randomly selected from the corresponding board, were sawn to the required length outlined by ASTM D790-10 (2010). Each specimen of pine and UUF WPC had final dimensions of $38.1 \times 10.2 \times 203.2$ mm. For the GTF-HDPE and the GUF-HDPE formulations, the specimens were cut to remove part of the edges (due to surface imperfections) leading to final dimensions of $25.4 \times 7.6 \times 177.8$ mm. The specimens were then conditioned at 25°C and 55% relative humidity for 48 hours, and the composite densities were determined prior to flexural tests. For the tests, a universal test frame equipped with a 907 kg load cell, with a support span of 157.5 mm and a crosshead speed of 4.2 mm min^{-1} (ASTM D790-10, 2010), was used. The test consisted of applying load to the specimens until the breaking point to determine MOR, MOE, and strain at break.

For water sorption tests, the thermoplastic-rich skin of the composites was removed using a knife planer, following a previously described procedure (Pelaez-Samaniego et al., 2013a). Three randomly chosen specimens of UUF and pine formulations (with dimensions $25.4 \times 6.3 \times 127.0$ mm) and GUF and GTF composites (of $25.4 \times 7.6 \times 127.0$ mm) were conditioned at 25°C and 50% relative humidity for 48 hours. The test consisted of immersing the specimens in distilled water at room temperature. The specimen thickness and weight were measured before the test started and periodically during the test, according to ASTM D570-98 (2010). The diffusion constant is a parameter that is used to evaluate the rate of moisture sorption for a specific material. The diffusion constant was calculated using Fick's law of diffusion in the hygroscopic range (from zero percent moisture to the fiber saturation point) taking into account diffusion through the material edges (Rangaraj and Smith, 2000; Stokke et al., 2014).

Analysis of variance was carried out for mechanical properties and water sorption results using SAS® statistical software (Cary, North Carolina, U.S.). The same software was used for analysis of covariance to evaluate the influence of density on other mechanical properties.

After flexural tests, cross sections of the GTF and UTF boards were analyzed by Scanning Electron Microscopy (SEM) to visualize the composites and determine the possible effects of HWE on fiber.

7.4 Results and discussion

7.4.1 Properties of liquid products

As expected, mass loss was greater with higher treatment temperatures of HWE (Table 7.1). During HWE, acetic acid is formed from the acetyl groups bound to hemicellulose (Borrega et al., 2011). Thus, it was expected that the pH of the aqueous phase would decrease with an increase in the treatment temperature.

Table 7.1: pH, mass loss, and sugar content for the aqueous phase of each hot water extraction (HWE)

HWE Temperature (°C)	pH	Mass loss (%)	Sugars (%)*
120	6.44	11.8	0.00
140	5.97	16.3	1.21
160	5.44	21.0	2.04
180	4.62	31.6	3.18
200	4.62	36.0	0.06

* Percentages refer to the corresponding ash-free material

7.4.2 Properties of solid products

7.4.2.1 Proximate analysis

The percentage of ash in the solid product decreased as the HWE temperature increased, reaching a minimum at 180°C (Figure 7.1). The amount of fixed carbon increased and, as expected, the presence of volatiles decreased. A slight decrease in ash content was observed as the temperature of HWE increased, as observed in previous studies (Chaffee, 2011; Pelaez-Samaniego et al., 2015).
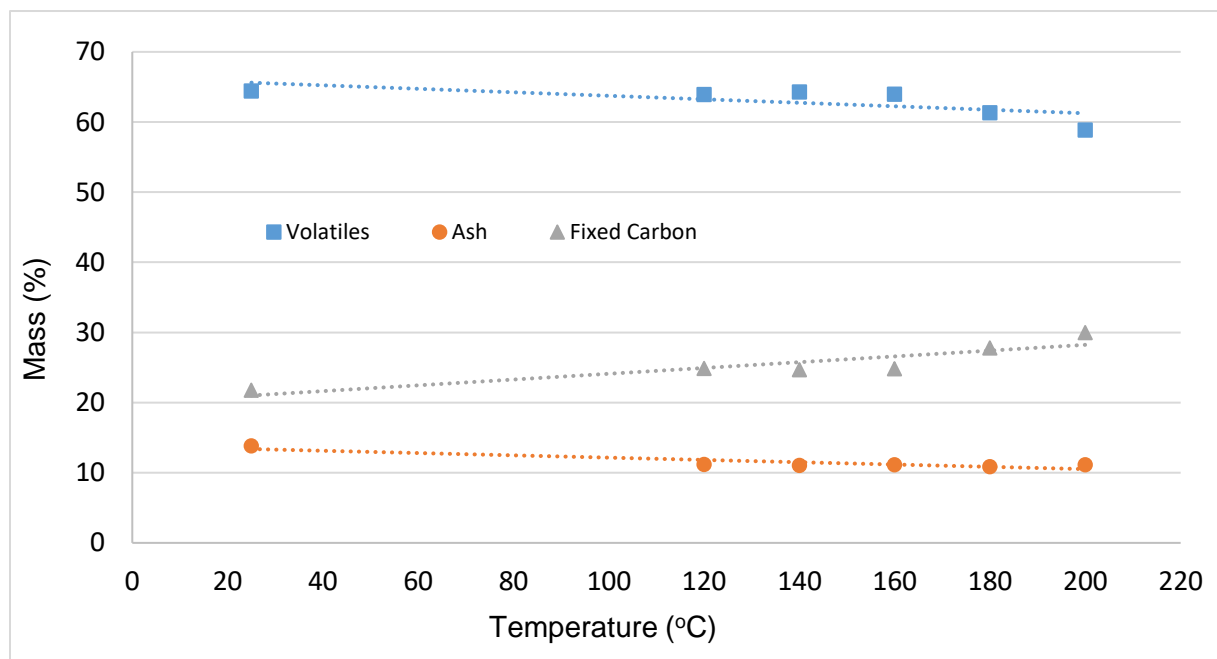
Figure 7.1: Percentage of volatiles, ash, and fixed carbon for each treated and untreated fiber following hot water extraction (HWE)

The TGA curves showed that untreated fiber and fibers treated at 120°C, 140°C, and 160°C started to degrade at lower temperatures than fibers treated at 180°C and 200°C (Figure 7.2). The differential thermogravimetric curve of untreated material (Figure 7.3) presented a perceptive shoulder, which was less visible with increases in HWE temperature. After 160°C, the shoulder in the differential thermogravimetric curve was no longer visible. This result is in agreement with the expected reduction of hemicellulose content in the solid fraction, as this shoulder is

associated with the hemicellulose content (Pelaez-Samaniego et al., 2013a). The peak corresponding to cellulose degradation shifted slightly to a higher temperature as the HWE temperature increased (Figure 7.3). This result suggests that inorganic salts in the AD fiber cause cellulose decomposition to occur at a lower temperature. As HWE removed those inorganic salts, cellulose thermal stability increased (Várhegyi et al., 1997; Benitez-Guerrero et al., 2014).
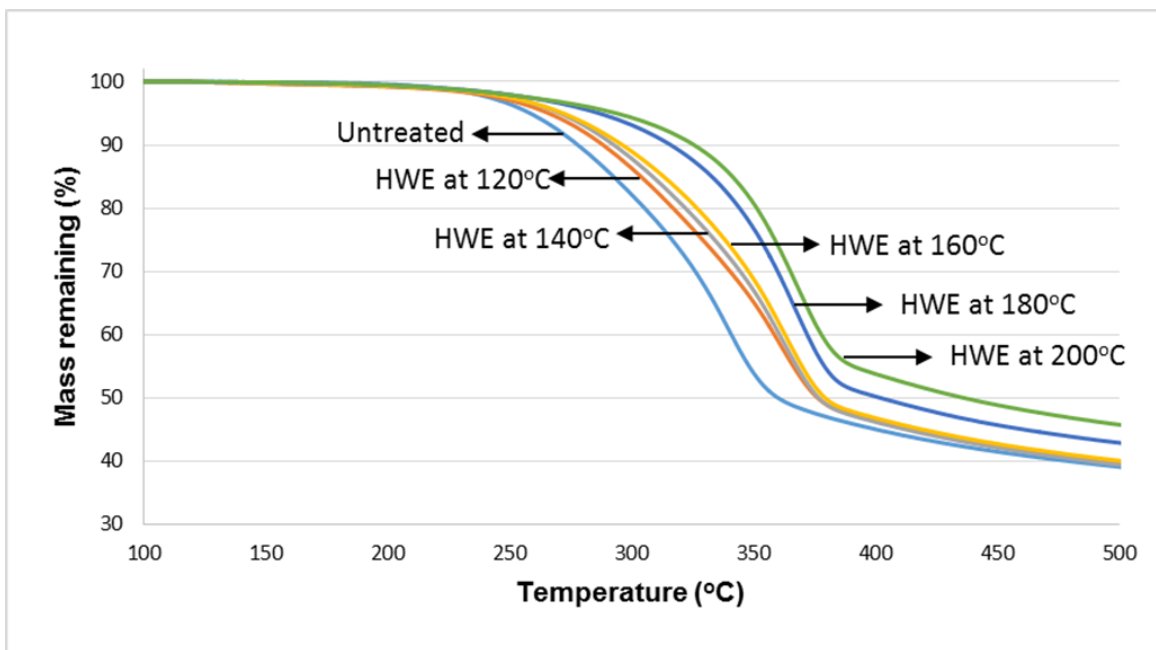
Figure 7.2: Thermogravimetric Analysis (TGA) curves of untreated and treated materials at different conditions
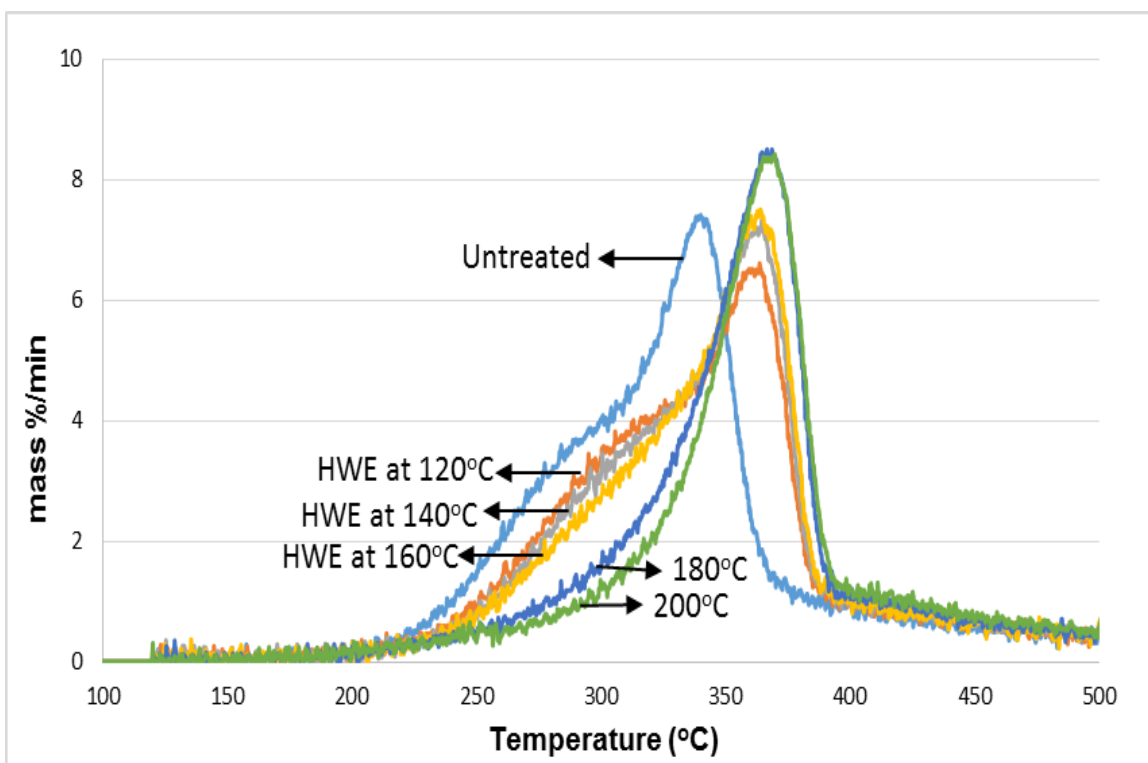
Figure 7.3: Derivative of thermogravimetric analysis (DTA) for untreated fiber and fiber treated at each hot water extraction (HWE) temperature

