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THE DEVELOPMENT OF CARBOXYLIC ACID SEPARATION BY NANOFILTRATION MEMBRANE FOR CARBOXYLATE PLATFORM USING LIGNOCELLULOSIC BIOMASS

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Boya Xiong

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The thesis of Boya Xiong was reviewed and approved* by the following:

Tom Richard
Professor of Agricultural and Biological Engineering
Thesis Advisor

Manish Kumar
Assistant professor of Chemical Engineering

John Regan
Professor of Environmental Engineering

Ali Demirci
Professor of Agricultural and Biological Engineering

Virendra Puri
Distinguished professor of Agricultural and Biological Engineering
Agricultural and Biological Engineering Graduate Program Coordinator

*Signatures are on file in the Graduate School

ABSTRACT

The carboxylate platform utilizes acidogenic digestion, converting lignocellulosic biomass anaerobically into short and medium chain carboxylic acids, which serve as reactive intermediates for downstream biofuel production. As a mixed-culture, non-sterile process, acidogenic digestion may provide a low-cost strategy for high substrate conversion rates and high product yield coefficients from lignocellulosic biomass. However, carboxylic acids in high concentrations are toxic to microorganisms, and can rapidly build up to inhibitory levels. For this process to achieve high conversion rates at an economical biomass loading rate, there is a need to separate and recover carboxylic acids during acidogenic digestion. Very limited research has been done to test different acid recovery technologies that can be applied to this carboxylate bioconversion platform. In this context, the goals of this study were to 1) identify the correlation between solid loading rate and acid yield 2) separate carboxylic acids from acidogenic digestion liquor using nanofiltration and 3) integrate this NF acid removal process into a batch digestion system, with system performance evaluated by the acid yield. The biomass substrate for the digestion studies was mainly hot water pretreated willow wood. The effects of pH and feed pressure on the acid and sugar rejection were investigated with 10 day old willow digestion liquor using two commercialized NF membranes, GE Desal-DK and Desal-DL membrane. In general, NF membrane achieved 0% to 40% rejection of carboxylic acid with the exception of butyric acid (>99% rejection), and > 90% rejection of sugars. The high rejection of butyric acid may be due to butyric acid's intermolecular interaction with the complex compounds in the digestion liquor. The lactic and acetic acid rejection decreased with pH which was correlated with the degree of dissociation of the acid, while the sugar rejection was not changed significantly by pH. Raising the feed pressure increased the permeate flux and slightly increased sugar rejection but unfortunately also increased the acid rejection. It is concluded that low pH and low pressure are

the favored operational parameters to separate lactic and acetic acid. To integrate the processes of separation and digestion, the pretreated willow was digested and the acids were separated from the liquor intermittently by short term NF. After separation the retentate was recycled back into the digestion reactor with water added to maintain the original solids loading rate. The integrated digestion system was able to remove 86.8% of total acid produced and lowered the acid concentration by 87% compared to a control digestion system without acid removal. The acid yield was also enhanced by separation, but not by a statistically significant amount. This study provides preliminary engineering design data to accelerate the development of a robust and scalable digestion and separation process for the carboxylate platform of lignocellulosic biofuel production.

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I did not quite understand when a former Ph.D. student told me that it is not hard to have an idea, what is really hard is to figure out experiments to implement and prove it. It was my first semester at Penn State, when I was just excited about working on biofuel and did not understand what the process of finishing a research project independently would really cost me. Now I would say, finding the novel research topic was not my best luck. My real best luck was that I had tremendous technical and intellectual guidance and support to realize this idea by an extraordinary group of people, whom I deeply benefit from in many ways.

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Chapter 1

Introduction

Lignocellulosic biofuel production has long been focused on two reactive intermediates: sugar and syngas. The sugar platform utilizes purified enzymes to hydrolyze biomass into five and six carbon sugars, while the syngas platform converts biomass into syngas (i.e. CO, H_2, CO_2) through pyrolysis and gasification. Both intermediates can be converted into advanced fuel by biochemical fermentation or thermochemical catalysis (Agler et al., 2011; Holtzapple and Granda, 2009). This research focuses on a third important platform of reactive intermediates, the carboxylate acids. Acidogenic microorganisms can convert lignocellulosic biomass feedstock in to short- and medium- chain carboxylic acids, which can then serve as a feedstock for chemical or biochemical conversion to fuels or chemicals. This platform integrates hydrolysis and acid fermentation into one single step, using undefined mixed cultures under anaerobic conditions, and is termed acidogenic digestion (Holtzapple and Granda, 2009). The main carboxylic acid products are lactic, acetic, propionic, butyric, valeric and hexanoic acid, which by themselves are valuable products, but also can be processed into fuels and chemicals (i.e. alcohols, esters, and alkanes) via biochemical, thermochemical and electrochemical processes (Agler et al., 2011). As commercial scale-up of the sugar and syngas platforms continue to be challenged by high costs to produce these intermediates(Fehrenbacher, 2014), the simplicity of the carboxylate platform has received increasing attention. Acidogenic digestion integrates enzyme production, hydrolysis, and acid fermentation into one single process, and it thus has the potential to reduce the capital and operational costs and increase overall conversion efficiency (Chan et al., 2011). The carboxylate platform can utilize waste materials as feedstock (e.g. wastewater, agricultural residues, animal

waste, paper industry waste, and municipal waste) thus minimizing feedstock cost. This process utilizes undefined mixed microbial consortia that may be derived from rumen fluid (Matei and Playne, 1984), wastewater (Agler et al., 2011), and/or marine sediment (Chan et al., 2011) or other inexpensive sources instead of pure cultures, and thus there is no need for sterilization. Furthermore, a diverse undefined mixed culture can easily adapt to a wide range of feedstocks, naturally co-fermenting a mixture of substrates, which is challenging for a pure culture (Matei and Playne, 1984). In summary, acidogenic digestion is a simple, cheap and robust process. Most research so far has focused on using waste material as feedstock. The research reported in this thesis utilizes willow wood biomass, a promising lignocellulosic feedstock for biofuel production.

Acidogenic digestion with high solids loading involves less process volume and energy use for operation, and yields high acid concentrations (Fu and Holtzapple, 2010). However, a high acid concentration has an inhibitory effect on microbial activity, thus reducing the overall acid yield and conversion rate (Kleerebezem and van Loosdrecht, 2007). Several studies have shown direct evidence of concentration dependent inhibition of microbial growth, specifically from lactic acid, acetic acid, propionic acid and butyric acid (Cheung et al., 2010; Nanba et al., 1983b; Roddick and Britz, 1997; Zeng et al., 1994). Therefore, strategies that reduce exposure to these carboxylic acids during digestion have the potential to improve system efficiency. One implementation of this approach is the MixAlco process, which minimizes acid inhibition by employing countercurrent fermentation, allowing the most digested biomass to contact the lowest acid concentration . However, operating this system in a stable fashion is challenging and time-consuming due to the multiple stages of fermentation involved (Chan et al., 2011). Other groups utilized separation technology to realize continuous removal of acids during fermentation. Nuchnoi *et al.* (1987) reported on-line acid extraction of acids from pure culture acid

fermentation using supported liquid membranes. This process requires direct contact between the fermentation broth and organic solvents that are not biocompatible, which may affect microbial activity in an integrated system. Roddick *et al.* (1997) demonstrated in-situ hexanoic acid removal using ion exchange resins that had fewer adverse effects on microbial growth. However, resins require preparation and regeneration using additional alkali and acid solutions, adding cost and complexity to the process. Both of these separation technologies generate acid products in salt forms.

Another potential separation strategy is pressure-driven membrane separation, which does not require organic solvents and thus avoids the associated toxicity concerns. Well established membrane separation technologies utilize low pressure porous membranes including microfiltration (MF) and ultrafiltration for particulate solute removal and high pressure non-porous membranes including nanofiltration (NF) and reverse osmosis (RO) for dissolved solute removal. The advantages of the these technologies include simplicity, high selectivity, high energy efficiency, and low chemical usage compared to conventional separation technologies such as extraction and adsorption (Cho, 2012). NF separation is versatile and is governed by size sieving, solution-diffusion, as well as Donnan exclusion (Van der Bruggen et al., 1999). Donnan exclusion occurs when the charged solutes are excluded by the fixed ions on the membrane with the same charge. Previous studies have reported that NF can reject glucose and xylose at 90 to 99%, and reject acetic acid and butyric acid at less than 5% under optimal conditions (Cho, 2012; Weng et al., 2010; Zhou et al., 2013a). RO has a denser active layer and the separation mainly depends on size, diffusivity and solubility of the solute. Thus RO separation demonstrates a higher rejection of acetic acid (50 to 100%) and butyric acid (60 to 100%) compared to NF separation (Cho, 2012; Zhou et al., 2013b). Zhou *et al.* (2013a) concluded RO is a better

membrane for acetic acid separation and sugar concentration in lignocellulosic biomass hydrolysate even if acid was rejected more by RO than NF, but their application of the research prioritized sugar concentration over acid separation. Cho (2012) on the other hand concluded NF is more attractive for butyric acid separation due to its higher flux and higher acid recovery than RO, even if RO performs better in product purity. The purpose of the present research is to remove sufficient acid from the digestion liquid to reduce or eliminate acid inhibition. Therefore, NF was selected for further study in this research.

Prior NF membrane research related to lignocellulosic biofuels has focused on two applications: 1) removal of inhibitors and concentration of sugar from enzymatic hydrolyzate and 2) separation of acetic acid from the aqueous fraction of pyrolysis (He et al., 2012; Teella et al., 2011). To the best of our knowledge, no published studies have reported the use of NF for acid separation from lignocellulosic biomass digestion. When reviewing the literature more broadly on acid separation from aqueous solutions, there are limited studies on separating multiple carboxylic acids (C1-C6) simultaneously, and there is no data on hexanoic acid separation using NF membranes. Furthermore, most of these prior studies utilized synthetic model solutions to conduct separation experiments (Cho, 2012; Weng et al., 2010; Zhou et al., 2013a). In a biomass digestion system there are a variety of complex and undefined molecules derived from plants that might interfere with the transport behavior of acids and sugars through membranes, thus changing separation behavior.

In this study we first investigated the relationship between solid loading rate and acid yield. In a second trial, we demonstrated the feasibility of NF for separating mixed carboxylic acids and retaining sugar substrates (i.e. arabinose, galactose, glucose and xylose) with two NF membranes. Specifically the effects of feed pressure and pH on solute rejection were investigated.

Although it is known that temperature also significantly affects membrane flux, the biological digestion process is mesophilic so the separation experiments were all conducted under room temperature conditions. The third trial evaluated acid removal in the integrated digestion system, comparing final acid yield (g acids / g dry biomass fed) of an integrated digestion process with NF acid removal with a batch digestion process without separation. The results reported here provide a proof of concept for acidogenic digestion with integrated carboxylic acid production and separation. This study also provides preliminary engineering design data for a membrane bioreactor setup that can accelerate the development of a robust and scalable process for the carboxylate platform of lignocellulosic biofuel production.

Chapter 2

Literature Review

With the increasing challenges of climate change and national energy security, the world is experiencing a rapid reconfiguration of energy production and resource utilization. These changes are driving rapid growth of alternative and clean energy, which is likely to continue for the next few decades. In this context, a new bio-based industrial sector is aggressively seeking alternatives to petroleum for production of chemicals and liquid transportation fuels (Richard, 2010). These modern biorefineries are being developed with multiple bio-based feedstocks and processing strateiges. However, there is a continuing societal concern about the competitive use of food crops as biorefinery feedstocks, so biorefinery feedstock sourcing is increasingly leading toward lignocellulosic biomass: dedicated energy crops, forests residues and agricultural wastes. Compared to a petroleum refinery, a biorefinery is a very recent industrial model. Without a clear picture of a cost effective path forward, it is especially important at this time to explore a diversity of technology platforms. Disruptive technologies may be crucial to long term development of this sector, especially when competing against inexpensive fossil resources.

Three Platforms of Lignocellulosic Biorefineries

The term “platform” in the chemical and bioprocessing sectors refers to a foundational feedstock that can be made into many different products. In lignocellulosic biorefineries, the major platforms are defined by classes of reactive intermediate chemicals: the syngas and sugar platforms that have received extensive research and development investments for several decades, and the much less explored carboxylate platform. The term “platform” is conceptual and used for describing these key intermediate compounds involved in the biorefinery process, produced by

gasification (syngas), enzymatic hydrolysis (sugar) or acidogenesis (carboxylic acids) from lignocellulosic feedstocks (Agler et al., 2014). Other potential biorefinery platforms include mixed alcohols (also from fermentation) and pyrolysis oils. These intermediates are subsequently converted into valuable end products by a downstream conversion process. The syngas platform utilizes a thermochemical conversion process, gasification to generate syngas (CO, H2, CO2, CO4, etc.) and other carbon containing by-products (tars, solid char) that often time are converted into alcohol or other hydrocarbons (Dutta, 2011). The sugar platform utilizes a biochemical conversion process, with enzymatic hydrolysis depolymerizing the carbohydrate components of biomass into five- and six- carbon sugar intermediates, and microbial fermentation metabolizing the sugars into ethanol (Lynd et al., 2005). Although the sugar platform has been the focus of most biochemical research efforts for biofuel and biochemical production to date, the costs of enzymatic hydrolysis continue to be prohibitive. The carboxylate platform also utilizes a biochemical conversion process, acidogenic digestion, integrating biomass hydrolysis and acid fermentation into one single step using a mixed culture to produce a mixture of carboxylic acids as intermediate compounds (Agler et al., 2011). The process is a variant of anaerobic digestion, truncated before methanogenesis, and employs undefined mixed cultures from a diverse inoculum source like compost or sewage sludge. The use of an undefined mixed culture is a key feature of the process because the diverse organisms involved can adapt to the complexity and diversity of feedstock substrates (Agler et al., 2011), making use of almost all the non-lignin parts of the biomass including cellulose, pentosans, hemicellulose, pectin and proteins (Datta, 1981; Holtzapple and Granda, 2009). This broad and nearly complete conversion of various substrate components is very difficult for pure culture fermentation to realize. In addition, the process is simplified because there is also no need for sterilization, leading to reduced capital and energy

investment (Datta, 1981; Holtzapple et al., 1999). The carboxylate platform can be applied to waste feedstocks (i.e. wastewater, agricultural residues, animal waste, paper industry waste, and municipal waste), thus further minimizing the overall process cost (Agbogbo and Holtzapple, 2007; Aiello-Mazzarri et al., 2006; Domke et al., 2004; Fu and Holtzapple, 2010). Therefore, the carboxylate platform has a great potential to become a cheap and simple, robust and efficient option for lignocellulosic biorefineries. Carboxylic acid products include short-chain acids formic, acetic, propionic, lactic, butyric acids from primary fermentations, and medium-chain acids valeric and hexanoic acids from secondary fermentations that use the short-chain acids as substrates (Agler et al., 2011). These are all valuable chemicals by themselves, and can also be converted by biochemical, electrochemical and thermochemical steps (Agler et al., 2011). The upstream side of this platform, converting biomass to carboxylic acids, involves multiple metabolic pathways giving the platform mixture a complicated composition. In addition to the acid product complexity, the feedstock substrates also have a complex composition compared to the pure substrates used in conventional sugar and starch fermentations. Liquor derived from lignocellulosic biomass generally contains sugars: xylose, arabinose, galactose (hemicellulose-derived) and glucose (hemicellulose and cellulose-derived); inhibitors of ethanol fermentation: furfural (hemicellulose-derived), hydroxylmethyl furfural (cellulose-derived), and phenolic compounds (lignin-derived) such as ferulic acid and vanilic acid and other acids such as acetic acid and formic acid (Maiti et al., 2012; Weng et al., 2010). The exact portion of each component varies widely and depends on both the feedstock and the type of conversion process.

Acid Inhibition and Solid Loading Rate

Establishment of a competitive biorefinery platform for lignocellulosic biomass requires high productivity, high product concentration, and high yield (Playne, 1981). However, the

improvement of acid production from organic materials has always been constrained in yield and productivity due to the toxicity of the acid products (Kleerebezem and van Loosdrecht, 2007). This phenomenon of product inhibition has been reported for several different acid production processes and their corresponding microbial producers (Nanba et al., 1983a; Wu and Yang, 2003; Zeng et al., 1994). One of the theories used to explain end-product inhibition of anaerobic acidogens is that when pH is reduced by the accumulation of acids, the protons might disturb the pH gradient generated by proton pump at the cell membrane interface. As a result, cells have to spend extra energy to maintain the pH gradient at the membrane, leaving less energy for biosynthesis and thus reducing the microbial growth rate (Herrero, 1983). Nanba *et al.* (1983a) reported a stronger suppression of *Propionibacterium Shermanii* specific growth rate by propionic and acetic acid under acidic conditions (pH 5.2) than neutral conditions (pH 6.8). But it was also observed that the specific growth rate decreased considerably even at pH 6.8 with an increase of propionate concentration. At pH 6.8, inhibition was observed starting at 9 g/L acetic acid and 7 g/L propionic acid, indicating that the acid in dissociated form may directly impose an inhibitory effect on microbial growth. This hypothesis was further supported by an independent observation of lower acid yield at higher acid concentration, also at near-neutral pH (6.5) (Golub et al., 2012).

This product inhibition could become more severe when a high substrate loading rate is applied. Since acids are the desired fermentation product, in a normal batch or continuous mix reactor higher substrate levels mean the process would be operated at elevated acid concentrations. These higher acid concentrations would presumably result in a lower yield and productivity at those higher solid loading rates (Datta, 1981; Golub et al., 2012; Ross and Holtzapple, 2001). Nevertheless acidogenic digestion with high solid loading appeals has

important advantages, giving less process volume and energy use for operation, while high product concentration is also favored for downstream users (Fu and Holtzapple, 2010). Although product inhibition suggests that high substrate loading and high yield will be mutual exclusive, innovative reactor configurations and/or separation strategies can resolve this apparent contradiction. Achieving *in situ* product removal during production has a great potential to enhance the system efficiency. In the next few sections, different digestion and separation strategies to reduce inhibition and enhance production will be reviewed.

MixAlco Process

The MixAlco process has been studied for a few decades and has been tested with many different feedstocks. The process typically uses a countercurrent fermentation set-up employing multiple fermenters. By transferring the liquid phase and solid phase in a countercurrent manner, this configuration allows the most digested biomass to contact the lowest acid concentration, and the freshest biomass to contact the highest acid concentration. This design is able to achieve a high product concentration (51.8 g/L) because the liquid picks up all the acids produced from as much feedstock as possible (Agbogbo and Holtzapple, 2007), and also achieves a high product yield (0.55 g acids/ g biomass fed) because biomass is digested in the system for a very long time (Thanakoses et al., 2003). The main drawback of the system is that the total acid productivity with combined fermenters is only around 2 g L^{-1} day^{-1}, which is lower than other lignocellulosic fermentation processes, and a complete run requires months to finish (Fu and Holtzapple, 2010). In addition, it can take months for the system to reach steady state, which is a challenge for both research and commercial operations. The MixAlco process separates and concentrates the products by drying and calcium precipitation (Holtzapple et al., 1999).

Conventional Acid Separation Technology

Solvent Extraction

Solvent extraction makes use of the difference in physical solubility of the solutes into a solvent, or alternatively a chemical reaction that occurs at an interface. Solvent extraction is a well-established technology that has been used for centuries, and over recent decades inventors and innovators have created a variety of membrane-based solvent extraction and pertraction systems that are alternatives to the classical solvent extraction (Schlosser et al., 2005). For acid separations, the acids are extracted into a solvent from the fermentation broth, and then an alkaline stripping solution removes acids from the solvent realizing both solvent regeneration and acid concentration at the same time. Agler *et al.* (2014) utilized 3% tri-*n*-octylphosphineoxide and mineral oil solution as solvent and a pH 9.0 aqueous stripping solution, with hollow fiber membrane as contactors, to extract carboxylic acids from the fermentation liquor produced by corn fiber acidogenic digestion. The solvent is selective to compounds that are weakly acidic and hydrophobic. Under these conditions only undissociated hexanoic acid was fully recovered, while butyric acid was partially recovered and very little acetic acid was recovered. Wu *et al.* (2003) developed a extractive fermentation for butyric acid production, integrating fermentation with *ex situ* pertraction system, and achieved a butyric acid removal rate at 7 $gL^{-1}h^{-1}$. The pH of the fermentation was self-controlled at 5.5 by acid production and removal. Butyric acid yield and productivity improved 15 and 30% respectively, compared to fermentation at the same pH but without extraction. Solvent toxicity was found to be a problem in free cell fermentation, although the problem was solved by immobilized-cell fermentation. Choi *et al.* (2013) used *in situ* biphase extractive fermentation for hexanoic acid production, achieving a 5 g L^{-1} h^{-1} acid removal rate while enhancing yield and productivity by 16 and 10% respectively. Grzenia *et al.* (2008) was

one of the few researchers that investigated solvent extraction of lignocellulosic biomass material, reporting 60% acetic acid removal from corn stover hydrolyzates and also some removal of fufural and phenolics. In summary, solvent extraction has a high removal rate and is able to concentrate appreciable amounts of acids as salts in the strong alkaline stripping solution (Wu and Yang, 2003). Both solution pH and the acid form can affect the extraction efficiency. The biocompatibility of the selected solvent is always a concern and needs to be tested.

Ion Exchange Resin

Ion exchange resin has certain ions loosely attached to the surface that can be exchanged by the ions with stronger affinity in the product solution as it passes by. Roddick *et al.* (1997) employed an anion exchange resin in early stage of hexanoic acid fermentation for *in situ* product removal, realizing pH self-control at 7, increased sugar substrate utilization and productivity, and 9% yield enhancement. However, the system had challenges as well. The resin strongly and quickly absorbed nutrients in the medium once it was added; OH^- was also replaced thus elevating pH. This process also negatively affected cell growth, which was reduced by placing the resin into a dialysis bag. However, the yield and productivity of the process with the resin in a dialysis bag was not significantly enhanced, suggesting the dialysis bag reduced the transport of hexanoic acid onto resin. The effect of resin on cell growth might be alleviated by immobilizing the cells or setting ion exchanging resin in an *ex situ* product removal configuration. In addition, the ion exchange resin seemed to have limited capacity to concentrate the acids, with 3 liters of NaOH, water and HCl solutions required to elute the hexanoic acid from only 1 liter of fermentation broth. The process also required multiple repeated steps of washing with strong NaOH and HCl solution to get the resin activated and regenerated, indicating implementation of this technology would require more complicated procedures and a large process volume.

In summary, there has been limited development of methods to separate a mixture of carboxylic acids from the fermentation broth of lignocellulosic materials, yet there remains a recognized need to investigate the feasibility of different acid separation technologies (Agler et al., 2014; Agler et al., 2011). Membrane technology is an alternative technology to separate acid effectively, and is the focus of this study. However, the application of membrane separation in lignocellulosic biorefinery, especially in carboxylic acid product recovery, is still largely unexplored. The next section will discuss the background information and potential usage of membrane separation on carboxylic acid separation.

Pressure-driven Membrane Separation

In pressure-driven membrane processes, hydrostatic pressure is applied to the feed stream on one side of a permselective barrier membrane, providing the driving force to divide the feed stream into permeate and retentate (concentrate). Separation is achieved based on the differences in the permeation rates of the solute species across the membrane. The growth and expansion of applications using membrane separation technologies is primarily due to the low energy requirements and capital costs compared to conventional separation processes in some industrial applications. In addition, the process is simple, compact and relatively easy to control. Since the technology is modular, it can be easily scaled up and adapted for different applications (Cho, 2012; Teella, 2011).

Membrane performance is normally evaluated by measuring solvent flux and solute selectivity (rejection %). After decades of development, the cross-section structure of membranes has evolved from a uniform chemical composition into thin film composites (TFC) containing a thin dense layer with high selectivity and a porous substrate layer that acts as a mechanical support for the thin layer and maximizes membrane flux. Each layer utilizes different materials to

optimize membrane permeability, selectivity and stability. Membranes can be classified as porous and non-porous based on their permeability and pore size. For porous membranes with well-defined pore structure, the selectivity is governed by the sieving mechanism and flux is normally high. Transport through very dense or non-porous membranes is mainly described by a solution-diffusion mechanism where diffusion properties of the membrane material and solute species both decide membrane permeation and selectivity (Baker, 2000).

Common membrane separation processes include microfiltration, ultrafiltration, nanofiltration and reverse osmosis, categorized by the pore size and applied pressure range. Microfiltration membranes have pore sizes from 100-5000 nm and can reject suspended particles like bacteria cells. Ultrafiltration membranes have pore sizes in the range of 2-100 nm and can remove macromolecules like proteins. Nanofiltration membranes have pore sizes around 0.5-2 nm and can remove small molecules such as multivalent ions and monosaccharide. Reverse osmosis can reject monovalent ions (Na^+, Cl^-, etc.) with pore sizes less than 0.5 nm, and are thus appropriate for water desalination and purification. With pore size getting smaller and smaller, solute diffusion starts to act more and more significantly in membrane performance, especially in nanofiltration and reverse osmosis membranes. Due to the membrane structure, the denser membranes are normally operated at a higher pressure range in order to achieve a substantial flux. For example, reverse osmosis (RO) can be operated as high as 120 bar while the highest pressure for microfiltration is about 2 bar (Van Der Bruggen et al., 2003).

Among all the membrane processes, nanofiltration (NF) is a well-suited candidate for separating carboxylic acids from sugar nutrients in digestion liquor. Considering the particle size sieving mechanism, NF membrane pore sizes lie between the molecular sizes of sugars (150-160 g/mol) and carboxylic acids (46-116 g/mol). Thus an NF membrane should be able to achieve

high permeation of carboxylic acids and high retention of sugars. In addition to size exclusion and the solution-diffusion mechanism, Donnan exclusion can also have a crucial impact on NF membrane performance (Van der Bruggen et al., 1999). The commercialized polyamide NF membrane structure is modified with ionized groups, such as carboxylic groups and/or amine groups (Cho, 2012), thus leaving the membrane electrically charged under various pH conditions. Donnan exclusion describes a process in which the separation is achieved when the charged ions in solution have the same charge as the fixed ions on the membrane structure. While carboxylic acids can be charged in the dissociated form under high pH, the operating pH can significantly change the membrane performance in terms of acid permeation. But the NF membrane separation process only provides a selective separation and is not capable of concentrating the acid products, as most of the solvent will also pass through the membrane. Some previous research groups compared the performance of NF and RO for separating acetic acid and butyric acid (Cho, 2012; Zhou et al., 2013a). RO showed higher product purity than NF, but the acid rejection was also higher and it required higher pressure to maintain the same flux as an NF membrane. Thus, RO was considered a less energy-efficient process compared to NF and not studied in this research.