7.4.2.2 Elemental analysis

The relative amount of carbon increased as the HWE temperature increased (Figure 7.4). The amount of oxygen, on the other hand, decreased with an increase in HWE temperature. Hydrogen and nitrogen content remained approximately constant. These results are in agreement with those found in the literature (Pelaez-Samaniego et al., 2015).
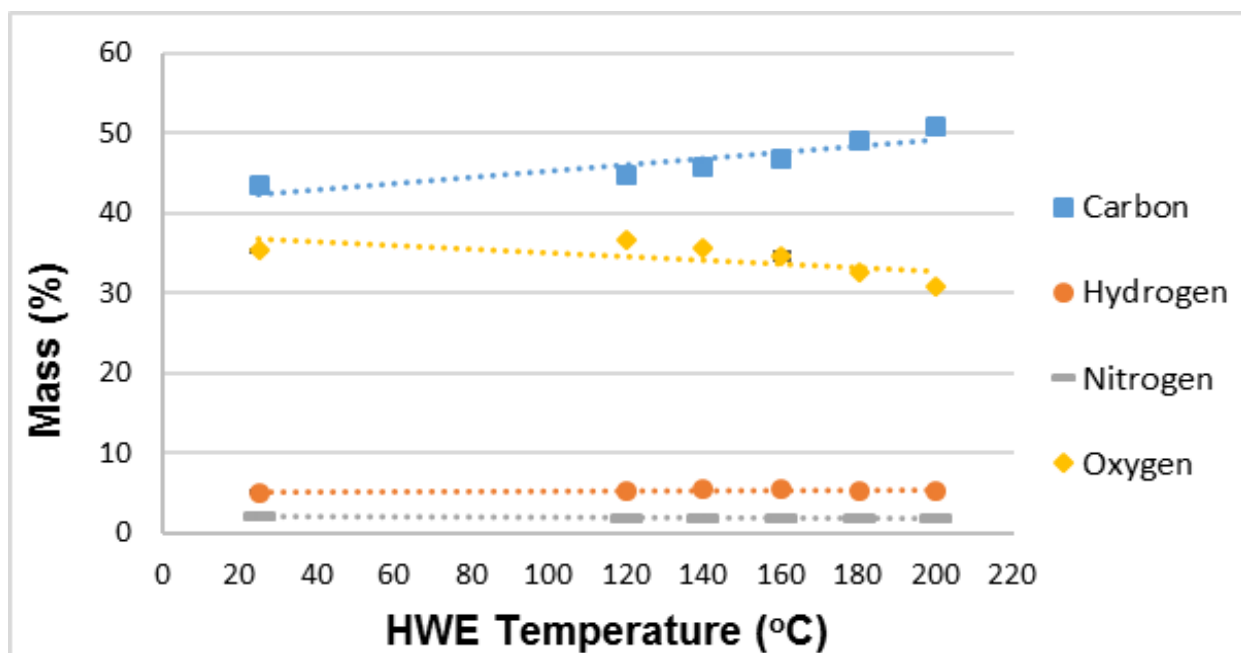
Figure 7.4: Elemental composition of fiber treated with hot water extraction (HWE) at different treatment temperatures

7.4.2.3 Chemical composition

The percentages of cellulose, hemicellulose, and lignin were multiplied by the mass percent remaining after each treatment (Figure 7.5). The content of each component decreased when the temperature of the treatment increased. As expected, hemicellulose content decreased faster than other constituents, indicating that greater amounts of hemicellulose were removed as the temperature of the HWE process was increased. The mass balance of the untreated material (i.e., at 20°C) was approximately 100% if the ash content (as per Figure 7.1) is added (Figure 7.5). The hemicellulose content in the material pretreated at 160°C was approximately 10%, and complete removal of hemicellulose was possible only by increasing the pretreatment temperature above this temperature.
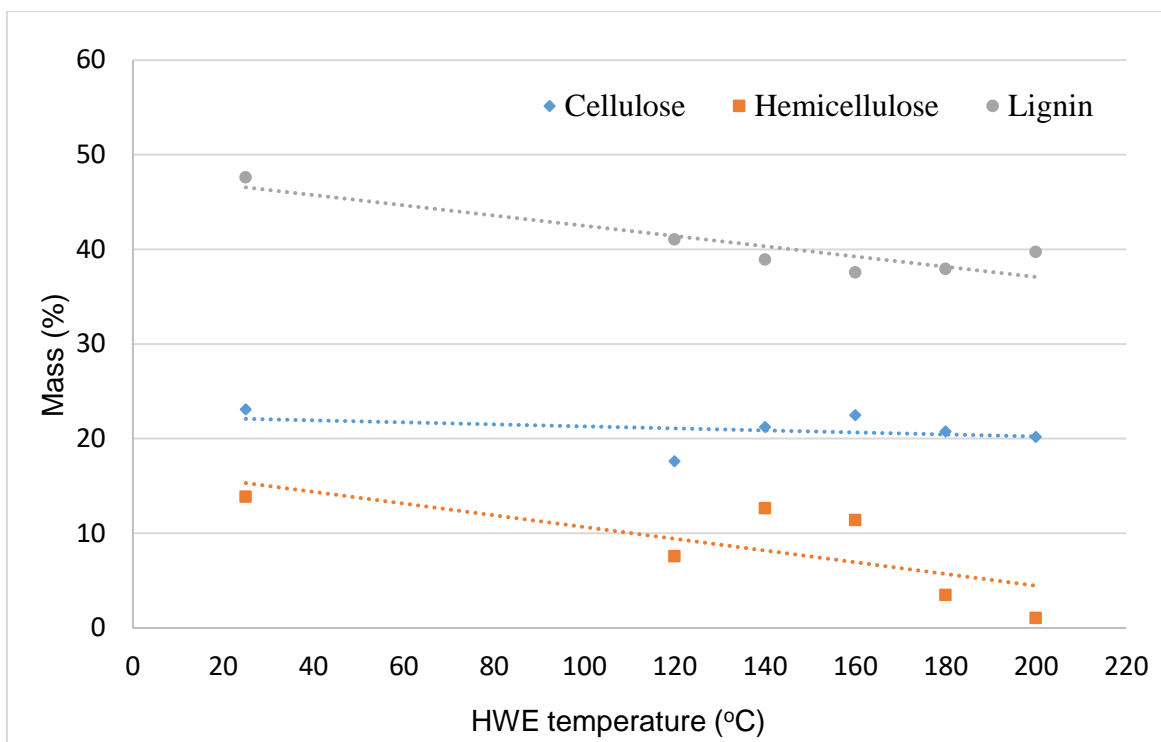
Figure 7.5: Cellulose, hemicellulose, and lignin content of untreated (20°C) and hot water extraction (HWE) treated fiber

7.4.2.4 Particle size distribution and bulk density

Pine, UUF, GUF, and GTF were analyzed for particle size distribution (Figure 7.6). Particle size of pine was distributed around 0.149 mm. Fiber 'as received' (UUF) presented a random particle size distribution with no pattern. For GTF and UTF, more than 90% of the particles were 0.149 mm or less. According to previous studies, thermochemical treatments facilitate wood grinding (Bridgeman et al., 2010; Pelaez-Samaniego et al., 2013a). This can explain why treated fiber had a smaller particle size than untreated fiber. The bulk density of pine, UUF, GUF, and GTF were 0.215 ± 0.004, 0.175 ± 0.005, 0.390 ± 0.001, and 0.324 ± 0.002 g cm^{-3}, respectively.
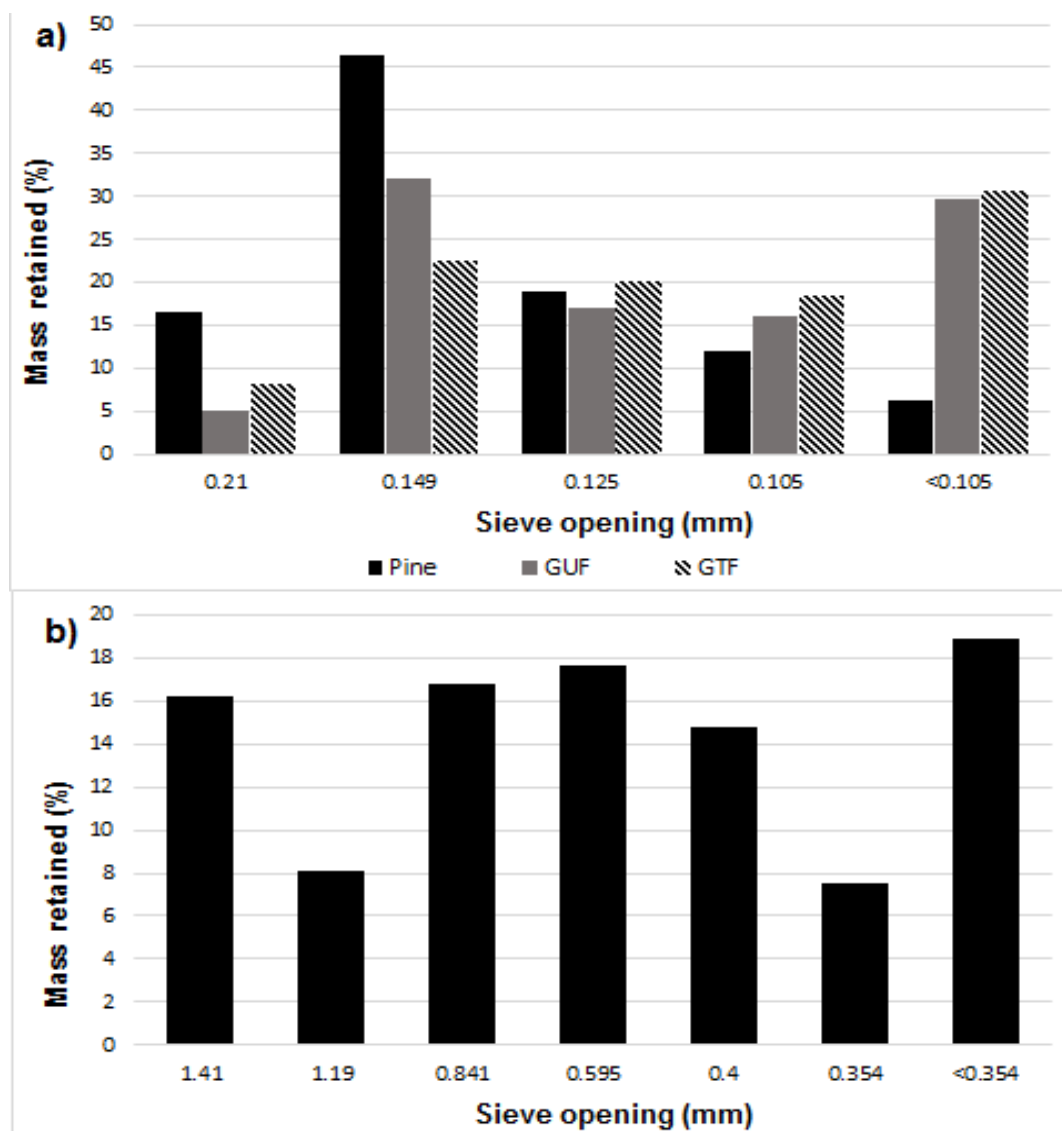
Figure 7.6: Particle size distribution of a) pine fiber, ground untreated (GUF), and ground treated (GTF) fibers, and b) fiber 'as received' (UUF)

7.4.3 Wood plastic composites properties

7.4.3.1 Torque rheology

Torque rheology results showed that the torque required for compounding treated fiber was higher than for the other fibers, which could result from the better adhesion between treated fiber and HDPE. High-density polyethylene has nonpolar characteristics, and treated fiber presents increased nonpolar characteristics (Mohanty et al., 2005). In the extrusion process, the extruder motor current intensity (amperage) was also different: it increased when switched from pine to fiber 'as received,' and from untreated to treated fiber.

7.4.3.2 Color

As observed in other studies, the color of the WPC boards produced with treated AD fiber was darker than those produced with untreated AD fiber. Oxidation of phenolic compounds, the presence of reduced sugars and amino acids, and the emanation of formaldehydes are some of the factors affecting the color of the fiber during thermochemical treatments (Pelaez-Samaniego et al., 2013a, b). Thus, it was expected that the WPC produced with treated fiber would be darker than that containing untreated fiber.

7.4.3.3 Surface irregularities

The composite produced with ground treated fiber (GTF) had rough and irregular surface and edges, with a snake-skin appearance in some parts of the boards. Despite the irregularities, all boards were adequate to prepare samples for mechanical testing and water immersion conditions. Some published articles report the same extrusion defects with maple-polypropylene composites (Slaughter, 2004), and wheat straw-HDPE composites (Schirp et al., 2006). It is possible that this problem could be fixed by changing the extrusion parameters or changing the formulations.