There has been some previous research using NF membranes to permeate carboxylic acids and retain sugars in idealized solutes and/or lignocellulosic hydrolyzates, although few if any have investigated these separations with lignocellulosic hydrolyzate that had been exposed to microbial fermentation, such a acidogenic digestion liquor. Weng *et al.* (2010), Maiti *et al.* (2012), and Zhou *et al.* (2013a) have used NF membranes to separate acetic acid and other inhibitors from sugars in pretreated lignocellulosic hydrolyzates in prior to fermentation. Negative rejection of acetic acid and furan was observed at very low pH and average xylose and glucose rejection was higher than 94%. By operating the membrane in batch filtration mode with

full recycle of retentate, the sugar concentrated in the retentate provided a more valuable intermediate for downstream processing in a biorefinery than typical of a lignocellulosic hydrolyzate. This concentration of sugars created an extra benefit for the process. It was also found that pH, pressure, temperature and feed concentration can affect membrane flux and retention of acetic acid and sugar. Acetic acid rejection was especially affected by pH. Freger *et al.* (2000) reported 60% lactic acid rejection with a synthetic industrial waste stream solution containing 2% (w/w) lactic acid and the rejection decreased to 35% with the presence of 4% salt in the solution. Cho *et al.* (2012) performed butyric acid separation by NF membrane from sugars, inorganic salts and other nutrient compounds in a synthetic fermentation broth. It was observed that butyric acid was rejected by 5% on average. Choi *et al.* (2008) studied organic acid removal from synthetic wastewater using NF membrane and reported 30% rejection of formic acid and propionic acid; both rejections were significantly affected by pH.

State-of-the-art of Membrane Separation Technology for Carboxylic Acid Removal from Acidogenic Digestion Liquor with Lignocellulosic Biomass

For both product recovery and elimination of product inhibition, separation of carboxylic acid products from acidogenic digestion liquor is a key to promoting the development of the carboxylate platform. Yet the application of NF membranes to separation challenges in lignocellulosic biorefineries, and especially for carboxylic acid removal, is still a very new area of research. To the best of our knowledge, no previous investigation has tested NF membranes for separating carboxylic acid products from lignocellulosic acidogenic digestion liquor. While a, few studies have explored the application of NF membrane on the separation of one single type carboxylic acid, very limited research has investigated the separation of a wide range of carboxylic acids such as will occur in an acidogenic digestate mixture. Research on separation of

larger carboxylic acid molecules is especially scarce, so for example we found no evidence NF membranes have previously been used to separate the six carbon hexanoic acid ($C_5H_{11}COOH$). Furthermore, almost all the previous research used a synthetic model solution for NF membrane separation experiments, yet the complex composition of real fermentation broths from lignocellulosic biomass or organic waste materials is unavoidable and can potentially alter membrane performance and optimum operational parameters. Here we propose the evaluation of NF membrane performance for removal of a mixture of 6 different carboxylic acids, with retention of the sugars in actual acidogenic digestion liquor derived from pretreated willow wood. The first set of experiments will investigate the relationship between solid loading rates and acid yield, to determine the solid loading rate at which inhibition begins to significantly diminish yield. A second set of experiments will investigate the effect of pH and pressure on acid and sugar transport across two commercialized NF membranes, Desal DK and Desal DL. Of the other operational variables, feed concentration was not considered in this study as it was previously found not to be a significant factor for acid (100-500 mg/L) (Choi et al., 2008) and sugar rejection (Weng et al., 2009). Temperature has been shown to affect both flux and solute rejection (Goulas, 2002; Zhou et al., 2013a), but also was not tested because the acidogenic digestion is mesophilic, so that raising the temperature during separation would impose an additional energy investment. In the final trials, a lab scale digestion was integrated with membrane separation and evaluated for acid removal capacity and yield. This study is intended to provide preliminary engineering design data for a continuous membrane bioreactor system designed for carboxylic acid production. The overall goal is to facilitate the establishment of an efficient, cheap, and robust carboxylate platform for lignocellulosic biomass.

Chapter 3

Materials and Methods

Overview of methodology

The methodology section of this thesis has been divided into three phases; (1) acidogenic digestion, (2) Nanofiltration, (3) integration of digestion with Nanofiltration.

In phase 1, acidogenic digestion was conducted in two batches with a different ranges of overlapping solid loading rates, followed by a third batch with different inoculum loading rates. Each trial was evaluated based on acid concentration and yield. The results were used to determine appropriate solid and inoculum loading rates for later experiments. Methane concentration in the gas phase was measured during digestion using Gas Chromatography (GC).

In phase 2, the Nanofiltration experiment was conducted under three pH and three pressure conditions with two commercialized NF membranes. Tests were run on a cross-flow filtration unit with actual willow biomass digestion liquor. The acid and sugar concentration in permeate and feed were measured using Ion Chromatography to calculate the acid and sugar rejection. Statistical analysis using ANOVA test was conducted to determine the significance of pH and pressure on solute rejection.

In phase 3, short term Nanofiltration was conducted on the supernatant liquid collected from digestion liquor intermittently during digestion. Permeate was collected to quantify the acid product recovered, while retentate was recycled back into the digestor. Water was added in order to maintain a constant solid loading rate. Acid removal rate and acid concentration reduction in the digestor was measured in both the integrated digestion – separation system and a control

digestor without separation. Acid yields were compared between this integrated digestion – separation system and digestion without separation using a two sample t test.

Methodology

Phase 1 Acidogenic digestion

Three batch digestions were conducted in total to establish appropriate digestion conditions. The first two batches examined solid loading rate with pretreated poplar wood as the feedstock and the last one examined inoculum loading rate effect with switchgrass feedstock. The feedstock used for the phase 2 separation experiment was pretreated willow, which was also used for the phase 3 integrated digestion – separation system trial.

Feedstock

There were three feedstocks in total involved in this study: poplar wood, switchgrass, and willow wood. Poplar wood was from a single *poplus* hybrid NE-388 tree, grown in a Penn State poplar field trial, and provided by Dr. John Carlson's research group at Penn State (Pandey, 2012). Switchgrass was harvested during the summer of 2012 from a field located at the arboretum of the University Park campus, Penn State University. Two sources of willow wood were used. Willow 1 was provided by Dr. Tim Volk's research group at State University of New York College of Environmental Science and Forestry, and was used for the Phase 2 trials. Willow 2, used for the Phase 3 trials, was provided by East Lycoming School District located in Hughesville, Pennsylvania. Both poplar and willow wood were air dried for long term storage prior to grinding with a Wiley no. 4 mill. Switchgrass was freshly ground after harvesting. All three feedstocks were pretreated prior to acidogenic digestion.

Hot water Pretreatment

The goal of pretreating the biomass was to break down the plant cell structure to separate lignin and hemicellulose from the cellulose and convert the biomass into a more readily digestible substrate for acidogenic digestion (Pandey, 2012). In the first batch experiment the poplar chips were ground to 2 mm particle size and then hot water pretreated in a one liter Parr 4842 Bench Top Reactor (Parr Instrument Company, Moline, IL) at 190℃ for 15 min. In the second batch experiment, poplar was pretreated with hot water in a 66 ml Zirconium cell using an automated Dionex Accelerated Solvent Extraction (ASE, Sunnyvale, CA) system at 190℃ for 15 min. In the third batch, switchgrass was ground to 2 mm and ensiled for one week prior to hot water pretreatment using a pilot scale SuPR2G Pretreatment Reactor (AdvanceBio Systems LLC, Milford, Ohio) at 180 ℃ for 15 min. Willow wood for phases 2 and 3 was ground into 1/8 inch and pretreated with the pilot scale reactor at 190℃ for 5 min.

Characterization of pretreated material

After pretreatment, the pretreated material was carefully mixed, with 3 gram subsampled and dried at 105 ℃ for 24 hours to measure moisture content, as calculated below. The dry biomass was then burned in furnace at 550℃ to determine the volatile solids content in the biomass. After being burned, the leftover ash, which is the inert fraction of the biomass, was weighed.

*Moisture content % (MC %) = [(wet weight-dry weight)/wet weight] *100%*

Solid % =100%- MC %

*Volatile solids content % (VS %) = [(dry weight-ash weight)/dry weight]*100%*

Compositional analysis of pretreated switchgrass and willow was conducted by a method that was adapted from the standard NREL protocol for biomass compositional analysis and

described by Sluiter *et al.* (2008). The first step was to determine the moisture, volatile solids and ash content of original material. Meanwhile, the original material was also extracted by water and ethanol using ASE-350 under the conditions of 100 ℃, 7 min static time, 150% rinse volume, 120 seconds purge time, and 3 static cycles. This extraction step separates the non-structural portion of the biomass from structural carbohydrates. Water and ethanol extractives are quantified during the process. The extraction is followed by a two-step complete acid hydrolysis of biomass into monosaccharide. First one hour incubation in 72% (w/w) sulfuric acid at 30℃ is followed by one hour autoclave at 121 ℃ in 4% (w/w) sulfuric acid. The acid hydrolysis removes all soluble carbohydrates and leave insoluble lignin and ash in the biomass. The sugars (glucose, xylose, arabinose, galactose and mannose) in the hydrolyzed liquid are measured using Dionex Ion exchange chromatography system, whose details will be introduced later in this chapter. Acid soluble lignin was measured using the LIGNIN STOVER smart start method on GENESYS™ 10S UV-Vis spectrophotometer (Thermo Scientific™, Waltham, MA). The remaining insoluble solids are dried in 105 ℃ and then ashed at 575 ℃ for acid insoluble lignin and ash quantification.

Inoculum preparation

The first two batches of inoculum (P1b1 and P1b2) were a mixture of silage and approximately 4-week old compost. These raw inoculum ingredients were procured at the Penn State Dairy Barns and Compost Facility, respectively, and collected fresh immediately prior to inoculum preparation. Raw inoculum was collected and placed into a sealed Ziplock® bag and returned to lab as quickly as possible (within an hour) to minimize exposure to oxygen. Phosphate buffered saline (PBS) buffer was freshly prepared and degassed for 15 min and then mixed with compost and silage in the ratio 3 g compost: 1 g silage: 8 ml PBS buffer. This ratio was

developed in preliminary experiments prior to the initiation of this research project. The PBS buffer and degassing by vacuum pump was added in order to enhance the preservation of bacteria and minimize the potential damage by oxygen from the buffer, as recommendations by Stieglmeier *et al.* (2009). After mixing, the mixture was filtered by a 90 μm screen with the liquid phase collected as inoculum. Because the heterogeneity of the inoculum liquid can bring inconsistency to the experiment, in the third batch (P1b3) the inoculum liquid was centrifuged at 4000 rpm for 10 min and the solid was collected as inoculum. In addition, in this third batch (P1b3) rumen fluid was also added into the mixture of inoculum the same portion as silage because it offers an adapted population of cellulolytic and hemicellulolytic anaerobic bacteria (Matei and Playne, 1984). Rumen fluid was collected from a fistulated cow in Penn State Dairy Barns, transported in a thermos and used within an hour for inoculum preparation. For all the willow digestion trials (phases 2 and 3), inoculum was prepared as 180 g compost, silage and rumen fluid each, mixed with 1 L degassed PBS buffer, and then mixed in a blender for 10 s before draining and centrifuging at 4000 rpm. After the inoculum solids were collected, moisture and volatile solids content was analyzed by the same method previously described for biomass moisture analysis, with the dry inoculum burned in a muffle furnace at 575 °C to determine the volatile solids content.

Acidogenic Digestion Preparation

The detailed information for each batch was summarized in Table 3-1.

Table 3-1. Summary of acidogenic digestion.

	Phase 1-1st batch (P1b1)	Phase 1-2nd batch (P1b2)	Phase 1-3rd batch (P1b3)	Phases 2 & 3- Digestion for separation (integrated digestions were noted as P3b1 and P3b2)
Feedstock	2 mm Poplar wood	2 mm Poplar wood	2mm Switchgrass (ensiled for one week)	1/8 inch Willow wood
Pretreatment method	Parr Bioreactor	ASE	SuPR2G	SuPR2G
Inoculum composition	3:1:8 (w/w/v*) compost/ silage/PBS buffer	3:1:8 (w/w/v) compost/silage/ PBS buffer	3:1:1:8 (w/w/v) compost/silage/ rumen fluid/PBS buffer	1:1:1:5.5 (w/w/v) compost/silage/ rumen fluid/PBS buffer
Inoculum form	Liquid	Liquid	Solid	Solid
Solid loading rate (g dry matter/L)	112 , 69 , 34 , 10	25, 10, 5, 2.5	10	75
Inoculum loading rate (g VS inoculum/ g VS biomass)	N/A	0.3	0.1, 0.03, 0.01, control (no inoculum)	0.1
Total amount of water in the digestor (ml)	448, 289, 234, 209	600	600	800
Sampling and mixing days	0, 4, 7, 10, 13, 14, 19, 22, 25, 33, 34	0, 2, 5, 9, 14, 21	0, 3, 6, 10, 13, 17, 21, 25, 29, 33, 37, 42	5, 10, 15, 20, 25 (Integration batch 1); 0, 1, 2, 3, 4, 5, 6, 7, 10, 13, 16, 19, 21 (Integration batch 2).
Digestion duration (day)	34	21	42	25 (Integration batch 1), 21 (Integration batch 2).
Replication	2	2	3	N/A
Total number of digestion bottles	8	8	12	8**

*w/w/v is in unit of g/g/ml.