7.4.3.4 Scanning electron microscopy (SEM) analysis

The cell wall of the untreated material was more homogeneous and smoother than that of the HWE-treated AD fiber (Figure 7.7). After treatment, fiber became rougher, with some coalesced droplets on the surface, which could be a lignin-rich material that migrated from the cell walls to the fiber surface (Selig et al., 2007; Sannigrahi et al., 2011; Pelaez-Samaniego et al., 2013a). Lignin softens and coalesces in the aqueous phase at temperatures within the range of the temperatures studied (120°C to 200°C) (Selig et al., 2007; Singh and Harvey, 2010), thus explaining the presence of droplet-like materials on the surface. This observation was confirmed by the scanning electron microscope pictures of the fibers in the WPC (Figure 7.8).
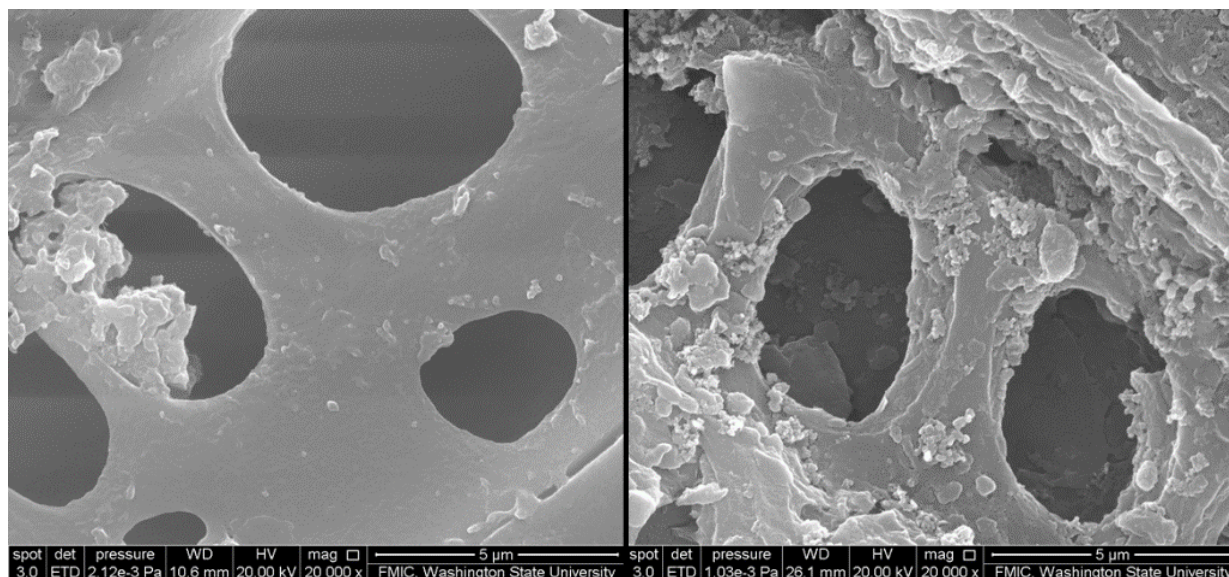
Figure 7.7: Scanning electron microscopy images of untreated anaerobic digester (AD) fiber (left) and 160°C hot water extracted (HWE) AD fiber (right) (20,000×). The image on the right shows rougher fiber with coalesced droplets of lignin-rich material, due to heat treatment
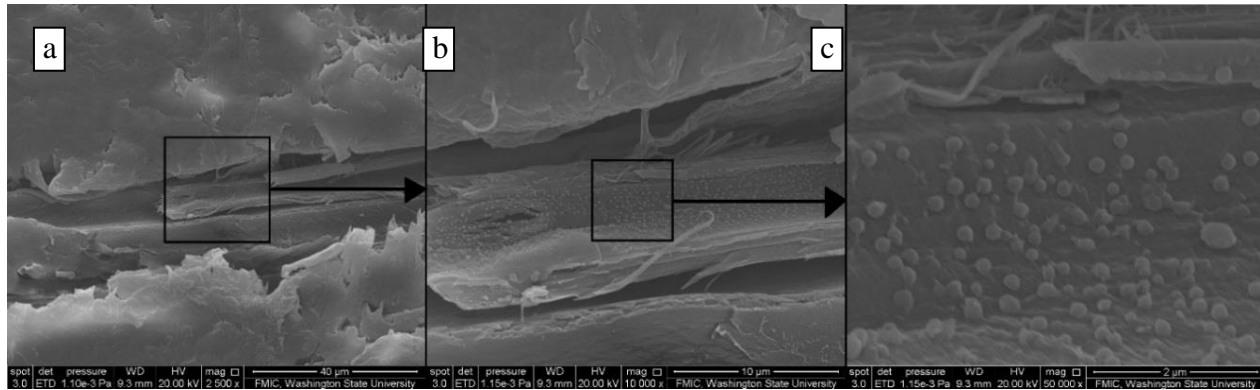
Figure 7.8: Scanning electron microscopy images of wood plastic composite (WPC) produced with ground treated AD fiber (GTF) at (a) 2,500×, (b) 10,000×, and (c) 50,000× magnification

7.4.3.5 Mechanical and physical properties

The calculated density of each material was measured at 25°C and 50% relative humidity. The density of the composites produced with pine and UUF were not statistically different (Table 7.2). The composites produced with GUF and GTF had a greater density than pine and UUF, but were not statistically different from each other. In all cases, the densities were close to those reported by Pelaez-Samaniego et al. (2013a). The modulus of elasticity and the modulus of rupture of WPCs produced with HWE AD fiber were better than those of composites produced from AD fiber without HWE pretreatment (Table 7.2). Analysis of covariance showed that density affected the MOR and MOE, but not the strain at break. The composite produced with HWE fiber showed increases in the MOR and the MOE of 36% and 30%, respectively, over the composite produced without HWE pretreatment. Strain at break was also reduced by 15.4% when the treated fiber material was used. These results suggest that treating the fiber via HWE prior to extrusion produced a composite with improved mechanical properties.

Table 7.2: Mechanical properties of each material compared with results from Pelaez-Samaniego et al. (2013a)

Filler	Density (g/cm³)		MOR (MPa)		MOE (GPa)		Strain at Break
Pine	1.15 ± 0.03 A		19.08 ± 1.15		2.94 ± 0.23		0.008 ± 0.000
UUF	1.12 ± 0.03 A		17.24 ± 0.83		2.09 ± 0.15		0.012 ± 0.000 C
GUF	1.20 ± 0.01 B	1.18*	15.85 ± 0.96	27.59*	2.16 ± 0.07	3.77*	0.013 ± 0.001 C
GTF	1.23 ± 0.01 B	1.18*	21.59 ± 0.58	31.70*	2.81 ± 0.11	3.75*	0.011 ± 0.001
*Pelaez-Samaniego et al. (2013a) values for wood plastic composites (WPC) using ponderosa pine untreated and treated via hot water extraction at 160°C for 90 minutes							

Both the water sorption (Figure 7.9) and the thickness swelling (Figure 7.10) of the composite produced with pine were the highest among the four tested formulations. Both composites produced with untreated fiber behaved very similarly. The ground treated fiber (GTF) composites showed the best performance.
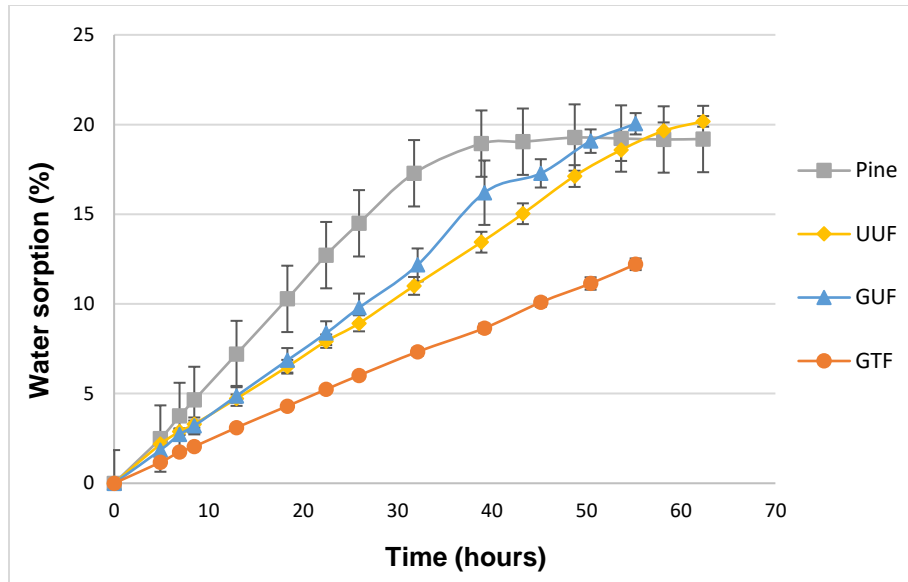
Figure 7.9: Water sorption as a function of time comparing pine, fiber 'as received' (UUF), ground untreated fiber (GUF), and ground treated fiber (GTF) composites
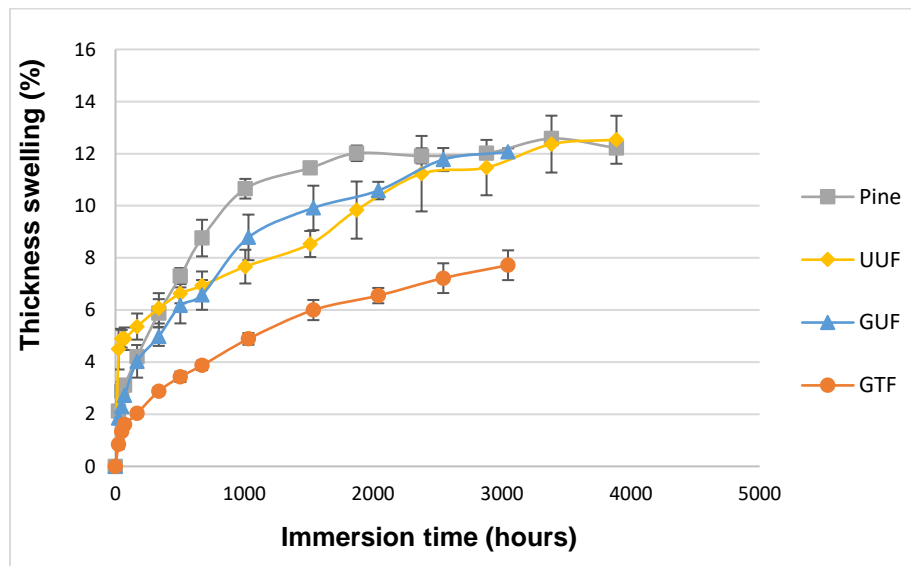
Figure 7.10: Thickness swelling as a function of time comparing pine, fiber 'as received' (UUF), ground untreated fiber (GUF), and ground treated fiber (GTF) composites

Water sorption in the GUF composite was significantly different from that of GTF, showing that treating the fiber improved this property. The thickness swelling of both untreated AD fiber materials (UUF and GUF) was not significantly different. However, the composite produced with treated fiber (GTF) absorbed less water and swelled less than all other WPCs. Hot water

142

extraction reduced moisture uptake by 39.1%, and swelling by 36.0%, after 127 days of water immersion.

The diffusion constants of WPCs made from pine, AD fiber 'as received', ground untreated AD fiber, and ground treated AD fiber were 1.13×10^{-6}, 3.92×10^{-7}, 6.15×10^{-7}, and 2.23×10^{-7} mm^2 s^{-1}, respectively. These results, once again, showed that HWE treatment greatly decreased the water affinity of the WPCs by reducing the inherent hydrophilicity of the untreated material.

7.5 Conclusions and recommendations

Hot water extraction shows potential as a pretreatment operation for using AD fiber for the manufacture of WPC. Due to hemicellulose removal, HWE was an effective method for reducing the hydrophilicity (water affinity) of AD fiber and the resulting WPCs. Wood plastic composites produced with treated fiber and HDPE absorbed less moisture than those produced with untreated fiber and HDPE. The mechanical properties of WPCs produced with HWE-treated AD fiber were superior to those produced using AD fiber without the pretreatment step. Our results therefore confirm the potential to produce composite materials from hot water treated AD fibers.

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8. Proof of Concept Project: Tools to Evaluate Water Quality Impacts of On-Field Application of Anaerobic Digestion System Nutrient Products

Chad Kruger, Kirti Rajagopalan, and Giridhar Manoharan

8.1 Background

Over the past decade, farmers have noticed a subtle but impactful change in precipitation events. The cause of this change can be linked to a variety of factors, including climate change, the increase in atmospheric particles, and natural weather cycles. No matter the cause, dairy farmers in western Washington are significantly affected by seasonal rainfall patterns and intensity. Rainfall directly impacts crop planting and harvest timing, manure storage capacity and holding time, manure application timing, flooding, and the potential for having a runoff or leaching event. Dairy farmers have raised questions regarding the need for increased storage, trucking of manure to low-risk areas, modification of farm management practices (e.g., buffer strips), or other technology-based strategies to reduce the risk of water pollution (e.g., anaerobic digestion and nutrient recovery). This project will provide initial insight into the potential for voluntary implementation—rather than additional regulatory imposition—of a decision support system focused on improved manure management practices to improve water quality.

8.2 Objectives

The objectives of this proof of concept project were to:
1. Assess the forecasted change in annual and storage season precipitation for Whatcom, Skagit, and Snohomish Counties, and
2. Determine the relative demand for increased manure storage or nutrient recovery technology to reduce the risk of nutrient discharge.

8.3 Methods

Gridded historical meteorological data (1979 to 2005) and climate projections for three future time frames (2040s, 2060s, and 2080s) were used to estimate projected changes in annual and storage season (October through March) precipitation for the three northwest Washington counties with significant dairy production (Whatcom, Skagit, and Snohomish). The gridded historical meteorological observations are at 4-km resolution and based on Abatzoglou (2011). Future climate projections are also at 4-km grid resolution. The three future time frames were considered as 30 years of projections centered around 2040, 2060, and 2080, respectively. They

are Modified Multivariate Adaptive Constructed Analog (MACA) based (Abatzoglou and Brown, 2012), downscaled global climate model data from the Coupled Model Intercomparison Project 5 (CMIP 5; Taylor et al., 2012). We used six climate change projections based on the RCP8.5 concentration pathway in this exploratory analysis. Median and 75th percentiles of change in precipitation are plotted for annual and storage season precipitation. Statistical significance tests for differences have not been performed. All plotted and mapped data are archived on the Center for Sustaining Agriculture and Natural Resources' AgClimateTool webpage (http://agclimatetools.cahnrs.wsu.edu/users/giridhar/climate/).

8.4 Results and discussion

8.4.1 Changes in annual precipitation

Figure 8.1 shows the percentage change in median annual precipitation (which represents a proxy for an average year) and Figure 8.2 shows the percentage change in the 75th percentile of annual precipitation (a proxy for wet years). The ranges shown in the figures are a result of location-specific differences. Results are grouped by county and elevation bands to account for changes in average precipitation magnitudes between low and high elevation areas.
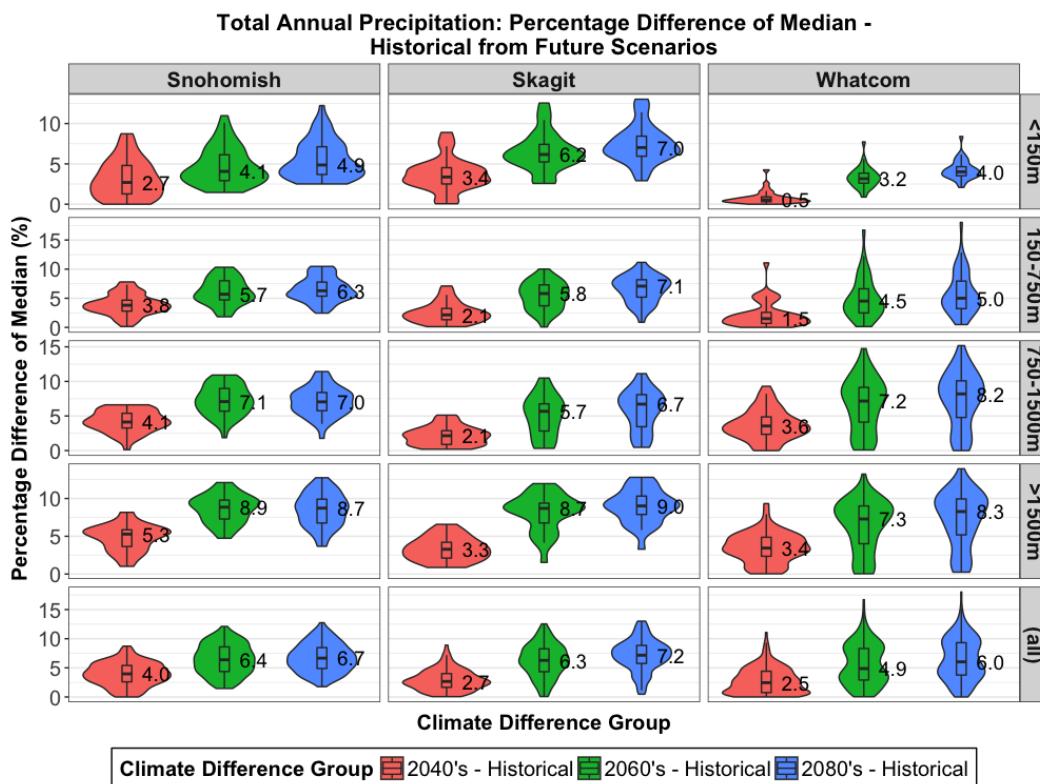
Figure 8.1: Changes between historical and future median annual precipitation, by county and by elevation band. The median difference is annotated in black text, and the ranges correspond to location-specific differences

147

Figure 8.2: Changes between historical and future values for the 75th percentile of annual precipitation, shown by county and by elevation band. The median difference is annotated in black text, and the ranges correspond to location-specific differences

In general, the differences in average year precipitation ranges from no change to 15% increase depending on the location and future time frame. The increases tend to be higher further out in the future.

8.4.2 Changes in storage season precipitation

Figure 8.3 shows the percentage change in median storage season precipitation (representing a proxy for an average year) and Figure 8.4 shows the percentage change in the 75th percentile of storage season precipitation (a proxy for wet years). The ranges shown in the figures are, as in the earlier figures, a result of location-specific differences. Results are similarly grouped by county and elevation bands to account for changes in average precipitation magnitudes between low and high elevation areas. In general, the differences in average year precipitation ranges from no change to 20% increase, depending on the location and the future time frame considered. The increases tend to be higher further out in the future and are generally larger than the changes in annual precipitation.

Maps of projected percentage changes between historical and 2080s values in median precipitation (average years) and 75th percentile precipitation (wet years) show location-specific differences across the three counties (Figure 8.5 and Figure 8.6). Projected increases in precipitation tend to be higher towards the east and higher elevation locations. Additional maps for all the scenarios are available at http://agclimatetools.cahnrs.wsu.edu/users/giridhar/climate/.

148
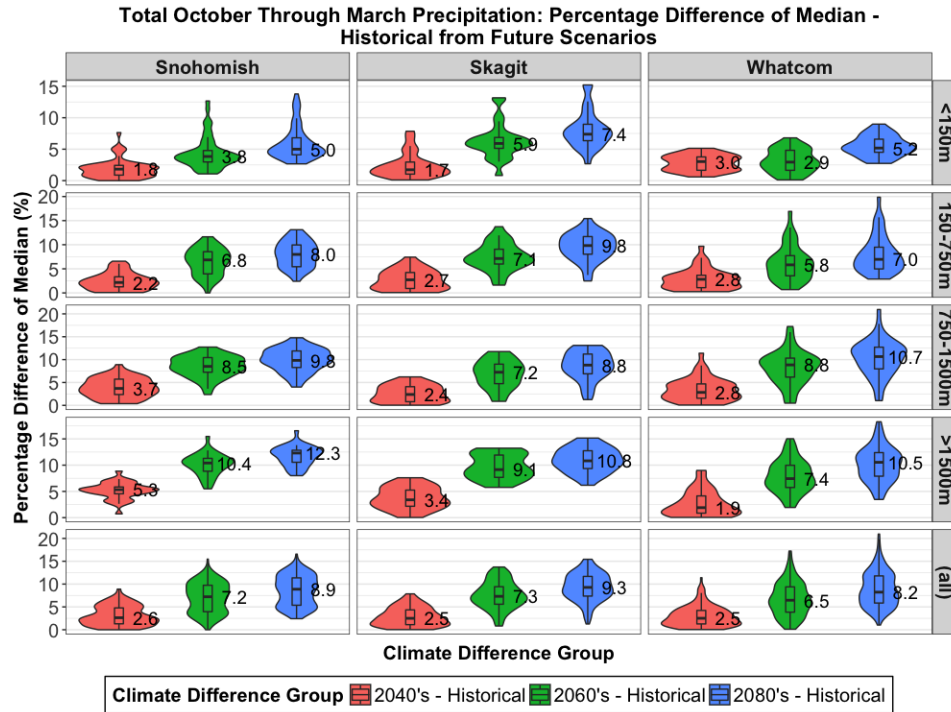
Figure 8.3: Changes between historical and future median storage season precipitation, by county and by elevation band. The median difference is annotated in black text, and the ranges correspond to location-specific differences
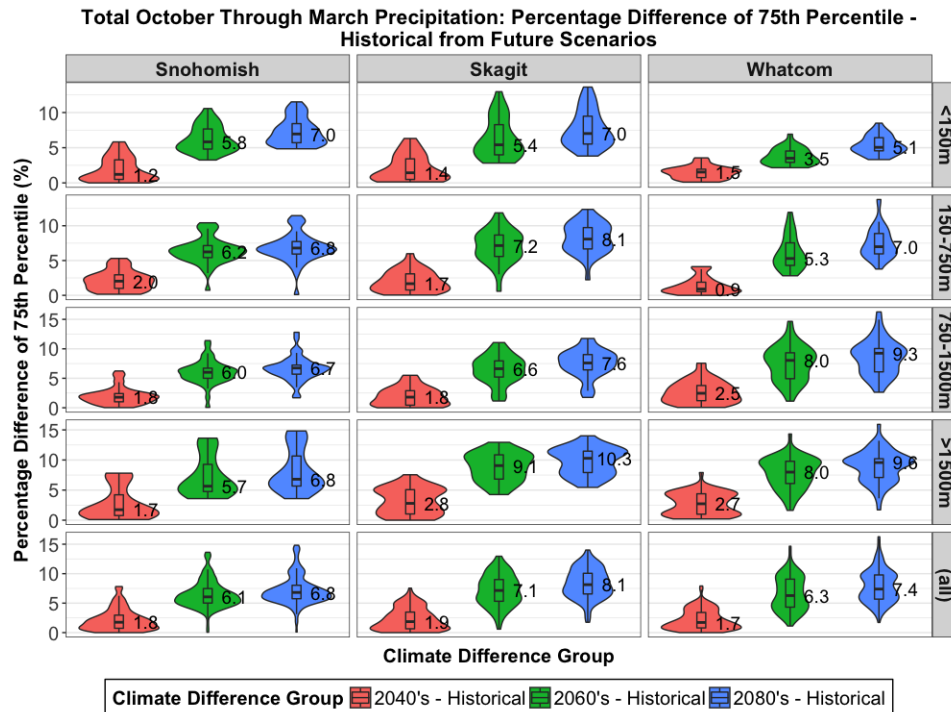
Figure 8.4: Changes between historical and future values of the 75th percentile storage season precipitation, by county and by elevation band. The median difference is annotated in black text, and the ranges correspond to location-specific differences
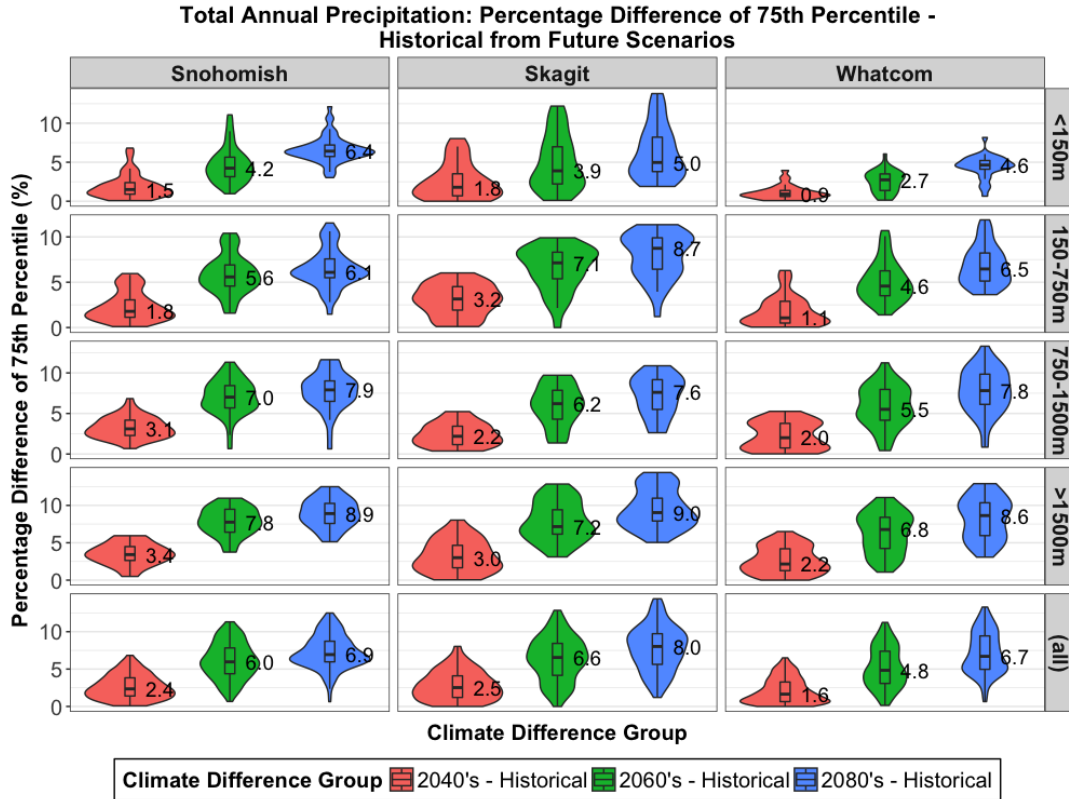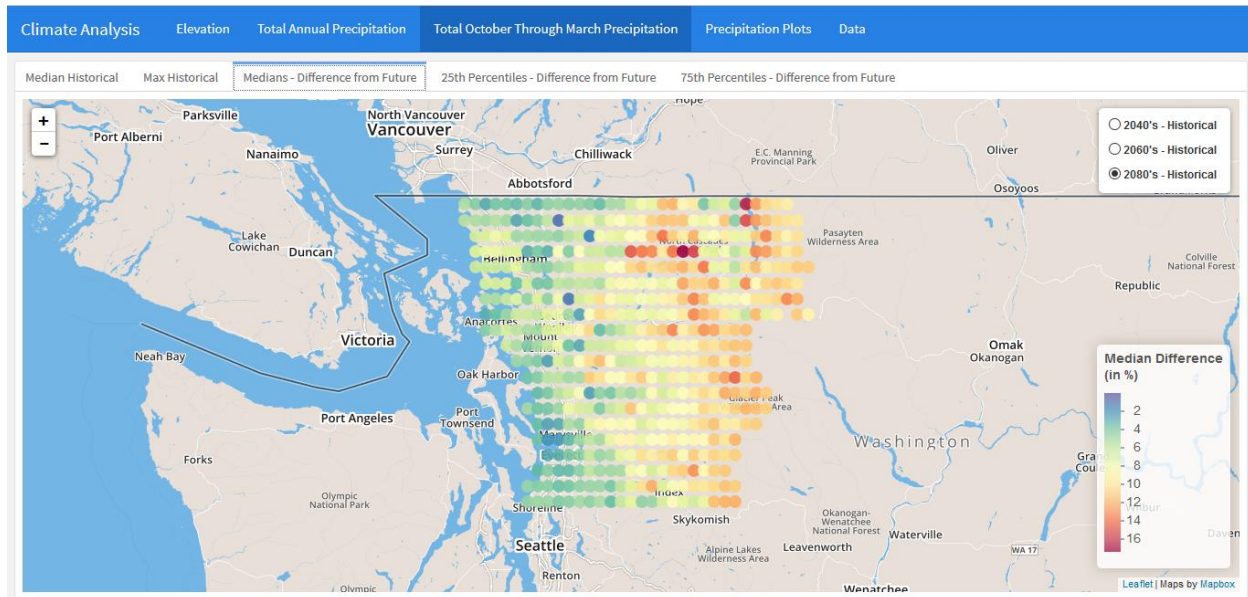
Figure 8.5: Difference between historical and future (2080) median (50th percentile) storage season precipitation across Whatcom, Skagit, and Snohomish Counties, Washington
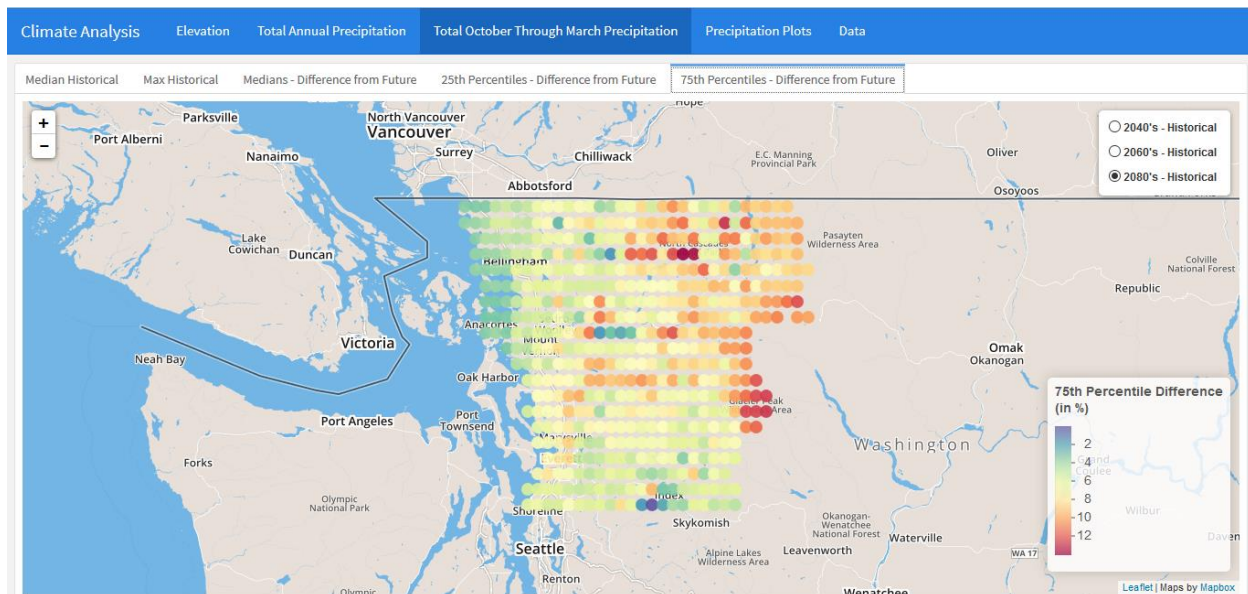
Figure 8.6: Difference between historical and future (2080) values for the 75th percentile of storage season precipitation across Whatcom, Skagit, and Snohomish Counties, Washington