** Identical bottles used to generate sufficient digestion liquor for the separation experiment.

For the 1st batch (P1b1), different amounts of wet poplar wood pretreated using the Parr bioreactor were mixed with 0.2 L water (fixed amount) and 0.02 L of liquid inoculum (fixed

amount) in order to test the effect of solid loading rates (g dry biomass (DM)/L) on acid concentration and yield. Solid loading rates used in this batch were 112 g/L, 69 g/L, 34 g/L and 10g/L (Table 3-1). Each level of solid loading was digested in duplicate reactors. The liquid inoculum was not analyzed, thus the inoculum loading rate on a dry matter basis was not available.

For the 2nd batch (P1b2), four lower solid loading rates were conducted with ASE pretreated poplar wood. Solid loading rates used in this batch were 25 g/L, 10 g/L, 5 g/L and 2.5 g/L (Table 3-1). Each level of solid loading was digested in duplicate. The total amount of water in each digestor was fixed during the initial reactor loading, while the dry biomass weight for each digestion was obtained by multiplying the total amount of water with specific solid loading rate. Wet biomass quantity was calculated by dividing dry biomass weight by the solid decimal fraction. Liquid inoculum was added using a ratio of 10 ml inoculum: 1 g DM, which was equivalent to 1 g VS inoculum for each 3 g VS biomass. After the wet biomass and liquid inoculum quantities were determined, the additional water required was calculated using the total water amount fixed in the beginning minus the water from biomass and liquid inoculum.

For the 3rd batch (P1b3), in order to test the minimal amount of solid inoculum needed for digestion, digestion was conducted with a fixed solids loading rate of 10 g DM/L and three different inoculum loading rates (g VS inoculum/g VS biomass) using switchgrass pretreated in the SuPR2G Pretreatment Reactor. Digestion was also conducted without inoculation as a control. Each level of inoculum loading used triplicate bottles for digestion. An example of the calculation process to determine wet biomass and inoculum quantity is presented in Figure 3-1. Dry biomass of feedstock was calculated as described in the 2nd batch (P1b2) experiment, and then the VS amount in the added dry biomass was calculated by multiplying dry weight by the biomass VS fraction. The inoculum VS amount needed was calculated based on the determined inoculum

loading rate, and dry inoculum needed was calculated using the inoculum VS amount divided by inoculum VS fraction. Wet inoculum requirements were calculated using dry inoculum amount divided by inoculum solids content on a decimal basis. After wet biomass and solid inoculum quantity was determined, the required water addition was calculated using the total water amount fixed in the beginning minus the water from biomass and solid inoculum.

Willow digestion used for the Phase 2 and Phase 3 experiments followed the same procedure as 3rd batch (P1b3) to determine wet biomass and solid inoculum quantity.

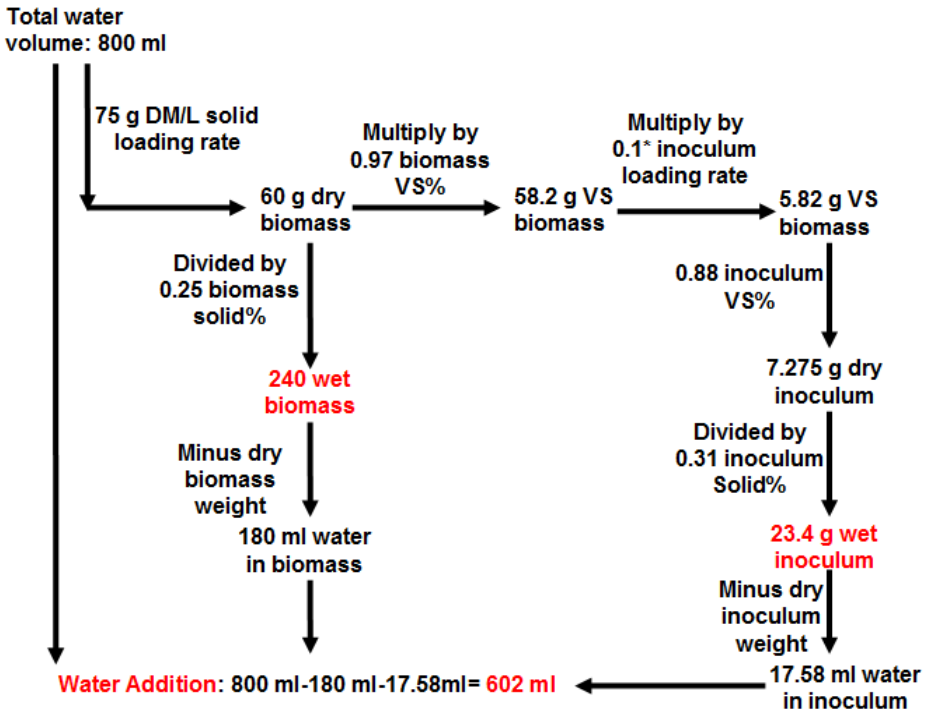


Figure 3-1. Flow chart of an example of determining wet biomass and inoculum quantity in prior to willow wood digestion. * Inoculum loading rate is in g VS inoculum/ g VS biomass. Solid% indicates dry biomass fraction, and VS% indicates volatile solids fraction on dry biomass basis.

Incubation condition and sample analysis

Each digestion was conducted as a batch, with all inoculum and substrate loading on day 0. Each reactor was a 1-L Schott bottle and was incubated in anaerobic chamber mixed with hydrogen and nitrogen gas at 25 to 30 ºC. The reactor pH was not adjusted in any batch digestion. The reactor was mixed manually every time liquid was sampled. The specific days for sampling and mixing for each batch were demonstrated in Table 3-1. Liquid samples (2 ml) were collected for acid characterization using a sampling syringe, then filtered with 0.2 µm PTEE syringe filter in prior to analysis. The carboxylic acids in the liquid were quantified on the Dionex Ion-Exchange Chromatography System ICS-3000 using an IonPac ICE-AS1 Ion exclusion guard (4×50 mm) and analytical (9×250 mm) columns for anion exchange chromatography (Dionex, ICS-3000, Sunnyvale, CA). Eluent was 100 mM methanesulfonic acid and flowed at 0.16 ml/minute at 30 ºC for 60 min during sample analysis. Carboxylic acids were detected by a UV detector at 210 nm wavelength. In the 3rd batch (P1b3), gas samples were collected for methane measurement by syringe and stored in saturated NaCl solution temporarily before methane analysis. Gas samples were analyzed using a gas chromatograph equipped with a 6-foot long molsieve-column (SRI 310C, SRI Instruments, Torrance, CA, USA) at an oven temperature of 80˚C.

Digestion was evaluated by acid yield which was calculated by

Yield = g total acids/ g dry biomass input

Phase 2 Nanofiltration Membrane Separations

Two repeated sets of sample preparation and filtration experiment were conducted to evaluate NF membrane performance on acid and sugar rejection with willow digestion liquor. The experimental variables for this trial were pH and pressure. Two commercialized NF

membranes were tested. All the centrifugation and filtration experiments were conducted in Dr. Manish Kumar's lab in the Chemical Engineering Department at Fenske Building.

Digestion liquor preparation

Digestion liquor was obtained from pretreated willow digestion at 75 g DM/L solid loading rate, with a 0.1 g VS/ g VS biomass inoculum loading rate as previously described. At day 10, digestion liquor was collected after centrifugation at 10,000 rpm for 30 min. In order to minimize nanofiltration membrane fouling, digestion liquor was filtered using vacuum filtration through Whatman No. 1 1.5µm glass fiber filter, 0.45µm cellulose acetate filter, and 0.2µm nylon membrane filter in series before nanofiltration. This series filtration process can accelerate the pre-filtration step compared to using 0.2µm filter directly. After microfiltration but before nanofiltration, the pH of digestion liquor was adjusted by 1 M NaOH and HCl solution to match the specified pH treatment levels for NF experiment.

Membrane characteristics

NF flat sheet membranes were purchased from Eastern Reverse Osmosis Systems (Wilmington, NC). The information of the membrane provided by manufacturer is summarized in Table 3-2. Desal-DL (DL) and Desal-DK (DK) membrane are thin film composites (TFC) comprising a top active layer (polyamide), an intermediate layer and a backing layer (polysulfone) (Teella, 2011). The molecular weight cut off (MWCO) of a membrane is defined as the molecular weight of the solute that has a rejection of 90% by the membrane (Van der Bruggen et al., 1999). Both membranes have used in previous studies for carboxylic acid recovery and sugar rejection (Cho, 2012; Teella, 2011; Weng et al., 2010). Thus they were selected in this study.

Table 3-2. Characteristics of the NF membranes used in this research. All the parameters were provided by manufacturer, except for the permeability constant which was measured in this study.

Name	Desal-DL (DL)	Desal-DK (DK)
Manufacturer	GE Osmonics	GE Osmonics
Configuration	Flat sheet	Flat sheet
Filtration area (cm^3)	138	138
Molecular Weight Cutoff (MWCO, Dalton)	150-300	150-300
Water permeability constant ($L/m^2*h*bar$)	5.90	4.46
Salt rejection (%, solute)	96, $MgSO_4$	98, $MgSO_4$
Maximum pressure (bar)	41	41
pH resistance	3-9	3-9

Filtration Set-up: cross-flow system

A Sepa® CF II membrane cell system from GE Osmonics Sterlitech (Kent, WA) was used to carry out the phase 2 filtration experiments. The system is featured as a lab scale cross flow filtration unit, presented in Figure 3-2. The cell unit consists of three major parts: cell body, cell holder and a hydraulic hand pump. Membrane with an active filtration area of 138 cm^2 was installed in the cell body. A feed spacer and permeate carrier were placed below and above the membrane in the cell body. Double O-rings secured the cell body from leaking. The cell body was then placed in a cell holder and pressurized using a hydraulic hand pump to above 900 psi against the feed pressure. The filtration was conducted in full recycle mode, so that all the retentate was returned to the reactor. The feed steam was pumped from the feed tank by a high pressure pump into the cell body bottom. Part of the feed permeated the membrane and reached the cell body top, was collected by permeate carrier and exited through the permeate outlet. The other part of the feed flowed tangentially across the membrane surface as retentate, alternatively called concentrate, and was recycled back into the feed tank after passing through a chiller for temperature control. A pressure gauge located on the retentate outlet monitored the

transmembrane pressure. The retentate flow control valve on the retentate outlet could also be used to control transmembrane pressure. Permeate was collected by a small container with tubing in the bottom connected with feed tank. Once the container reached a certain level, permeate flowed back into feed tank. The permeate container was so small that it would not change the concentration in the feed tank. The permeate container was also placed on a digital balance where permeate weight was recorded and displayed on computer in real time to obtain membrane flux data. A Labview program was used to control the pump to achieve the preset transmembrane pressure, and displayed real time pressure data. Changing the retentate control valve changes the transmembrane pressure temporarily, but the pressure builds up again under the Labview program's control. Cross flow velocity (retentate flow) was not controlled and changed with pressure change caused by the pump when the control valve was not interfering.

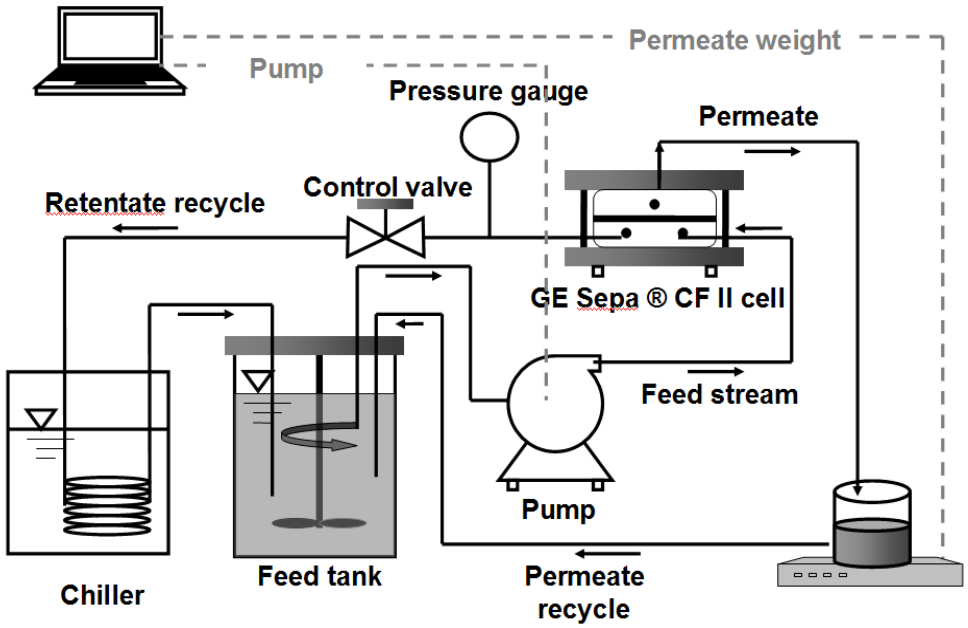


Figure 3-2. Schematic diagram of cross-flow filtration system in constant pressure operation mode.

pH and Pressure evaluations using cross-flow filtration system

Before loading with digestion liquor, the membrane was compacted with DI water at 16 bar until the membrane flux reached a stable level. The DK membrane was normally compacted for 36 h, while the compaction of the DL membrane only required 24 h. Once pressure became steady, pure water flux was measured while increasing the pressure from 8 bar to 24 bar. Each pressure level was maintained for 10 min after the pressure was stabilized. The flux was obtained by

$$J = V/At \quad (1)$$

V is the permeate volume; A is the effective filtration area 138 cm^2, which is fixed for the Sepa CF II cell; t is the time over V was collected. Permeate weight was recorded every minute automatically. The permeate solution density was assumed to be 1 g/ml and was used to convert permeate weight to permeate volume. The flux was averaged from the most recent 10 values once the flux became stable.

Table 3-3. Experimental variables tested in phase 2 experiment.

pH	Pressure
3	116 psi /8 bar
5	232 psi/ 16 bar
7	348 psi/ 24 bar

The effects of pH and pressure were tested with 6 filtration experiments using two types of NF membranes. The specific levels of pH and pressure tested were demonstrated in Table 3-3. Each filtration experiment was conducted at one solution pH and three applied pressures. Right before liquor loading, the membrane was filtered with DI water for a half hour with the same pH as the sample. The pH of the digestion liquor was adjusted before loading into the feed tank, while a 1.5 ml sample was collected as feed sample for analysis. Once the sample was loaded, the

system was run for 20 minutes at 8 bar to stabilize the temperature and pressure. Permeate was then collected by 1.5 ml and collected again after ten minutes. Then the pressure was increased to 16 bar and permeate was sampled twice at 10 min intervals after the system was stabilized for 5 min. After the sampling at pressure 24 bar, the digestion liquor was collected and the membrane was flushed with DI water for 2-3 hours before again measuring water flux with different pressures. A new membrane was used for each new pH experiment. There were six experiments in total for one complete set of treatments including the two NF membranes at three pH levels, with three pressures tested in each experiment. Another replicate of this trial was carried out with new replicated digestion material.

Synthetic model solution filtration

In addition to the filtration experiments using actual digestion liquor, one filtration experiment was conducted on the cross-flow filtration system using a synthetic model solution simulating the composition in actual liquor, but without the many other compounds found in actual digestion liquor that might interfere with separation. The filtration procedure was the same as when using actual digestion liquor as was described previously. The composition of the synthetic solution is specified in Table 3-4. This mixed acid solution was used as feed solution without pH adjustment, with the initial pH at 2.4. This experiment can provide information about hexanoic acid retention under conditions without the interference from digestion liquor complex, which had not previously been investigated, and can be used to compare to the acid rejection using actual digestion liquor.

Table 3-4. The acid concentrations (mg/L) in synthetic model solution and actual digestion liquor.

	Lactic	Formic	Acetic	Propionic	Butyric	Hexanoic
Synthetic model solution	1212	92	1055	678	1324	1482
Actual digestion liquor*	1335	157	828	1309	1032	1960

*The average concentration in feed solutions from six filtration experiments using Willow 1 as feedstock. The feed solution was from one batch digestion.

Membrane performance

Solute rejection was used to evaluate the NF membrane performance. The rejection was calculated by Eq. (1), defined as the ratio of concentration difference across the membrane divided by the bulk concentration in feed (Geankoplis, 2003). C_f and C_p represent the concentration of feed and permeate respectively.

$$R\ \% = (1- C_p / C_f) \times 100\% \quad (2)$$

The value *R%* ranges from 0% (complete permeation of solute, C_p equal to C_f) and 100% (complete rejection of solute, C_p equal to zero).

Sugar and acids in all the feed and permeate liquid samples were quantified using ion chromatography as previously described. Details on acid quantification were the same as used in Phase 1. Sugar measurement was carried out using CarboPac PA 20 guard (3×30 nm) and analytical (3×150 nm) columns. Eluent was 2 mM NaOH running at 0.5 ml/ minute at temperature 30 ºC for 30 minutes during sample analysis. Sugars were detected by pulsed amperometry with a gold working electrode.

Zeta potential measurement

Surface charge of the NF membrane has a significant influence on solute transport. To quantify this aspect of the treatment, zeta potential was measured at various pH values. A SurPASS® (Anton Paar GmbH, Graz, Austria) electrokinetic analyzer was used to measure the

streaming current as a function of pH. A 0.1 M KCl solution was used as the background electrolyte and HCl (0.1 M) and NaOH (0.1 M) were used to adjust the pH. The streaming current data was recorded at an applied pressure ranging from 0 to 300 mbar. The Smoluchowski equation was employed to calculate the zeta potential of the membrane. The detailed method was described by Xie et al. (2011).

Phase 3 Integration of digestion with membrane separation

This phase of the investigation evaluated bench-scale integration of willow digestion with NF membrane separation. A dead-end filtration set-up with low applied pressure was used for permeating acid and rejecting sugars. Two batches of integrated digestion (P3b1 and P3b2) were carried out with different acid removal frequencies, while the effect of acid removal on digestion was evaluated by comparing to a control digestion without acid removal.

Filtration Set-up: Dead-end filtration

An Amicon® Stirred Cell Model 8200 with 200 ml feed volume was used for NF separation. The filtration is a batch system where the feed sample was loaded in the cell at the beginning, and the permeate was collected without recycle. With this setup the feed volume, which was also the retentate volume, was reduced. The filtration cell was connected with a nitrogen gas tank as pressure supply and the maximum pressure capacity was 75 psi. Magnetic stirring was used to minimize concentration polarization. A reservoir was connected to the cell in order to process more than 200 ml liquid.

Integrated digestion

Figure 3-3 illustrates the integrated digestion system. Willow wood particles are insoluble and largely settlable solids, so that the digestion liquor can be fractioned into solid and liquid phases simply by gravity. After settling, the top layer liquid fraction, which was 250 ml to 300 ml and about 26% to 30% of the total digestion liquor was preprocessed prior to nanofiltration using the centrifugation and microfiltration steps previously described for Phase 2. After preprocessing the total feed weight was recorded. Prior to the liquid transfer, a small amount of feed liquor was sampled from the digestor for feed stream analysis. The cumulative volume removed for sampling was less than 2.7% of the total digestor volume and the resulting total volume reduction from the digestor was considering during the calculation of acid yield.

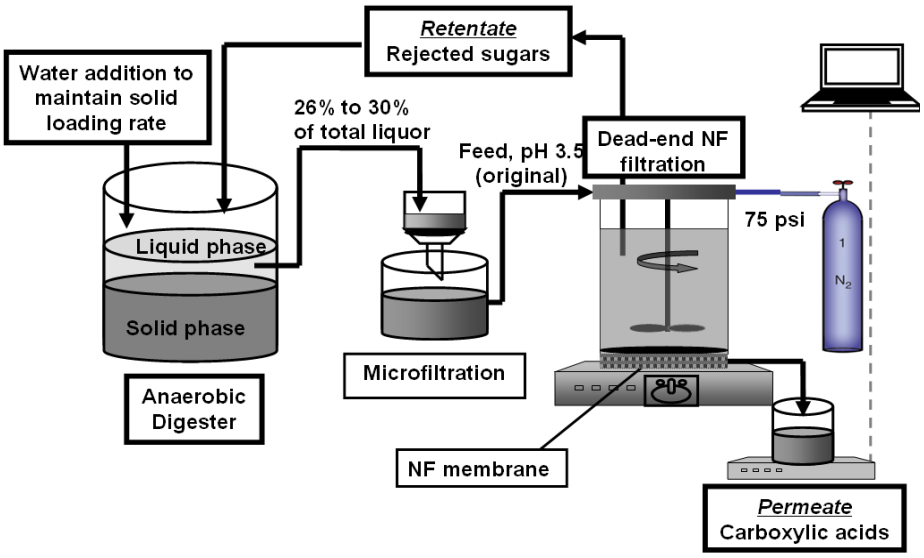


Figure 3-3. Flow chart of Phase 3 integrated digestion and separation.

The membrane was flushed with DI water before the filtration experiment, and filtration was conducted with original digestion liquor pH (3.5), 75 psi and at room temperature. Permeate

weight was also automatically collected by a digital balance connected to a computer. At the end of each filtration cycle, small fractions of retentate and permeate were each sampled for analysis, and their total weights were recorded.

Retentate was recycled back to digestor with the addition of make-up water. The amount of make-up water was calculated by the difference between the weight of feed collected initially and the weight of retentate recycled back. Then the digestor was mixed manually and sampled again. For batch one (P3b1), acid removal was carried out every five day. For batch two (P3b2), acid was removed every day starting from day 2 until day 7, and then every three days until day 21. The pH of the digestor was also measured in batch two (P3b2). The control digestion also experienced liquid phase removal on the same schedule as the integrated system, and that liquid was placed back into the control digestor when the retentate was recycled back into the integrated digestion to control for any effect on digestion due to the temporary solids loading rate increase.

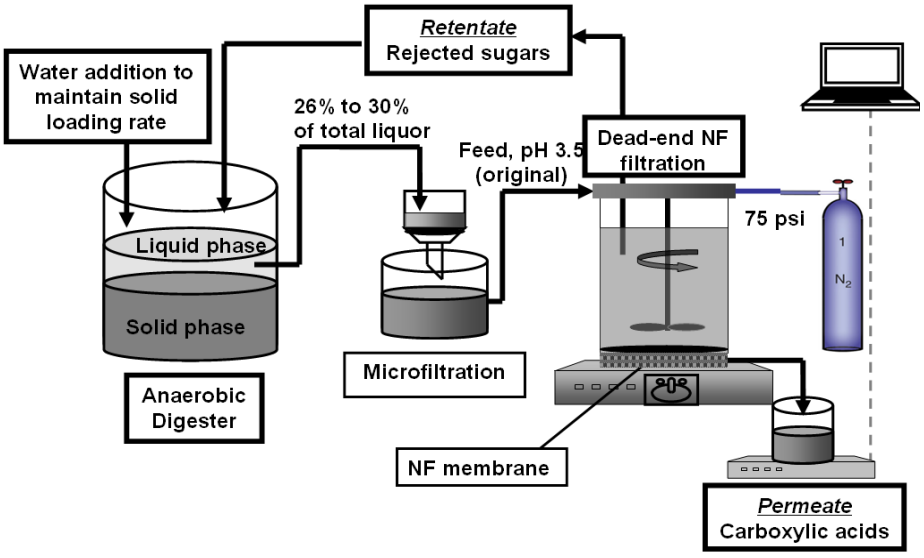


Figure 3-4. Flow chart of Phase 3 integrated digestion and separation.

Evaluation of integrated digestion

Five Phase 3 filtration experiments were conducted in one batch (P3b1) and used to evaluate dead-end filtration regarding membrane flux, the change of acid and sugar concentration in permeate, membrane fouling, acid mass recovery and sugar loss.

Acid mass recovery% and sugar mass loss% were calculated by

(Acid concentration in the permeate × permeate volume)/ (Acid concentration in the digestion prior to acid removal × total volume in the digestor) × 100%

(Sugar concentration in the permeate × permeate volume)/ (Sugar concentration in the digestion prior to acid removal × total volume in the digestor) × 100%

The average acid mass recovery and sugar mass loss of the five filtration experiments are reported. The total acid produced in the integrated digestion – separation system was the sum of the acid mass in all permeate collected and the acid mass in the digestor in the end of digestion. The small amount of acid collected during sampling was also accounted for in the total acid yield calculation.

Chapter 4

Results and Discussion

This chapter starts with the discussion of the influence of solid loading rate and inoculum loading rate on acid yield and concentration (Phase 1). Next, the results of NF membrane performance on separating acid from sugars in willow digestion liquor are described, and the effects of pH and pressure on membrane flux and solute rejection are discussed (Phase 2). The last part describes the acid removal capacity of the integrated digestion-separation system (Phase 3), and also compares the acid yield of the integrated system to a control digestion without acid removal.

Phase 1: Acidogenic digestion

Phase 1 results discuss the effects of solid loading rate and inoculum loading rate on acid yield and concentration.

Solid loading rate

Acid concentration increased with increased solid loading rate for both ranges of solid loading rate (Figure 4-1). There was no statistically significant differences within the four yields of 2.5 to 25 g DM/L solid loading rate (P value=0.570 at α =0.05). But from 10 g DM/L to 69 g DM/L, acid yield was decreased significantly from 0.14 to 0.04 g acid/ g dry biomass fed, and remained almost constant when solid loading rate was further increased to 112 g/L. The yields of 10, 34 and 69 g DM/L solid loading rate were also statistically different (P value=0.000 at α =0.05). Based on the Tukey's test results, the yields of 69 and 112 g DM/L solid loading rate were statistically the same.

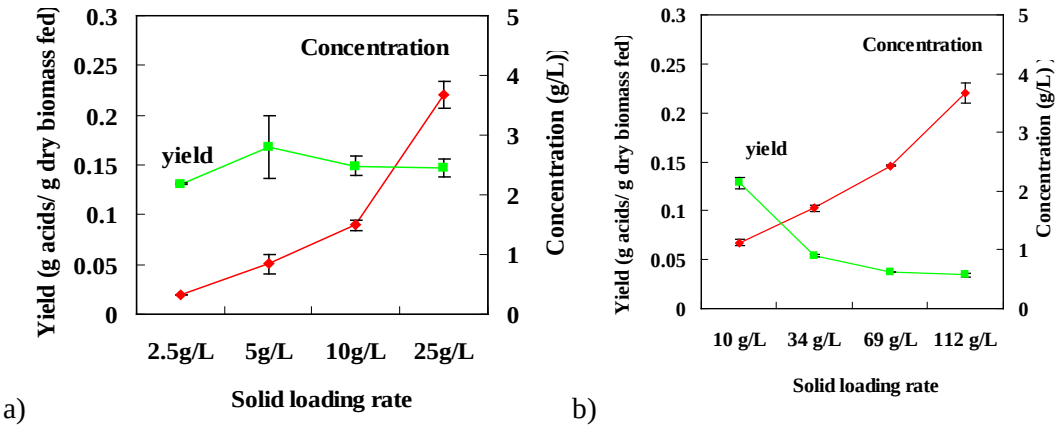


Figure 4-1. Total acid yield and concentration for two different ranges of solid loading rates at day 10. a). Low range: 2.5g/L to 25 g/L; b). high range: 10g/L to 112 g/L. Solid loading rate unit indicated g dry biomass/L.