8.5 Conclusions and recommendations

The first step of this proof of concept study indicates that for both average and wet years in the future, precipitation is expected to increase, both in annual precipitation and precipitation during the storage season. These changes suggest that increased storage or alternative management

150

strategies will be required to protect water quality in the three northwest counties of Washington State. This indicates the need for slightly increased storage capacity for most years, with significant additional storage in some years, or an alternative emergency management plan for accommodating wet years (such as the winter of 2016-17). Alternatively, opportunities for implementing additional treatment of stored manure, such as nutrient recovery technology, could be implemented.

The next step of this proof of concept study will focus on evaluating management options (storage vs. nutrient recovery), and will provide more information on the relative potential to implement safe manure storage management strategies in the future.

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SECTION 4

OUTREACH AND EXTENSION ACTIVITIES

9. Commercialization, Technology Transfer, and Extension

Georgine Yorgey, Chad Kruger, Timothy Ewing, Sonia A. Hall, Jingwei Ma, and Jim Jensen

9.1 Abstract

This chapter describes the commercialization, technology transfer, and extension work carried out with the support of Appendix A funding. This work focuses on supporting improved decision making about emerging technologies by dairy industry professionals, the manure management support industry, and others. In order to support the adoption and application of emerging technologies for waste management, the extension team carried out the following outreach activities:

- Delivered nine presentations at regional and national conferences;
- Provided technical support to regional stakeholders, including answering questions and pointing them towards appropriate additional resources.
- Participated in a federal advisory panel focused on furthering the adoption of technologies to recover nutrients and to control the production of greenhouse gases, and participated in the Washington State Department of Agriculture's (WSDA) Dairy Nutrient Advisory Committee, an effort organized to propose non-regulatory methods for improving dairy nutrient management in Washington State
- Published seven formal extension publications, with five additional peer-reviewed publications in progress, and seven other durable extension products, including webinars and blog articles.

Outreach work was aimed at (1) increasing awareness of the opportunities and potential surrounding an AD systems approach; and (2) sharing tools, resources, and successful experiences that can help diverse groups further develop and implement these technologies in their professional fields. Building awareness and making resources available are critical early steps that contribute to improving the economic viability and the environmental footprint of facilities processing organic wastes in Washington. Through these outreach activities, the team made an estimated 23,880 contacts with scientists, producers, industry professionals, regulators, policy-makers, and other interested parties across the country.

9.2 Technology transfer, outreach, and extension activities

The goals of improved management of concentrated livestock wastes and recovery of carbon, nitrogen, and phosphorus will only be achieved if the new and improved technologies, developed through funded research, are adopted and applied by processors, industry, and commercial producers. For this reason, outreach and extension are critical for achieving the Appendix A goals.

The team responsible for the delivery of outreach and extension materials for the biennium included Ms. Georgine Yorgey (Assistant Director, Center for Sustaining Agriculture and Natural Resources [CSANR]), Mr. Chad Kruger (Director, CSANR), Dr. Timothy Ewing (Research Engineer, CSANR), and Dr. Sonia A. Hall (Sustainable Systems Analyst, CSANR), with targeted support from several other individuals. Outreach and extension deliverables were in the form of conference presentations, technical support to multiple stakeholders, a field day, formal extension publications, and other durable extension products. These deliverables are outlined below.

9.2.1 Building relationships with clientele

In collaboration with others, Ewing and Kruger made numerous presentations during the biennium related to anaerobic digestion, pyrolysis, and nutrient recovery. These presentations included the following:

- Kruger, C.E., Yorgey, G., Ewing, T.W. (2016) Anaerobic Digestion Systems. MV REC Brownbag Series, Mount Vernon, Washington. 12/13/16. Followed by a question and answer session for Future Farmers of America students from Lynden Christian High School, chaperoned by Chris Clark from Whatcom Conservation District and accompanied by Washington State Representative Vincent Buys.

- Ewing, T.W. (2016) Panel discussion: Where Does Biochar Fit Within the Larger Biorefinery Concept? Biochar Workshop, Lopez Island, Washington. 10/28/16. Organized by the San Juan Islands Conservation District.

- Ewing, T.W. (2016) The Billion Ton Bioeconomy Initiative: Challenges and Opportunities. The Pacific Northwest Bioeconomy Forum. Seattle, Washington. 10/3/16. Organized by the U.S. Department of Agriculture (USDA). Ewing was an invited participant.

- Ewing, T.W., Kruger, C.E., Yorgey, G. (2016) From Dairy Farm to Bio-refinery: Developing Technologies to Produce Environmentally Friendly Fuels, Power, and Value-Added Products. USBI Biochar 2016, Corvallis, Oregon. 8/24/16.

- Kruger, C.E. (2016) Saving the Planet with Soil Amendments? Skagit County Master Gardener's Know and Grow. Mount Vernon, Washington. 8/16/16.

- Hall, S.A., Kruger, C.E. (2016) Renewable Energy Farm Walks (2 separate farm walks). Goldendale, Washington on 4/4/16, and Kennewick, Washington on 4/25/2016.

- Kruger, C.E. (2016) AD Technology and Small Farms. Small Farms and Community Food Systems Retreat. Mount Vernon, Washington. 3/21/16.

- Kruger, C.E. (2016) Extracting Value from Waste on a Small Farm: What's Realistic & What's Possible? San Juan Ag Summit. Camp Orkila, San Juan Islands, Washington. 2/13/16.

- Ewing, T.W., Kruger, C.E., Yorgey, G. (2016) Dairy Nutrient Recovery Technologies within an Anaerobic Digestion Bio-refinery. Anaerobic Digestion Webinar Series: Emerging Technologies to Improve Environmental and Economic Impact. 2/10/16.

In addition to these presentations, Appendix A funding was used to complement various other funding sources (including from USDA's National Institute of Food and Agriculture, the Environmental Protection Agency's [EPA] Water Environment Research Foundation, Washington Department of Ecology's [Ecology] Waste to Fuels Technology Partnership, and USDA's Natural Resource Conservation Service [NRCS]) to host an anaerobic digestion field day for more than 80 participants at Edaleen Dairy (Lynden, Washington) on June 9, 2016. The morning included presentations on these topics: dairyman's perspective, economic lessons learned, Renewable Natural Gas technologies, biochar and its integration with AD systems, fine solids separation, nutrient recovery approaches, water quality and application issues, and composition of nutrient recovery products. Additional lunchtime talks introduced the economic and environmental modeling tools that have recently been developed. The afternoon introduced participants to a new, three-year effort looking at the application of dairy manure-derived fertilizers to red raspberries and blueberries (funded by USDA NRCS).

9.2.2 Sharing research findings and providing technical support

Ewing and Kruger provided technical support to industry, academics, and various other stakeholders during the 2015-2017 biennium. Technical support aims to provide non-biased information and resources to specific individuals and support their decision-making around biorefinery-related issues. In addition to fielding many individual inquiries, major efforts included:

- Kruger and Ewing provided support to the U.S. EPA's Nutrient Recycling Challenge and arranged for Washington State University's institutional partnership in the challenge. This competition, hosted by EPA with a variety of industry, non-profit, and academic partners, awarded prizes for the most promising technologies to recycle nutrients from livestock manure.

- Kruger was invited to participate in the Washington State Department of Agriculture's Dairy Nutrient Advisory Committee, an effort organized to propose non-regulatory methods for improving dairy nutrient management in Washington State.

- Ewing has communicated with and provided technical support for approximately 10 consultants, companies, and community groups interested in developing anaerobic digestion or nutrient management related projects.

- Ewing provided mentoring and technical support for a group of seven engineering students completing their senior design project in anaerobic digestion at Gonzaga University.

- Ewing has collaborated with and provided technical support for project development ideas with crossover between biochar, the biorefinery concept, and composting to three faculty members at the WSU Puyallup Research and Extension Center.

- Working with Kruger and Yorgey, Ewing has gathered background information to support building a roadmap to guide research efforts related to the dairy biorefinery in Washington State. This information is attached as an appendix to this report.

- Along with Chris Clark from Whatcom Conservation District, Ewing attended and provided technical support for a test of Janicki Bioenergy's Omni Processor for treating dairy manure. Following this, Jensen, Ewing, and Yorgey collaborated with partners at the Stillaguamish Tribe and Janicki to submit an NRCS CIG proposal, in which WSU (led by Ewing) would provide independent evaluation of the Omni Processor on a dairy in Snohomish County. In June 2017, we were notified that funding has been awarded to this project.

9.2.3 Durable extension products

For the 2015-2017 biennium, three major, peer-reviewed extension deliverables formed the core expected scope of work for Appendix A. As of July 2017, one of these publications had been completed, and two were being revised after peer review:

- Hall, S.A., Benedict, C., Harrison, J., and Yorgey, G.G. In submission. Nutrient Recovery Products from Dairy Manure. Washington State University Extension Publication, Pullman, Washington (deliverable for Appendix A for 2015-2017 biennium).

- Ma, J., Frear, C.S., Yorgey, G.G. In revision. Approaches to nutrient recovery from dairy manure. Washington State University Extension Publication, Pullman, Washington (deliverable for Appendix A for 2015-2017 biennium).

- Jensen, J., Yorgey, G.G., Kruger, C.E., Frear, C.S. In revision. Completing a successful feasibility study for an anaerobic digestion project. Washington State University Extension Publication, Pullman, Washington (this publication covers the environmental credits concepts proposed as an Appendix A deliverable for the 2015-2017 biennium).

The following additional formal extension products, initiated in previous biennia or completed primarily with complementary funds, were in progress or published during the 2015-2017 biennium:

- Hall, S.A., Yorgey, G.G. In preparation. Peer reviewed video. Recovering Nutrients from Manure – New Tools for Maintaining Air and Water Quality. WSU Extension. Pullman, Washington.

- Kennedy, N., Yorgey, G., Frear, C., Kruger, C. In revision. The dairy manure biorefinery. Washington State University Extension Publication, Pullman, Washington.