This relationship was consistent with what has been reported before (Datta, 1981; Ross and Holtzapple, 2001). In acidogenic digestion with municipal solid waste, yield was decreased from 0.38 to 0.23 g acid/ g VS fed when solid loading rate was increased from 35 to 68 g VS/L, but the yield was only reduced from 0.23 to 0.21 g acid/ g VS fed when increasing solid loading from 68 to 88 g VS/L (Ross and Holtzapple, 2001). Ross and Holtzapple also reported a yield decrease less than 0.01 g acid/ g VS fed when solid loading of manure increased from 75 to 105 g VS/L. There are two hypotheses explaining this relationship of increased solid loading rate with decreased acid yield. The first hypothesis, which is the most common one, is that the elevated acid concentration had an inhibitory effect on microbial growth and acid production (Cheung et al., 2010; Ross and Holtzapple, 2001). The second hypothesis is that, at low solid loading rate, fewer nutrients and less of the easily digestible biomass is available to the microbes so they utilize more of the recalcitrant biomass than they do under high solids loading. Under low solids loading and thus substrate limited conditions, microbes utilize not only the easily digestible fraction of biomass but also the less available, more recalcitrant fraction of biomass. By utilizing

more of the biomass, microbes can produce more carboxylic acid per unit of biomass. In contrast, at a high solids loading rate, easily digestible biomass is abundant and available. The microorganisms only digest the easily accessible fraction of biomass and utilize part of the food to produce energy storage compounds in preparation for expected future starvation periods (Aiello-Mazzarri et al., 2006).

The acid inhibition challenge identified in the first hypothesis can potentially be solved by removing acids from the system. Based on the results reported here, no significant further inhibition was observed when increasing solids loading from 69 g DM/L to 112 g DM/L. A solids loading rate of 75 g DM/L was selected for further study to represent a high solids loading rate with high acid strength and a potentially inhibitory environment.

Inoculum loading rate and methane generation

Figure 4-2 shows acid concentration over time for digestion with different inoculum loading rates. A 10 g DM/L solid loading rate was used in this experiment to minimize the potential for acid inhibition to influence the effect of inoculum rate. From day 10 to day 25 the highest inoculum loading had the highest acid concentrations, but during other periods there was a smaller difference of acid concentrations between the different inoculum loading rates. By the end of the trial the control without inoculation had similar acid concentrations as the treatments with inoculation.

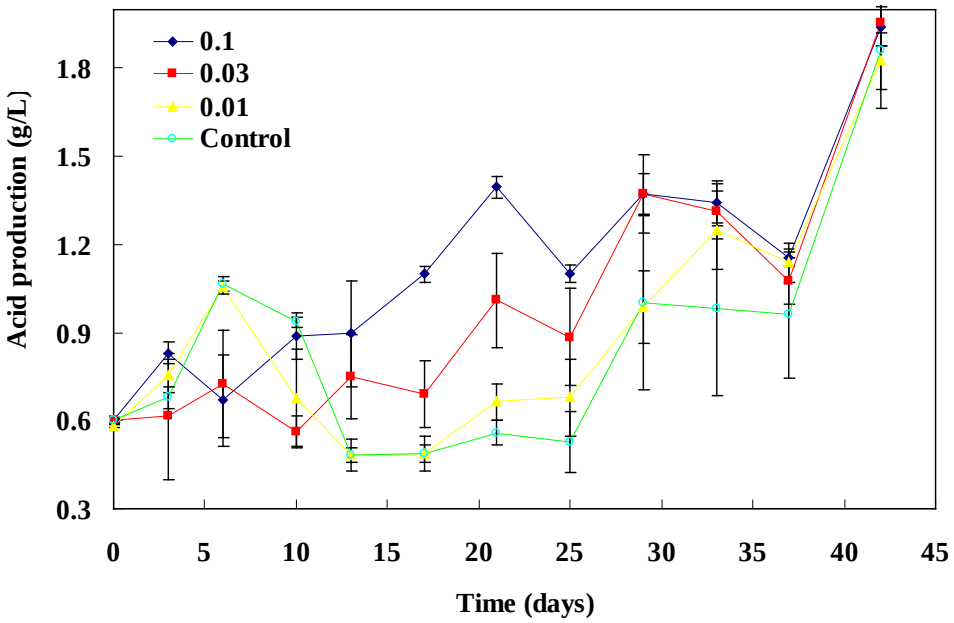


Figure 4-2. Acid concentration with different inoculum loading rates (g VS inoculum/ g VS biomass). The control was acidogenic digestion without inoculation. All trials used a 10 g DM/L solids loading rate. Error bars are the standard error of triplicate experiments.

It is important to note that this control was not sterilized. Switchgrass was frozen right after high temperature pretreatment, but it was still inevitable that ambient bacteria in the lab would be mixed into the digestor during preparation. Also, the concentration did not exceed 1.5 g/L acid until the very end of the 42 days of digestion and even then was less than 2 g/L. Since the system was so dilute, substrate for acid production was limited. This might have created the small differences in acid production between the different inoculum loading rates. This argument was supported by a later willow digestion experiment at a solid loading rate of 75 g DM/L, comparing 0.1 g VS inoculum/ g VS biomass (10%) with a control treatment without inoculant. This later trial had a significantly higher acid concentrations in the treatment with inoculation (Table 4-1).

Table 4-1. Total acid concentration (g/L) in Willow 2 digestion with 75 g DM/L solids loading rate.

Days	W/O inoculum	10% inoculum
0	3.4	3.5
6	3.3	33.6
16	4.7	35.6

During the 42 day digestion trial the pH was fairly stable, dropping from 4.2 to 4.0. Methane concentration in the gas phase was below the detection limit throughout the entire digestion period. It was speculated that the naturally low pH in the system suppressed methanogenesis (van Kessel and Russell, 1996).

Phase 2: Nanofiltration Membrane Separation

Digestion liquor composition and compound properties

Carboxylic acid and monosaccharide composition

Acidogenic digestion liquor produced from a lignocellulosic biomass feedstock has a complex composition. Multi-component solutions of this sort can result in molecular interactions that change the solute rejection for NF membranes (Bargeman et al., 2005). Thus it is important to have an understanding of the carboxylic acid and monosaccharide composition in willow digestion liquor.

Figure 4-3 and Table 4-1 illustrate the carboxylic acid and sugar composition and concentration in willow digestion liquor. Willow 1 and Willow 2 produced the same acid and sugar species, but there was an obvious difference in the percentages of each acid with the two feedstocks. It is clear that Willow 2 had a higher digestibility and generally produced a higher concentration of most of the acids (Table 4-1). Willow 2 also contained 10 times higher hexanoic

acid concentrations than Willow 1 (23.6 and 2.1 g/L respectively). Because of the hexanoic acid formed such a large fraction of the carboxylic acids products of Willow 2, other individual acids were a smaller fraction of the total acid portfolio even when their volumetric concentrations were greater than with Willow 1. The rank order of concentrations of the major carboxylic acids stayed consistent among the two feedstock treatments, with hexanoic acid> propionic acid >lactic acid> butyric acid> acetic acid> formic acid. Hexanoic acid was the largest component of the carboxylic acid profile for each feedstock, comprising 29 and 76% of total carboxylic acids for Willow 1 and Willow 2, respectively. Lactic, propionic, and butyric acid comprised the next largest fractions, in the range of 14-22% of the total carboxylic acid portfolio for Willow 1 and 6-8% in Willow 2. The acetic acid fraction of total carboxylic acids was 17% for Willow 1 and only 2% for Willow 2. Of the acids measured, formic acid had the smallest share of the total acid portfolio (< 2%) which made it the least important acid. The acid composition from willow digestion is different from the acid composition when switchgrass is used as feedstock, when valeric acid is also one of the major products (see Appendix A, Figure A-3).

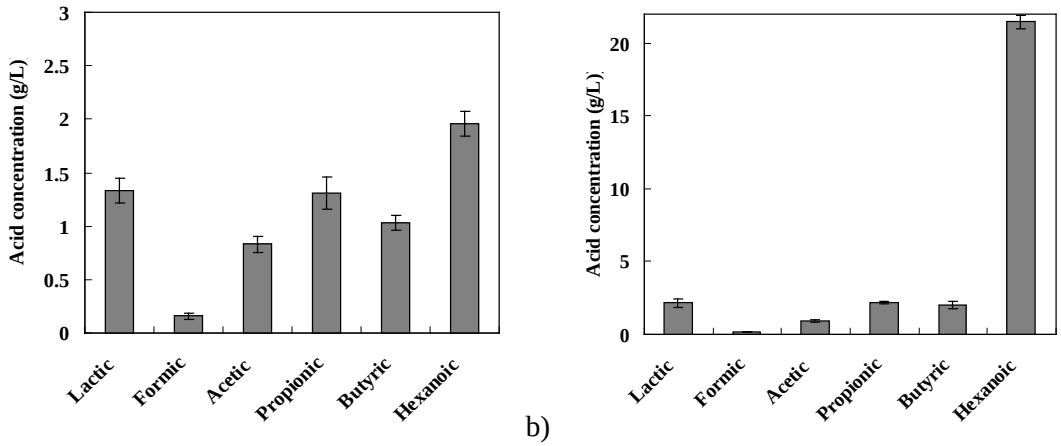


Figure 4-3. Carboxylic acid concentration in 10 day old digestion liquor of pretreated a) Willow 1 and b) Willow 2. The digestion was with 75 g DM/L solids loading rate.

The Holtzapple group has studied acidogenic digestion of a wide variety of lignocellulosic feedstocks and reported only a minor variation in acid composition. Their results generally demonstrated acetic acid (40-77%) and butyric acid (15-31%) as the two major components of the carboxylic acid profile, followed by propionic, valeric, and hexanoic acids, each making up less than 20% (Chan et al., 2011; Domke et al., 2004; Fu and Holtzapple, 2010). It is worth noticing that these previous researchers did not report production of lactic acid or a large quantity of hexanoic acid. Choi *et al.* (2008) studied acid removal by NF membrane from wastewater containing formic, acetic, propionic, succinic and citric acid, with concentrations of 50-500 mg/L. These concentrations are considerably lower than the acid strength in this study. Teella *et al.* (2011) reported acid removal by NF membrane from the aqueous fraction of fast pyrolysis oil containing 7 wt% acetic acid. Weng *et al.* (2010) utilized hydrolyzate from rice straw as a feed solution, with the hydrolyzate containing 2.5 g/L acetic acid. To the best of our knowledge, an acid composition similar to that reported in this study has not previously been tested using an NF membrane for acid removal.

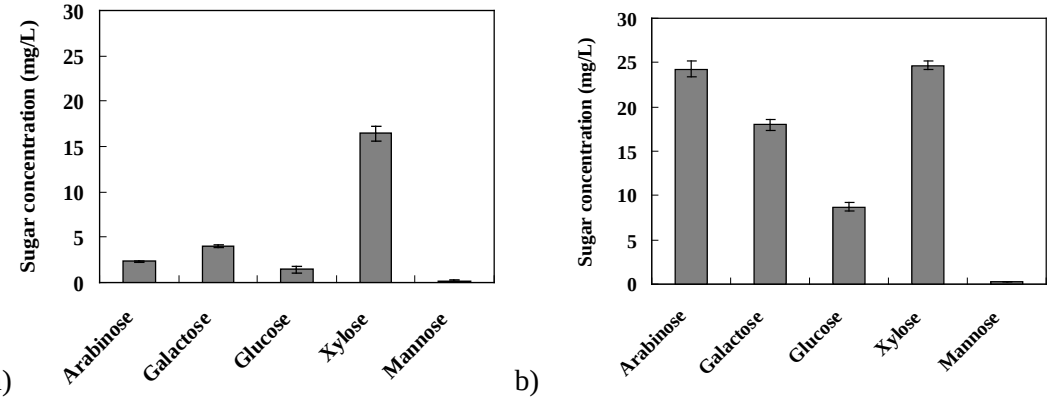


Figure 4-4. Monosaccharide concentration in 10 day old digestion liquor of pretreated a) Willow 1 and b) Willow 2. The digestion was with 75 g DM/L solids loading rate.

If all the hemicellulose and cellulose in pretreated willow were all hydrolyzed and released immediately, the digestor initially would theoretically contain 21.75 g/L glucose, 3 g/L xylose, and the rest of the sugars less than 0.7 g/L. Thus the sugar dissolved in the system at any one time represents only a small part of the total monosaccharide in the biomass. Because the sugars are released slowly through hydrolysis and then utilized by acid fermentation nearly immediately, this sugar concentration reflects both hydrolysis (increasing concentrations) and acid fermentation (reducing concentrations). The sugar concentration at any one time can also be very dynamic. In the beginning of the digestion, arabinose and glucose had concentrations of 350 and 320 mg/L respectively, which were the two highest among all the acids. Xylose and galactose concentrations were 240 and 160 mg/L respectively, and mannose had the lowest concentration (60 mg/L). After two days of digestion, arabinose and galactose concentration dropped by around 22% of its initial amount, and glucose and mannose concentration were reduced by 50 and 60% of their initial amounts respectively. Xylose concentration stayed the same in the first two days but decreased by half on the 3rd day. Other sugar concentrations also experienced dramatic decreases (61-94%) on the 3rd day. The sugar concentrations on day 10 are indicated in Table 4-1 and Figure 4-4, for both Willow 1 and Willow 2. All the sugar concentrations on day 10 were lower than 30 mg/L. Mannose concentration was lower than 1mg/L. The rank order of sugar content in the original pretreated willow is glucose 29%, xylose 4%, then galactose, arabinose and mannose which together are less than 1% of total dry biomass (Appendix A, Figure A-1). Thus, these results indicate that glucose was most consumed among the sugars hydrolyzed.

Weng *et al.* (2010) used a NF membrane to concentrate 24 g/L xylose, 3 and 5 g/L glucose and arabinose from biomass hydrolyzate after acid hydrolysis. The glucose concentration in the aqueous fraction of fast pyrolysis bio-oils reported by Teella *et al.* (2011) was 15 wt%, which was significantly higher than the sugar concentrations reported by other lignocellulosic

biomass researchers using NF membranes. The sugar concentrations in this study are lower than those that have been previously reported in the literature.

Physical properties of target compounds

The properties of solutes are crucial in determining their transport behavior through a membrane. Table 4-2 shows the key solute properties that are of interest in this study. The Stokes diameter is another molecular size parameter other than molecular weight and was calculated by Stokes-Einstein equation (Hiemenz, 1997):

$$d_s = \frac{K_B T}{3\pi D} \tag{3}$$

In this equation, d_s is the Stokes diameter, K_B is Boltzmann's constant (1.38×10^{-23} m^2 kg s^{-2} K^{-1}), T is temperature in Kelvin, μ is the coefficient of viscosity of water (0.0089 m^2 s^{-1}), D is the diffusion coefficient. All the D values displayed in Table 4-2 are at a standard temperature of 25℃ and were obtained from literature. The equation assumes that the molecule is in spherical shape. This equation indicates that the Stokes diameter is inversely related to the diffusion coefficient at any given temperature.

Both molecular weight and Stokes diameter provide information about molecular sizes which is helpful for understanding the molecule transport through NF membrane. All the sugars have molecular weights larger than 150 g/mol and Stokes diameter >0.63 nm. Since the selected NF membrane has a MWCO of around 150 Dalton according to the vendor (GE-OSMONICS), it was expected that the sugars would be highly rejected by the NF membrane. In contrast, carboxylic acids have molecular weights ranging from 46.3 (formic acid) to 116.15 g/mol (hexanoic acid), and Stokes diameters in the range of 0.31-0.62 nm. When only considering the

physical sieving effect of the membrane, the acid molecules are small enough they should permeate through the NF membrane. Hexanoic acid has only a slightly lower Stokes diameter than xylose and arabinose, although the diffusion coefficient values were obtained from different sources and thus may vary in their absolute and relative values.

Table 4-2. Solute properties in willow digestion liquor.

Components	Molecular weight (g/mol)	Stokes diameter (nm)	Diffusion Coefficient (10^{-6} cm^2s^{-1})	Dissociation constant (pK_a)	Concentration in digestion at day 10
Xylose	150.13	0.638	7.69 [d]	12.15 [c]	15.6[a], 24.7[b] mg/L
Glucose	180.15	0.725	6.76 [d]	12.28 [c]	1.4 [a], 8.7 [b] mg/L
Arabinose	150.13	0.634	7.73 [d]	12.43 [c]	2.3 [a], 24.3 [b] mg/L
Galactose	180.15	0.725	-	12.39 [c]	4.0 [a], 18.0 [b] mg/L
Mannose	180.15	0.725	-	12.08 [c]	0.2 [a], 0.2 [b] mg/L
Formic acid	46.03	0.314	15.6 [f]	3.75	0.1 [a], 0.1 [b] g/L
Acetic acid	60.05	0.389	12.6 [f]	4.75	1.2 [a], 0.7 [b] g/L
Lactic acid	90.08	0.494	9.93 [g]	3.86	1.2 [a], 2.5 [b] g/L
Propionic acid	74.08	0.485	10.1 [f]	4.88	1.6 [a], 2.4 [b] g/L
Butyric acid	88.11	0.534	9.18 [f]	4.82	1.0 [a], 2.0 [b] g/L
Hexanoic acid	116.16	0.625	7.84 [f]	4.85	2.1 [a], 23.6 [b] g/L

a: average concentration in Willow 1 digestion.
b: average concentration in Willow 2 digestion.
c: (Bhattacharyya, 2012).
e: Assume the same with glucose.
d: (Weng et al., 2010)
f: (Bidstrup and Geankoplis, 1963)
g: (Ribeiro et al., 2005)
-: not available.

As previously mentioned in the literature review, electrostatic repulsion also has a large effect on solute rejection by NF membranes. Therefore, the charge of the solute plays a crucial role in determining the actual rejection efficiency. The dissociation constant determines the solute charge state under various pH conditions. Sugars have a pKa around 12, which is much higher than the pKa of carboxylic acids which ranges from 3.7 to 4.9. Therefore the charge of the acids

will vary significantly across the pH range tested (pH 3 to pH 7) while the charge of the sugars will be nearly constant. At pH 3, most of the acetic acid was still in the undissociated form. At pH 5, around 50% of the acetic acid changed into the dissociated form, and almost 100% of the acetic acid was in the dissociated form at pH 7. In contrast, xylose stayed completely in the undissociated form below pH 10.

Pure water flux

A NF membrane, like a reverse osmosis membrane, is a diffusion-type membrane so the transport of solvent and solute are governed by a diffusion-type model (Geankoplis, 2003). Water flux increases linearly with pressure, which can be described by the Equation (4).

$$J_w = A\,(\Delta P - \Delta\pi) \tag{4}$$

In this equation J_w is the membrane flux ($Lh^{-1}m^{-2}$), A is the water permeability constant ($Lh^{-1}m^{-2}\ bar^{-1}$), ΔP and $\Delta\pi$ are the applied pressure (Bargeman et al., 2005) and osmotic pressure (Bargeman et al., 2005) differences between the feed and permeate respectively. The osmotic pressure $\Delta\pi$ is zero when the feed is pure water, thus the value of A for pure water can be obtained from the slope of pressure and flux data. In this study the water permeability constant is reported as the average of the A values from six individual membranes. The permeability constant of the DK membrane is 4.46 $Lh^{-1}m^{-2}\ bar^{-1}$ ($R^2 = 0.99$), which is significantly lower than the 5.60 $Lh^{-1}m^{-2}\ bar^{-1}$ permeability constant of the DL membrane ($R^2 = 0.99$). These values are consistent with what Tella *et al.* (2011) and Cho *et al.* (Cho, 2012) have reported in their study. Because higher flux means less time required for the separation process, the DL membrane has an advantage over the DK membrane when only considering this aspect.

Effect of pH on solutes retention and sample flux

Since NF membranes have charged groups on the active surface layer, pH plays a very important role in solute transport in these membranes, especially for ionic species (Cho, 2012). Each filtration experiment was conducted with one pH under various pressures, and a new membrane and new digestion liquor were used every time the pH was changed.

Solute rejection

Carboxylic acid rejection

Hexanoic and propionic acid are the first two most common acids. From these results, NF membranes can achieve hexanoic acid rejection of 4-18% of for the DK and 6-11% for the DL membranes, and propionic acid rejection of 22-40% for DK and 31-34% for DL using actual digestion liquor (Figure 4-5). Figure 4-6 shows the solute rejection using a synthetic acid solution at an initial pH of 2.4. Compared to the hexanoic acid rejection using the synthetic solution, the rejection using actual digestion liquor is slightly higher. The propionic acid rejection using digestion liquor was about three times higher than that for the synthetic solution. This level of propionic acid rejection using digestion liquor is consistent with a study by Choi *et al.* (2008) using wastewater, indicating that the digestion liquor matrix increased the propionic acid rejection.

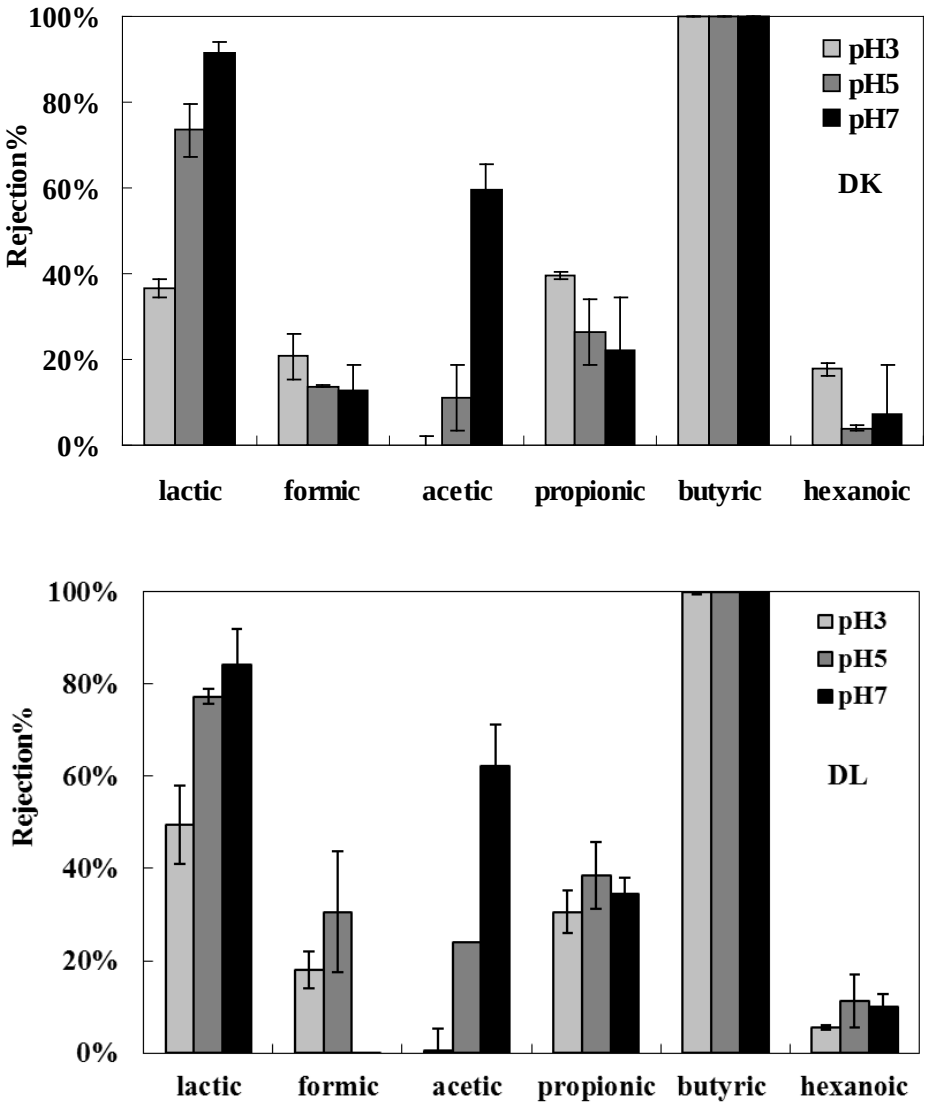


Figure 4-5. Carboxylic acid rejection at three different pH values, a pressure of 8 bar, and a temperature of 25 °C using two NF membranes, DK and DL. Error bars of carboxylic acid rejection indicate standard error for duplicate sets of filtration experiments.

Both propionic and hexanoic data indicated the rejection of those acids was not largely affected by pH, with an ANOVA analysis giving P values of 0.094 and 0.277 for these two acids respectively (α =0.05). According to the Figure 4-5, the rejection for propionic and hexanoic acids did not follow a consistent trend with respect to pH. This suggests that electrostatic

repulsion did not play a role on the rejection of these two acids. However, given the large variations in the data these results are not conclusive.

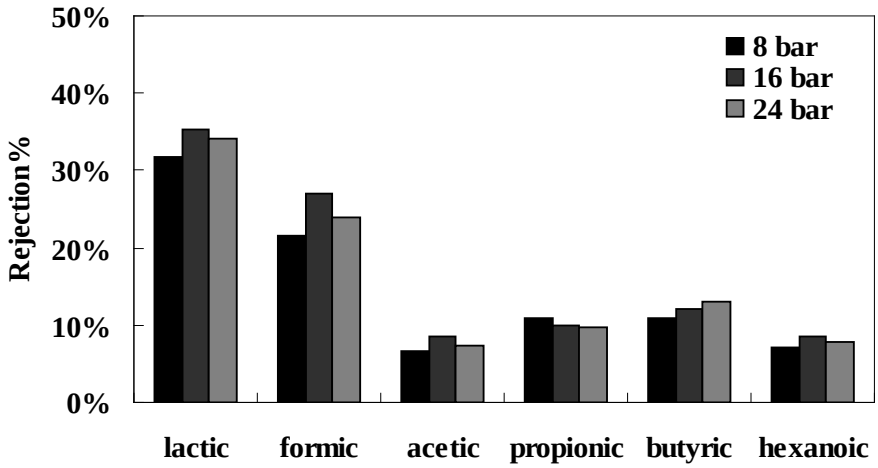


Figure 4-6. Carboxylic acid rejection using a synthetic model solution with initial pH 2.4, at three different pressures, and a temperature of 25 °C, using DL membrane. Data was from one set of filtration experiment.