- Jensen, J., Frear, C., Ma, J., Kruger, C., Hummel, R., Yorgey, G. 2016. Digested fiber solids: Developing technologies for adding value. Washington State University Extension Publication FS235E, Pullman, Washington. http://pubs.wpdev.cahnrs.wsu.edu/pubs/fs235e/

- Galinato, S., Kruger, C.E., Frear, C.S. 2016. Economic feasibility of anaerobic digester systems with nutrient recovery technologies. Washington State University Extension Publication TB27, Pullman, Washington. http://cru.cahe.wsu.edu/CEPublications/TB27/TB27.pdf

- Kennedy, N.P., Yorgey, G.G., Frear, C.S., Kruger, C.E. 2016. Considerations for building, operating, and maintaining anaerobic co-digestion facilities on dairies. Washington State University Extension Publication EM088, Pullman, Washington. http://pubs.wpdev.cahnrs.wsu.edu/pubs/em088e/

- Mitchell, S.M., Kennedy, N.P., Ma, J., Yorgey, G.G., Kruger, C.E., Ullman, J.L., Frear, C.S. 2015. Anaerobic digestion effluents and processes: The basics. Washington State University Extension Publication FS171E, Pullman, Washington. http://pubs.wpdev.cahnrs.wsu.edu/pubs/fs171e/

- Kennedy, N.P., Yorgey, G.G., Frear, C.S., Kruger, C.E. 2015. On-farm co-digestion of dairy manure with high energy organics. Washington State University Extension Publication FS172E, Pullman, Washington. http://pubs.wpdev.cahnrs.wsu.edu/pubs/fs172e/

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In addition, in early 2016, we produced a five-part Anaerobic Digestion Systems webinar series titled "Emerging Technologies to Improve Economic and Environmental Impact." Recordings from the series can be accessed at http://csanr.wsu.edu/webinars/anaerobic-digestion/. Though the coordination of the webinars was funded by other sources, the content of the presentations is

relevant to Appendix A and was supported by the ongoing work of this project team. The five webinars included:

- Dairy Nutrient Recovery Technologies within an Anaerobic Digestion Bio-refinery (Dr. Ewing)
- Anaerobic Digestion Bio-refinery: Potential for Biochar Production and Utilization (Dr. Manuel García-Pérez)
- Agronomic Evaluation of Anaerobic Digestion System Recovered Fertilizers (Dr. Hal Collins)
- An Introduction to the Anaerobic Digestion System Enterprise Budget Calculator (Dr. Greg Astill)
- Insights for Anaerobic Digestion from Dairy-CropSyst, a Decision Support Tool for Gaseous Emissions and Nutrient Management (Mr. Bryan Carlson)

9.3 Impacts of technology transfer, outreach, and extension activities

The team estimates 23,880 scientists, producers, industry, regulators, policy-makers, and other interested parties across the country were reached through the core outreach activities described above: conference presentations, technical support, and formal and other extension publications. These impacts include:

- 80 participants attended the AD Systems Field Day.
- An estimated 190 people attended presentations by the team during this biennium.
- Webinars and extension publications, including those published during the current and previous biennia, were viewed or downloaded an estimated 7810 times during the 2015-2017 biennium.
- The blog articles and CSANR's webpages on Appendix A topics have been viewed almost 15,800 times cumulatively during the 2015-2017 biennium.

These statistics do not include views or downloads of the additional research products posted on webpages hosted by EPA, eXtension, and Ecology, so represent a conservative estimate of the impact of outreach activities. The project team has increased awareness around the potential and opportunities surrounding biorefinery technologies, and has shared tools, resources, and successful experiences that will help diverse groups further develop and implement these technologies.

APPENDIX

Background for Agricultural Anaerobic Digestion Systems Roadmap for Washington State

Near-term Research to Promote Technology Commercialization

Disclaimer

This Roadmap is not intended to support technology purchase or investment decisions. Description or discussion of commercial technology vendors or products does not constitute or imply endorsement by authors, or State government. Content may include information and data from sources including peer reviewed literature, technical reports, project feasibility studies, and unpublished data from academic and industry projects and personal interviews. Any economic estimates, scale-up factors, or performance indicators that are provided are for informational purposes only.

List of abbreviations

AD	Anaerobic Digestion
ARC	Agricultural Research Center
BSysE	Department of Biological Systems Engineering
CH_4	Methane
CNG	Compressed Natural Gas
CO_2	Carbon Dioxide
CAFO	Concentrated Animal Feed Operation
CSANR	Center for Sustaining Agriculture and Natural Resources
EPA	Environmental Protection Agency
FOG	Fat, oil, grease
GHG	Greenhouse Gas
H_2S	Hydrogen Sulfide
N	Nitrogen
NH_3	Ammonia
NR	Nutrient Recovery
P	Phosphorus
RDD&C	Research, Development, Demonstration, and Commercialization
TS	Total Solids
US	United States

USDA	United States Department of Agriculture
USGS	United States Geological Survey
VS	Volatile Solids
WA	Washington State
WA-ECY	Washington State Department of Ecology
WSDA	Washington State Department of Agriculture
WSU	Washington State University

Background

Appendix A research

In the 2007–09 biennium the Washington State Legislature funded a joint Washington State University (WSU) and Washington State Department of Agriculture (WSDA) program targeting applied bioenergy research. The funds were requested by WSU and WSDA to undertake near-term, applied research (~5 years to commercialization) needed to successfully implement the Energy Freedom program and bioenergy initiatives enacted in 2006. Examples of projects to be funded were listed in *Appendix A*, which was attached to the funding request.

Since 2007, WSU's Agricultural Research Center (ARC) and WSDA have collaborated on this research effort. In the area of cropped biofuels, *Appendix A* funds have been directed to research projects coordinated by the WSU Department of Crop and Soil Sciences, as the Biofuels Cropping Systems project. In the area of energy conversion and nutrient recovery from agricultural wastes, funds have been directed to research projects coordinated by the WSU Department of Biological Systems Engineering (BSysE), while the WSU Center for Sustaining Agriculture and Natural Resources (CSANR) has provided extension and outreach, and support for commercialization efforts.

During the 2015-2017 biennium, Washington State University researchers continued anaerobic digestion systems related research with support from *Appendix A* funds. This Roadmap Background was developed with those funds, with a goal of informing the development of a Research Roadmap in summer of 2017, to guide future projects that promote near-term technology transfer and commercialization of AD systems.

For more information about Appendix A projects, contact:

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Roadmap intent

There are three main goals of the Roadmap effort:

1. Introduce anaerobic digestion (AD) as the central unit operation of a larger system of processes and technologies used to help manage manures and other agricultural organic wastes while producing renewable energy and other value-added bioderived products.
2. Provide an outline of current AD systems in Washington State and highlight past research that has both advanced the understanding of these systems and pushed processes and technologies towards commercialization.
3. Guide development, selection and funding of future *Appendix A* research projects, improving our ability to increase the understanding of AD systems and offering a path to near-term technology transfer and commercialization.

Combined, these three goals outline a Roadmap that is intended to meet the specific needs of the *Appendix A* program while also being accessible to a large and diverse audience in Washington State interested in furthering energy conversion and nutrient recovery from agricultural wastes, including manures. This Roadmap Background serves a few purposes for various stakeholders:

- For Washington stakeholders, it provides a condensed introduction to AD systems, and an overview of previous *Appendix A* research. This Background sets the stage for productive stakeholder discussions to develop a Roadmap, motivating individuals to foster community engagement and awareness around the improvement of AD systems.
- For agricultural producers and the agricultural equipment and management industry, it highlights previous *Appendix A* projects, informing the refinement of current strategy for *Appendix A* investments.
- For university researchers, it provides information that assists in the formulation of proposals that clearly target solutions to identified problems or limitations. There is a strong incentive for university researchers to collaborate closely with both agricultural producers and industry in order to best identify current near-term opportunities.
- For Washington government policy makers and agencies, it provides a living document which can be used to highlight current progress, to frame future goals, and to leverage additional federal, state, and private resources.

By promoting projects which lead to state-of-the-art improvements to AD systems in Washington State, a Roadmap informed by this background can contribute to reducing project costs, reducing operating energy usage, reducing application of industrial non-renewable chemicals and fertilizers, producing renewable energy, producing renewable bio-based chemicals, fertilizers, and other co-products, and overall improving air, water, and soil quality in agricultural areas.

Roadmap limitations

This Roadmap is not intended to promote any specific or general legislation, to suggest changes in existing legislative policy, or to define changes in State Agency policy or rulemaking.

The scope of this Roadmap is limited to research based projects to address current problems and limitations. Issues concerning regulatory policy, permitting, and statutory fees are not considered. Likewise, building materials and equipment costs are not considered.

Anaerobic digestion systems overview

The storage, safe treatment, and disposal of agriculturally derived organic waste is a major focus for Washington agricultural producers (Chen et al., 2016; Goldstein et al., 2015; US EPA, n.d.). These producers rely on a variety of management processes and treatment technologies to ensure the environmentally safe and economically efficient end utilization of organic waste (Burton and Turner, 2003; Dick et al., 1998; Sims and Wolf, 1994). AD systems is the descriptive title for the integration of multiple processes and technologies focused on treating organic waste while also producing bioenergy and value-added bioproducts (Bujoczek et al., 2000; Holm-Nielsen et al., 2009; Ward et al., 2008). A simplified process flow diagram for an AD system is shown in Figure A- 1: Collected organic waste is fed to an anaerobic digester to produce biogas, treated solids, and nutrient-rich liquids.
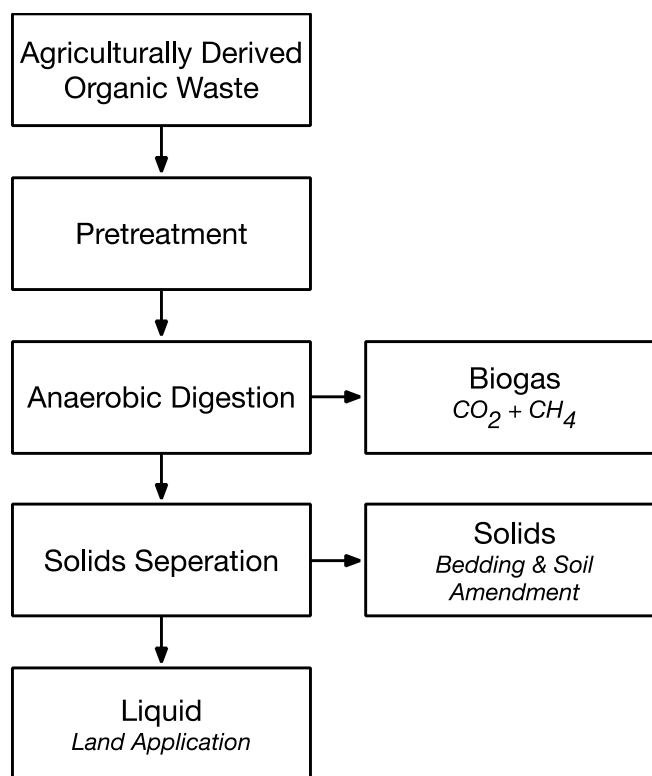

Figure A- 1: Simplified Anaerobic Digestion System [adapted from US EPA, 2013]

Additional resources
- Overview of Anaerobic Digestion Systems

- Introduction to Washington Dairies and Digesters

Anaerobic digestion core principles

Anaerobic digestion is a natural biological process that treats the organic matter in agricultural wastes, reduces pathogens and odors, and produces biogas (Hills and Roberts, 1981; Martin et al., 2003; Kaparaju and Rintala, 2008; Kim et al., 2002; Salminen and Rintala, 2002). Biogas is produced when microorganisms catalyze the conversion of organic carbon to methane (CH_4) and carbon dioxide (CO_2) in the absence of oxygen (O_2) (Weiland, 2010). Produced biogas generally contains ~60% methane, ~40% carbon dioxide, water vapor, and trace sulfur and nitrogen containing compounds (Abatzoglou and Boivin, 2009). Agriculturally derived organic waste is biologically active and contains pathogens such as bacteria and viruses (Kearney et al., 1993; Lund et al., 1996; Smith et al., 2005). When conducted at mesophilic (>35℃) temperature, AD can reduce common pathogens, such as fecal coliform bacteria and *E. coli*, by as much as 98% (Forbis-Stokes et al., 2016; Pandey and Soupir, 2011; "Pathogen Reduction in Anaerobic Digestion of Manure - eXtension," n.d.). The production of organic acids (including volatile organic compounds) during the natural anaerobic degradation of agricultural wastes contributes to on-farm odor issues. Controlled and contained AD reduces odors by coupling four categories of microorganisms to break down, process, and convert organic waste to biogas (Powers et al., 1997; Van Horn et al., 1994) (Figure A- 2). This treatment results in the reduction of total solids, the conversion of organic nitrogen to ammonium, and a low-odor effluent (Uludag-Demirer et al., 2008, 2005).
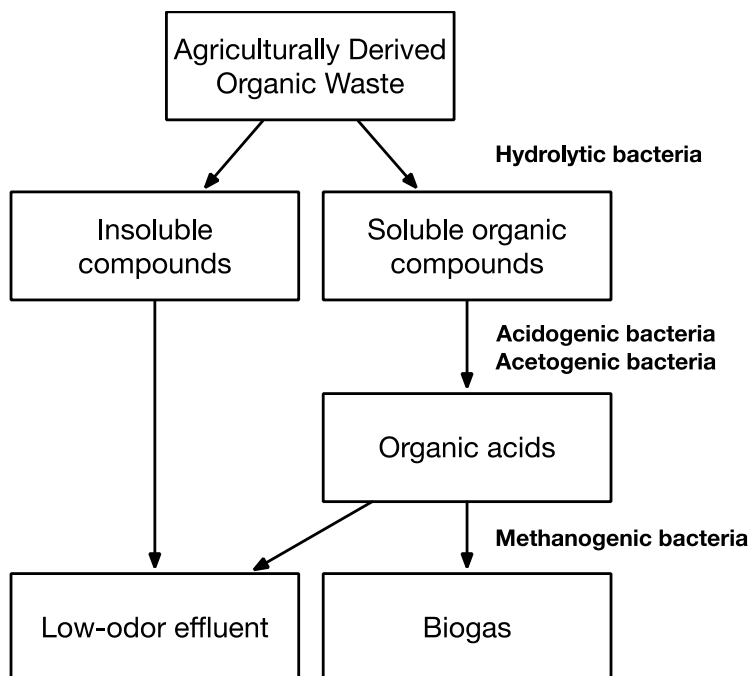

Figure A- 2: Anaerobic digestion metabolism [adapted from Gerardi, 2003; Stams, 1994]

Additional resources
- Introduction to Anaerobic Digestion
  http://cru.cahe.wsu.edu/CEPublications/FS171E/FS171E.pdf

Agricultural feedstock (agriculturally-derived organic wastes)

Agriculturally derived organic wastes can vary widely in applicability as a feedstock for AD. Feedstocks rich in lipids and easily-degradable carbohydrates generally produced more methane compared to those composed mostly of recalcitrant lignocellulosic-materials (Nallathambi Gunaseelan, 1997; Noike et al., 1985; Siegert and Banks, 2005). As with formulating a livestock diet, proper consideration must be made for the variety of substrates that make up the feedstock for AD. Dairy, poultry, and swine animal manures are often utilized as primary sources of feedstock for AD. Because of its neutral to basic pH (6.5-8.5) and large buffering capacity, dairy manure has been highlighted as especially suitable for co-digestion (El-Mashad and Zhang, 2010; Macias-Corral et al., 2008). Examples of co-digestion substrates include corn processing by-product, pre-consumer food scraps, and corn stover (Figure A- 3). The process of co-digestion involves mixing co-substrate(s) with the primary feedstock (i.e., dairy manure) before feeding to the digester to increase methane production. Co-substrates that contain more chemical energy per unit mass compared to primary manure feedstocks are categorized as high strength wastes (Hartmann and Ahring, 2005; Murto et al., 2004). A limit in Washington State of 30% by volume co-substrate addition was set to prevent chemical and biological inhibition of the digestion process, which could lead to reduced methane production and only partial stabilization of resulting effluent. Since the goal of co-digestion is to increase methane production compared to the primary manure feedstock, it is important to choose co-substrates with higher methane potentials. For example, more methane would be produced by co-digestion of dairy manure with pre-consumer food scraps; however, less methane would be produced because of dilution by co-digesting dairy manure with corn processing by product (Figure A- 3).
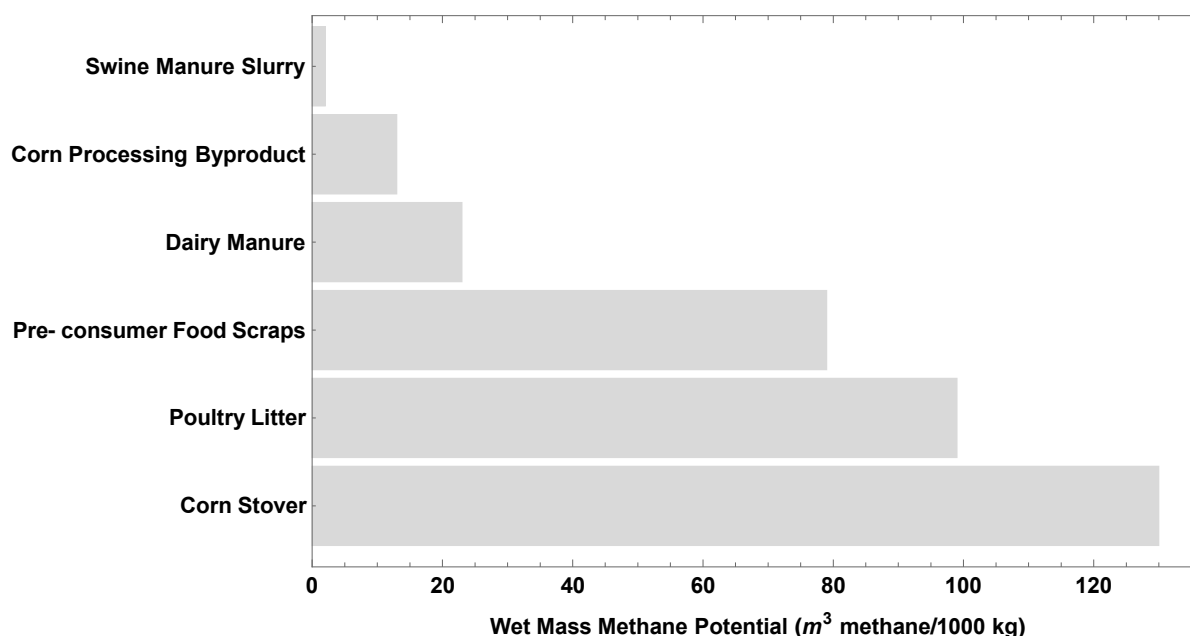
Figure A- 3: Select wet mass methane potentials [adapted from Hamilton, 2012]

Additional resources
- On-farm Co-digestion of Dairy Manure with High Energy Organics.
  http://cru.cahe.wsu.edu/CEPublications/FS172E/FS172E.pdf

- Considerations for Building, Operating, and Maintaining Anaerobic Co-Digestion
  Facilities on Dairies
  http://extension.wsu.edu/publications/wp-content/uploads/sites/54/publications/em088e.pdf

- Washington State Biomass Inventory
  https://fortress.wa.gov/ecy/publications/documents/0507047.pdf

Anaerobic digestion systems engineering and technologies

Types of digesters
There are two main categories of anaerobic digesters: wet digesters and dry digesters (Mitchell et al., 2015). Wet digesters are classified due to the ability to mix and pump the feedstock and effluent in the same manner as other liquids. Feedstock used in wet digesters contains 3-15% total solids. Dry digesters have feedstock that remains in a stackable pile, which is usually >25% total solids. Due to the high liquid content in the manures, dairy and swine facilities would generally consider wet digesters. For poultry and other CAFO facilities, availability of dilution liquids from co-substrates or processing residues should be considered to determine the applicability of wet vs. dry digestion.

Beyond the two main categories, multiple configurations of AD systems exist, primarily differing in terms of operating characteristics and temperature. Wet digesters are classified as either batch (where the feedstock is loaded all at once then effluent is completely removed after a

165

specified period of time) or continuous (where feedstock is loaded at one end while effluent is removed at the other end). Continuous systems can further be divided as continuous stirred tank reactors (CSTRs) and plug flow reactors (PFRs) (Nasir et al., 2012; Yu et al., 2013) (These terms are defined more fully in the glossary, at the end of this document.). The most common dairy digesters in Washington State are based on the PFR design.

Along with the physical configuration and operation of these digesters, there are three common temperature ranges: psychrophilic, mesophilic, and thermophilic (Chae et al., 2008). Psychrophilic digesters are often simply passive covered lagoons that operate between 20 – 30℃. The most common types of dairy and swine digesters operate in the mesophilic rage of 30 – 40℃. Digesters operating in the thermophilic range of 50 – 65℃ have both advantages and disadvantages. Advantages include increased volatile solids (VS) reduction, shorter hydraulic retention time (HRT), and more effective pathogen removal; however, disadvantages include higher energy requirements for heating, more odor, and greater sensitivity to temperature variation compared to psychrophilic and mesophilic digesters (Angelidaki and Ahring, 1994; Chae et al., 2008; Gavala et al., 2003; Kim et al., 2006).

Additional AD systems equipment and processes
Anaerobic digestion systems include additional unit operations beyond the central anaerobic digester, such as pre-treatment and primary solids separation (Figure A- 1) (Ariunbaatar et al., 2014; Møller et al., 2002). Pre-treatment units can be as simple as mixing pits to ensure thorough mixing of feedstocks being used for co-digestion, or as advanced as physical, chemical, or biological processes to enhance the digestion of the given feedstock. Primary solids separation generally occurs immediately after AD. This step is targeted to remove coarse fibers and any large contaminants that may have entered the digester with the feedstock. Primary solids separation can be followed by a number of unit operations to treat the resulting effluent including suspended solids removal, nitrogen removal, phosphorus removal, and filtering capable of producing drinking water suitable for livestock (Kaparaju and Rintala, 2008; Rajeshwari et al., 2000; Sooknah and Wilkie, 2004).

Additional resources
- Rationale for Recovery of Phosphorus and Nitrogen from Dairy Manure
  http://cru.cahe.wsu.edu/CEPublications/FS136E/FS136E.pdf

Agricultural value-added products and end-utilization of organic wastes
With the relatively low cost of energy in WA, partially due to the abundant availability of hydroelectric power, and the lack of premium sales contracts from local utilities, production and sale of electricity from biogas alone is no longer sufficient to meet the capital expenditure and subsequent return on investment (ROI) for an agriculturally based anaerobic digester (Bishop and Shumway, 2009; Carley, 2009; Stokes et al., 2008). To address this issue, various unit operations have been developed to work in conjunction with anaerobic digesters. These unit operations include: solids separation, nutrient recovery processes, water treatment, and biogas upgrading systems. When combined into one facility, AD with these unit operations is defined as AD Systems. Therefore, to promote the economic feasibility of facilities deploying AD systems, both value-added products and associated markets must be developed to support full utilization

of generated organic wastes (Bozell and Petersen, 2010; Cherubini, 2010; Fernando et al., 2006; FitzPatrick et al., 2010; Kamm and Kamm, 2004). The following list provides a brief introduction to common AD Systems unit operations.

Primary solids separation: This is a mature technology utilized at facilities both with and without AD. Common equipment includes sloped screen separators. After separation, digested fibrous solids can be used as animal bedding or as value-added soil amendment. Accounts for approximately 40% total solids (TS) removal and contains both 10-20% total nitrogen and total phosphorus.

Advanced solids separation: This is a developing technology in the agricultural area but a mature technology in municipal wastewater treatment. Common equipment includes dissolved air flotation (DAF), belt presses, and screw presses. Suspended solids are associated with majority of the phosphorus in the digested effluent. This process removes about 97% total suspended solids (TSS) which contain 30-35% of the total nitrogen and 70-90% of the total phosphorus in the effluent.

Nutrient recovery – Struvite crystallization: This is a relatively new technology used on dairy manure, but it is a mature technology in the municipal wastewater treatment area. This process uses a polymer to bind with suspended solids. Once small particles are bound and aggregated to form larger structures, gravity settling or centrifuge can be used to remove the resulting struvite. Used in place of advanced solids separation, this technology produces slow-release crystalline fertilizer containing magnesium, nitrogen, and phosphorus. This process removes approximately 10% of the total nitrogen and 70-80% of the total phosphorus in the effluent.

Nutrient recovery – Ammonia stripping: This technology has been demonstrated with dairy and poultry manures. This technology can be implemented before or after advanced solids separation. The process requires aeration of the digested effluent followed by a blower and acid tower. To reduce the input of consumable chemicals (such as sulfuric acid) carbon dioxide stripping can be used. Ammonia stripping removes about 50-75% total nitrogen in the effluent, but effectively no phosphorus is removed.

Nutrient recovery – Vermicomposting: This technology has been demonstrated with dairy manure. The primary removal action is similar to treatment with a biological filter or packed bed reactor. There is some potential for value-added sales of castings as well as worms. Vermicomposting is best to use after primary solids separation. Removes approximately 70-90% total nitrogen and 5-10% total phosphorus from the remaining wastewater.

Water treatment: While a mature technology in municipal systems, the complexities and costs have until recently kept these systems out of agricultural facilities. Common processes include reverse osmosis, UV treatment, distillation, and molecular sieves. Typical performance yields 50-60% clean water with remaining volume containing removed solids. The solids fraction will contain about 96% of total nitrogen and 100% of total phosphorus.

Additional resources
- Digested Fiber Solids: Developing Technologies for Adding Value
  http://cru.cahe.wsu.edu/CEPublications/FS235E/FS235E.pdf

Anaerobic digestion systems in Washington State

Current agricultural waste management strategies lead in at least some cases to overloading of nutrients in soils, leaching of nutrients and pathogens into waterways, and greenhouse gas emissions. While steady incremental progress has been made over the past 13 years to improve agricultural waste management practices and to develop and deploy new waste management technologies such as anaerobic digestion, the overall rate of change has been relatively slow. At the same time, the awareness of the environmental, social, and economic issues related to current manure management practices by both the general public and the scientific community has grown considerably. The Center for Sustaining Agriculture and Natural Resources, with funding from *Appendix A* and support from WSDA, is thus providing this background to assist in the development an Agricultural Anaerobic Digestion Systems Roadmap that leverages the collective insights of the various stakeholders that have an interest in wider deployment of improved manure management technologies.

Since 2011, *Appendix A* has funded thirteen major research projects, three ancillary AD projects, and provided WSU Extension and Outreach capacity to enable technology transfer and provide general industry AD systems support. A brief summary of each project follows:

Nutrient recovery within anaerobic digestion and biochar platforms
Based on current manure management practices, nutrient overloading with associated negative air, water, and soil impacts is an issue on some Washington dairies. Nutrient recovery based on manure contact with biochar as a filter media has the potential to reduce these negative impacts. Biochar is produced using various thermochemical processes, including pyrolysis. Both thermal and chemical modifications can be made to develop biochar with specific characteristics. With the intent to utilize on-farm materials, biochar was produced from AD separated fiber using pyrolysis. In one set of trials, addition of calcium during production of biochar resulted in 53% removal of phosphate after 12 hours of contact with a liquid waste stream. In another set of trials, post-pyrolysis air oxidation at 250°C resulted in improved ammonium removal. To effectively treat liquid waste containing nitrogen and phosphorus from a 1000-cow dairy, it was estimated that between 5 – 115 tons of biochar per day would be needed.

Flush dairy and anaerobic sequence batch reactor approach
Flush dairy manure presents a challenge when considering treatment by anaerobic digestion because of the large volume of added water which correspondingly requires a large digester. Existing separation technologies exist to thicken the manure, which would reduce the size and capital cost of the digester; however, these unit operations result in significant loss of digestible organic material to the lagoon. A potential solution is to use multiple small reactors (small scale digesters) with reduced hydraulic retention time (HRT) positioned in series arrangement. Testing of a system with HRT of 4 to 6 days and organic loading rate (OLR) of 0.5-1.3 g VS/L/day at 22°C resulted in stable operation. Microbial analysis indicated that the population in the reactors was dominated by *Methanosarcia* spp., which is a beneficial methane producer.

Biogas purification within the anaerobic digestion/nutrient recovery platform
As produced biogas from AD of animal manures is primarily composed of methane (~60%) and carbon dioxide (~40%) but also contains impurities such as hydrogen sulfide. The value and end uses of biogas can be increased by removing impurities and reducing the concentration of carbon

dioxide. During the process of removing nitrogen from AD effluent, in the form of ammonia sulfate, the liquid effluent pH is raised to approximately 9.7 and significantly reduced in carbon dioxide. Since commercial biogas purification processes are expensive and often utilize caustic (high pH) consumables, several trials were conducted to demine the feasibility of using post-AD, post-NR effluent for biogas stripping. Using a bubble column reactor, it was determined that nearly 100% of hydrogen sulfide and a large percent of carbon dioxide were removed from raw biogas. Based on these trials, near-term commercialization of the technology is expected.

Anaerobic digestion on small-sized dairies

The use of AD as part of the manure management plan for small-scale dairies (<200-400 cows) has been challenging due to the unfavorable economics of small-scale electrical generation. To address this limitation, a novel small-scale vertical mixed plug flow digester was built and coupled with a boiler and condenser to produce thermal energy for heating and chilling of a processing facility. Feedstock for the digester consisted of approximately 200 gallons per day of manure and periodic doses of milk parlor washwater. After about 8 months of operation, the digester was only producing about 100 cubic feet of low quality biogas per day. Troubleshooting indicated that variations in temperature and inconstant feeding could have contributed to poor system performance.

Pretreatment of fibrous feedstock for entry into digester

The use of plant matter as a feedstock for AD has the potential to open additional waste streams for energy production. Many field residues, lawn clippings, and targeted growth energy crops have specific methane potential greater than dairy manure; therefore, co-digestion with these feedstocks could provide an overall increase in biogas production. Unfortunately, lignocellulosic materials can take up to 5-7x longer to digest compared to animal manures and other organic fraction of municipal solid wastes such as food scraps. One possible method to address this issue is to convert the lignocellulosic material to simple sugars before feeding to the digester. Three types of pretreatment were explored: soaking in aqueous ammonia, exposure to ozone, and a mixture of aqueous ammonia and ozone. It was found that the mixture (10 minutes of ozone and 6 hours of soaking aqueous ammonia) used on lawn clippings resulted in the highest sugar recovery, which would correspond to the highest biogas production. Although the mixture provided the best results, the soaking aqueous ammonia only pretreatment results were similar enough that it is recommended to further investigate this method due to the reduced handling and storage concerns.

Anaerobic digestion of algal biomass residues with nutrient recycle

The extraction of lipids from microalgae for the production of biofuels has resulted in the generation of a new waste stream that may be of interest as an AD feedstock. The residue resulting from the extraction process essentially contains all of the cellular material from the microalgae except for the targeted lipids along with a mixture of extraction solvents and some remaining long chain fatty acids. Through multiple trials with five microalgae species and five extraction solvent mixtures, it was found that biogas production was inhibited by the presence of extraction solvents and long chain fatty acids. As a follow-on, some of this inhibition could be overcome by increasing the inoculum-to-substrate ratio in the test digesters; however, this was not seen as a viable workaround for large scale production and waste treatment.

Nutrient recovery technologies for dairy application and characterization of the performance of engineered biochars as soil amendments

The over application of nitrogen and phosphorus during the spreading of manure on agricultural lands is a problem for some producers in WA. This over application can lead to negative air, water, and soil impacts. One potential solution is to remove these nutrients from the manure effluent before application to agricultural lands. The development of engineered biochars can be utilized to capture both excess nutrients and pathogenic microorganisms in the effluent. The initial materials and production temperature conditions of biochar relate directly to the engineered characteristics and ability to capture nutrients and act as a soil amendment. After the biochar is "charged" with nutrients from treated dairy manure effluent, it can be applied as a soil amendment. In addition to the use as a soil amendment, it was found that biochar improved the water holding capacity of sandy soil. It was also determined that mixing charged biochar with composting did not contribute to nutrient exchange with the compost.

Gypsum as a replacement for sulfuric acid in bio-ammonium sulfate production in dairies

Nutrient recovery from AD effluent is becoming an essential component in the overall management of manure. Current industrial nutrient removal technologies utilize potentially hazardous chemicals such as sulfuric acid. To reduce the need of dangerous chemicals on agricultural facilities, this research proposed to utilize gypsum slurry as the contacting agent to produce ammonium sulfate from ammonia and carbon dioxide gasses. It was found that while the gypsum slurry was effective at producing the liquid fertilizer, there were significant concerns that techno-economic analysis would show that a commercial-scale unit was not feasible.

Expansion and scale-up of hydrogen sulfide scrubbing using bubble column and high pH ammonia-removed effluent

Previous research showed that post-NR effluent could be used to upgrade biogas by removing nearly 100% hydrogen sulfide and some carbon dioxide. To continue to build on the previous findings, a commercial-scale reactor was fabricated and tested. This reactor demonstrated 95% hydrogen sulfide removal along with some carbon dioxide. The removal of impurities in the biogas improves the value and potential uses. A companion lab-scale test showed that multi-stage sequential biogas purification is possible, which could lead to reduced cost and increased value of biogas stripping at the commercial scale.

Improving pretreatment technologies of manure fiber and crop residues for enhanced methane production

Anaerobic digestion and the resulting biogas production from lignocellulosic materials can take up to 5-7x longer compared to animal manures. To reduce the need for larger or multiple digesters, pretreatment processes can reduce lignocellulosic materials to simple sugars, which are faster to digest. Previous research has indicated that a mixture of soaking in aqueous ammonia and exposure to ozone was effective for pretreatment of lawn clippings. To extend on this finding, straw and separated manure fiber were chosen as test feedstocks. After pretreating individually with either aqueous ammonia or ozone, it was again found that a mixture provided the best pretreatment. In addition, it was found that longer soaking in aqueous ammonia produced more biogas compared to longer exposure to ozone with straw. Using the same parameters to test separated dairy fiber resulted in much less biogas production, which indicates that pretreatment parameters need to be determined for each type of lignocellulosic material.

170

Production of microbial biofertilizer from wheat straw
The reduction of soil health has been suggested as the result of over 50 years of industrial growing practices. With the more recent advent of removing crop residues for use as AD or biofuel feedstock, the problem may only be getting worse. To return nitrogen and phosphorus nutrients to the soil, a portion of the harvested crop residue could be converted to biofertilizer that also contains beneficial microbes. This study investigated the pretreatment of crop straw by soaking in aqueous ammonia and exposure to ozone to release simple sugars. The resulting sugars were then treated with hydrolyzing enzymes and utilized by *Azotobacter vinelandii* as a growth media. It is suggested that biofertilizer application to agriculture lands could improve soil health and lead to significant fertilizer cost-savings.

Utilization of wheat straw for the production of lipids
The question over growing crops for food or fuel has recently become a central area of conversation in U.S. agriculture. The production of biodiesel from plant oils is reaching a feedstock limitation plateau. This study suggests that crop residue from wheat straw can first be pretreated and then used as a feed for different oleaginous yeasts to produce lipids. One trial showed that post growth lipids accounted for 42% of the yeast dry cell weight. It was suggested that additional research be conducted to evaluate scale-up and assess economic feasibility.

A comprehensive techno-economic model to evaluate different anaerobic digestion options for various applications
The adoption of AD Systems technology is primarily dependent on favorable economic outcomes. Since there are a number of integrated technologies associated with AD Systems and nearly infinite number of combinations of feedstocks for co-digestion, it is necessary to utilize modeling to predict both process and economic outcomes. This study developed a comprehensive techno-economic model. The model was used to evaluate AD add-on technologies such as pretreatment and nutrient recovery processes. It was also used to evaluate use of various feedstocks such as food scraps and crop residues. Finally, the model indicated that economic outcomes improved with larger scale facilities.

Smaller projects on various anaerobic digestion topics
The use of AD in colder climates requires an increased portion of the generated energy, termed patristic energy, to be returned to the digester in order to maintain the necessary mesophilic temperatures. Operating a mesophilic digester at lower temperatures results in significantly reduced performance due to reduced activity of resident mesophilic bacteria. To reduce parasitic energy usage, it is suggested that psychrophilic (low temperature) digesters be developed. Due to the lower activity by low temperature bacteria, microbial analysis was used to isolate suitable higher performing psychrophilic bacteria. This research could lead to higher performing psychrophilic digesters.

The growth and harvesting of algal biomass for biofuel production is a promising research area; however, the treatment of the resulting residue from cell bodies and extraction solvents with AD does not produced expected biogas due to various inhibitions. This study investigated the production of carboxylic acids from microalgal biomass using anaerobic sequence batch reactors. After multiple trials, it was found that 12 days of hydraulic retention time resulted in the highest

production of carboxylates. It is suggested to conduct genetic community analysis to determine the major bacterial population in the batch reactors.

Some methane remains suspended in the liquid effluent after AD. This reduces the efficiency of the digester and can also lead to later release of the methane directly to the atmosphere. This study investigated using a vacuum pump and high temperature for degassing the liquid effluent. The process resulted in the release of 10 mL of methane/L effluent at 55°C. An assessment of the economics of the process is necessary to determine scalability.

Anaerobic digestion extension-technology transfer project
Commercialization, industry support, and extension
Improvements in the processing of dairy manure in Washington will only be made if advanced technologies are adopted and applied. Multiple extension and outreach activities were carried out during this biennium to encourage and facilitate such adoption. These activities included:
- A major field day event held at two dairy farms in Northwest Washington, showcasing applied research and demonstrations of anaerobic digestion.
- Presentations at multiple national and regional conferences where the extension team shared the results of anaerobic digestion, pyrolysis, and nutrient recovery-related research to extension, engineering, industry, regulatory, and educational professionals.
- Technical support to nine stakeholder groups ranging from federal agencies—for example, through the Nutrient Technology Challenge and the Biogas Opportunities Roadmap—to state agencies—such as the Washington State Departments of Agriculture, Ecology, and Commerce—to non-profit entities—including Sustainable Conservation, in California.
- Training of young professionals who are beginning their professional careers across three continents.
- An anaerobic digestion curriculum for training anaerobic digestion technicians.
- Publication of six formal extension publications.

An estimated 12,000 scientists, producers, industry specialists, regulators, policy-makers, and other interested parties across the country were reached. These activities led to increased awareness of biorefinery technologies, tools, resources, and successful experiences. Such awareness and resources are critical to the advancement and adoption of technologies and processes in Washington State that create energy from livestock manure and other organic residues.

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Glossary

Agricultural producer: A farm operator. Examples of *agricultural producers* include crop growers, operators of dairy and poultry farms and CAFOs.

Anaerobic digestion (AD): A process utilizing microorganisms to breakdown organic material in the absence of oxygen. Feedstock can include animal manures and food scraps. A renewable energy source, biogas, containing methane (CH_4) and carbon dioxide (CO_2) is a primary product.

Batch: Material does not enter or exit the system boundary during a given process. A batch system is also defined as a closed system.

Biogas: A renewable energy source produced by anaerobic digestion. *Biogas* is primarily composed of methane (CH_4) and carbon dioxide (CO_2) with small amounts of hydrogen sulfide (H_2S) and water vapor (H_2O).

Continuous: Material enters and exists the system boundary in equal amounts during a given process. A continuous process is also defined as an open system or a steady-state system.

Continuous stirred tank reactor (CSTR): An open system at steady-state that is well mixed.

Feedstock: A raw material. Examples of agricultural *feedstocks* include animal manures, waste crops, and milk and egg spoilage.

Plug flow reactor (PFR): An open system at steady-state with no axial mixing.

Process flow diagram (PFD): A diagram commonly used to indicate the general flow of material and energy in a system. The *PFD* displays the relationship between major equipment of a facility. Other commonly used terms for a *PFD* are flowsheet or flowchart.

Roadmap: A plan or strategy intended to achieve a particular goal.

Unit operation: A basic step in a process that involves a physical change or chemical transformation of the feedstock material. A *unit operation* is often denoted on a process flow diagram as a single boxed icon.