Butyric acid is the 4th most prevalent acid in the acidogenic digestion liquor, and has a molecular weight 88.11 g/mol. This is less than the MWCO of NF membrane and has pK_a 4.82, so was expected to have low rejection at low pH. Cho et al. (Cho, 2012) also studied butyric acid permeation with a pure butyric acid solution at less than 5 g/L, reporting around 5% rejection. However, in the present trial the concentration of butyric acid in the permeate was under the detection limit, indicating almost 100% rejection even under pH 3. To explore this effect, a pure 2 g/L butyric acid solution was filtered through the DL membrane at original pH 3.24 under 8 bar, giving only 6% rejection. This was interpreted to mean that the DL membrane is able to permeate butyric acid efficiently at this given condition when there are no interfering compounds. Intermolecular interactions between organic acids increasing acid rejection was reported before (Laufenberg et al., 1996), but the 11% butyric acid rejection in a synthetic model solution (Figure

4-6) indicated that the presence of other carboxylic acids was not the primary cause of butyric acid rejection. There is currently no scientific consensus on a mechanism to understand and predict solute rejection in a multi-component system containing organic acids, especially for a complex multi-component system like digestion liquor. This solution contains a wide variety of sugars, Na^{+}, Mg^{2+}, Cl^{-} and SO^{-}_{4} ions, and other undefined compounds from pretreated willow wood material that might interact with butyric acid, and it appears that this digestion liquor composition can have a significant effect on butyric acid rejection. It is hypothesized that certain compounds might have been adsorbed by the membrane and acted as another rejection layer for butyric acid. Other research has previously found that a wastewater matrix significantly reduces the estrone rejection, with no clear reason (Bellona et al., 2004). To the best of our knowledge, no previous study has reported this significant increase in butyric rejection due to the feed organic matrix.

Lactic acid and acetic acid are the 3rd and the 5th most prevalent carboxylic acids in this trial. The rejection of lactic and acetic acids dramatically increased from 37 to 91% and 0 to 60% with the increase of pH from 3-7 on DK membrane. For the DL membrane, lactic and acetic acid rejection increased from 49 to 84% and 0 to 62% respectively with the same increase of pH. Compared to the rejection results using the synthetic solution at pH 3.2, with actual digestion liquor the lactic acid rejection increased while the acetic acid rejection decreased. The presence of xylose may have reduced the acetic acid rejection, as has previously seen observed (Weng et al., 2009) although the reduction was very minor. ANOVA analysis indicates the significance of pH on these lactic and acetic acid rejection, with R squares of 71.36 and 91.35%, respectively, with a P-value 0.00 (at α =0.05) (see Appendix A, Table A-2).

The Donnan exclusion effect is known to be a dominant factor affecting solute transport behavior variation through a NF membrane as a function of pH. Bellona et al. (Bellona and

Drewes, 2005) reported that the surface charge of membrane and the degree of ionization of solute largely affect the transport of organic acids, and both factors vary by pH. Figure 4-7 shows the zeta potential of DK and DL membranes at different pH. Both DK and DL membranes have isoelectric points around 3 or 4. When pH is higher than the isoelectric point of an NF membrane, the membrane is negatively charged (Figure 4-7) resulting from deprotonation of carboxyl groups on the membrane surface. As pH rose from 5 to 7, the charge of the membrane decreased from -20 mV to -50 mV for DK and to -40 mV for DL. Meanwhile, because lactic and acetic acid have pK_as of 3.86 and 4.75 respectively (Table 4-2), so as pH increases the acid molecule transitions into its dissociated form and becomes negatively charged at high pH. At high pH, electrostatic repulsion effects occurred and the negatively charged membrane rejects negatively charged acids. On the other hand, at low pH (~3), the NF membrane is either neutral or positively charged, and acid molecules are largely protonated resulting in high percentage of the uncharged form. As a result, acid molecules pass through the membrane with either minimum electric interaction with the membrane (Childress, 2000), or possibly the slight positive charge on the membrane could attract the negative charge from small amount of dissociated acid, which could further enhance the permeation assuming the acids were not actually absorbed. Timmer *et al.* (1993) reported the mass transfer through an NF membrane of undissociated lactic acid was five times greater than that of dissociated lactic acid due to electrostatic repulsion. Various other studies concluded consistently that 2.9-3 is the optimum pH for NF to achieve low rejection of acetic acid (Cho, 2012; Maiti et al., 2012; Zhou et al., 2013a).

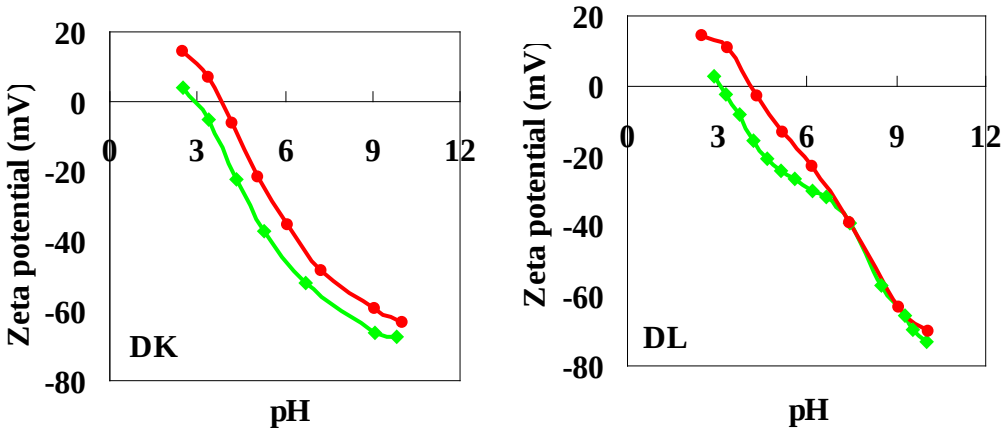


Figure 4-7. Zeta potential measurements for DK and DL membranes. Background electrolyte: 1 mM KCl. The two lines on each graph indicate measurements on two individual membranes that are the same type. Because the equipment is not able to set pH accurately to a fixed value, zeta potential of each membrane was measured at different pH points within the same range. The pH value where zeta potential reaches zero indicates the isoelectric point of the membrane. The isoelectric points for DK membranes are 2.9 and 3.8; for DL membrane these were 3.1 and 4.1 for the two membranes tested.

Among the carboxylic acid products measured in this study, formic acid is the least prevalent acid in this particular carboxylic acid portfolio. It had a rejection around 20% for both the digestion liquor and the synthetic solution. Formic acid rejection was significantly affected by pH according to ANOVA analysis, with an R squared term of 59.83% and a P value of 0.002 (α =0.05). According to Tukey's test result, the highest average rejection occurred at pH 5 and there was no significant difference between the formic acid rejection at pH 3 and 7. The low rejection at pH 3 is likely because of electrostatic repulsion, while at pH 7 there may be a more severe concentration polarization effect compared to other pH values. Choi *et al.* (2008) concluded formic acid rejection increases with a pH increase, which was explained by electrostatic repulsion. This effect is expected to be stronger for formic acid than other acids because of the small size of formic acid. However, the results from this study did not support this argument. Although there is contrary evidence, due to the high variability of formic acid rejection values the results are

inconclusive. Because of the low concentration of formic acid, the quantification was more likely to be affected by the organic matrix in the willow digestion liquor during analysis on the IC, thus generating a larger variation in the results.

Another interesting and important observation is that the order of acid rejection did not follow the order of molecular weight of the carboxylic acids. The rank order of the molecular weights of these acids is hexanoic> lactic> butyric> propionic> acetic> formic; while the order of rejection at pH 3 was butyric> lactic> propionic> formic> hexanoic> acetic. These results indicate that the sieving mechanism was not the sole or even necessarily dominant factor in determining the acid rejection by NF membrane. This discrepancy may be because the size of the acids is much smaller than the pore size of the membrane. Additionally, hexanoic, butyric and propionic have pK_as around 4.8, acetic acid's pK_a is 4.75, while formic and lactic have a pK_a around 3.7. This indicates the degree of dissociation of formic and lactic acid at pH 3 is higher than that of hexanoic, butyric and propionic acid. The acids with lower pK_as would generally have higher rejection due to Donnan exclusion than the acids with high pK_as. This can explain why formic and lactic acid can have a higher rejection than hexanoic acid even these two acids have lower molecular weight. But in general, the order of rejection also did not match the acid pK_a order either, which indicates the charge effect resulted from the dissociation of acid is not the sole player in determining the solute rejection. The mobility of the acids is not significantly different between undissociated and dissociated forms (Albery, 1967) thus the Stoke diameter does not alter during the dissociation of the acids. It is possible that dimer formation among acid molecules increased Stoke diameter thus increased the rejection (Dunn and Stokes, 1965), or perhaps there is another mechanism that explains these results. Further investigation is needed to understand the transport of individual acids through NF membranes in this multi-component system.

In conclusion, the NF membrane is able to achieve low rejection for hexanoic (15%), propionic (35%) and formic (20%) acids, none of which seem to be strongly affected by pH. Although low rejection rates for lactic acid were more difficult to achieve, a low pH condition was favorable for lactic and acetic acid permeation, achieving 40% lactic rejection and 0% acetic rejection at pH 3. The differences in acid rejection between the DK and DL membranes were significant in the rejection of lactic (P value= 0.000), acetic (P value= 0.029) and propionic (P value= 0.048), according to ANOVA analysis. The mean lactic acid rejection using DK was 75% and only was 45% with DL. For acetic and propionic acid rejection, DL had only 5% higher rejection than DK for both acids. Formic and hexanoic acid rejection did not differ significantly between the two membrane (P value =0.740 and 0.437).

Sugar Rejection

Figure 4-8 showed sugar rejection at 8 bar pressure and various pH levels using two NF membranes. All the sugars, which have molecular weight of 150 or 180 g/mol, were highly rejected by both NF membranes. Both NF membranes achieved arabinose, galactose and glucose rejections greater than 97.5%, and xylose rejection of 93%. The mannose had a concentration as low as 0.2 mg/L in the feed and was not detected in the permeate. Therefore, the mannose rejection data was not available. Because the size of the sugars was equal or greater than the nominal pore size of the DK and DL membranes, it is reasonable to assume that the sieving mechanism plays a dominant role in determining sugar rejection. This is also consistent with previously reported results using the same NF membranes (Weng et al., 2010; Zhou et al., 2013b). The lower xylose rejection relative to glucose and galactose rejection can be explained by the difference in molecule sizes, although it is unclear why the similarly sized arabinose had a higher rejection than xylose.

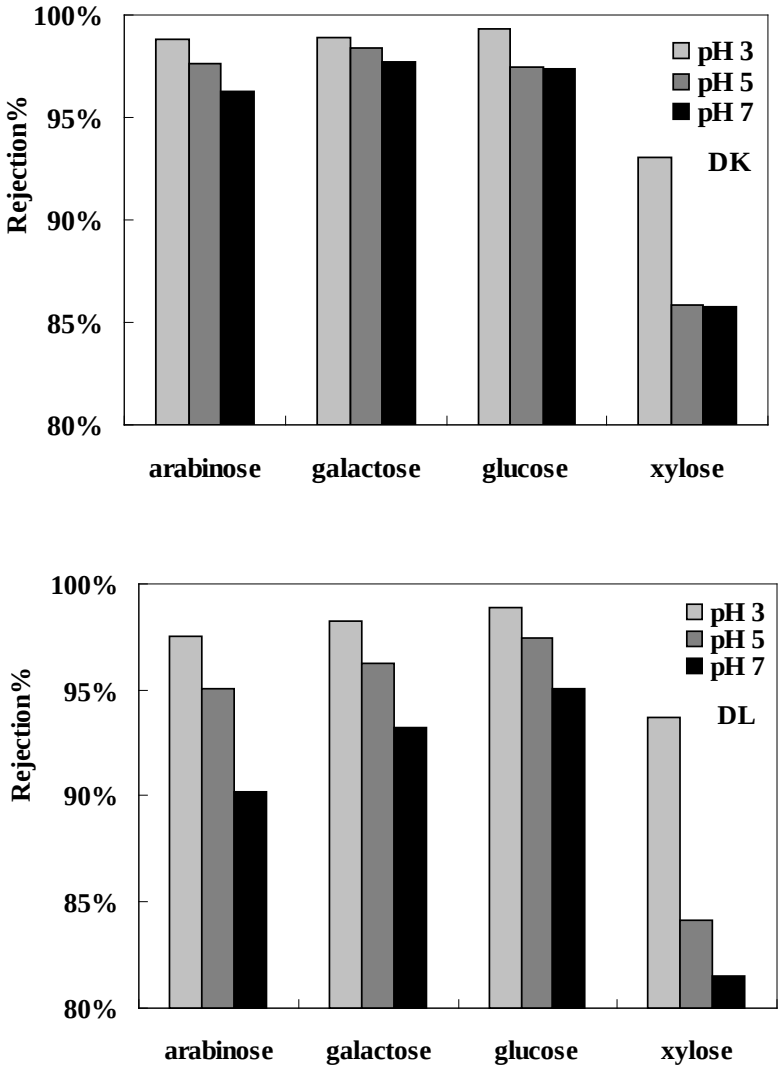


Figure 4-8. Sugar rejection at three different pH values at 8 bar, 25°C with two NF membranes. Arabinose, galactose and glucose data were from one single set of experiments. Xylose data was from duplicate experiments. Data with standard error of the xylose are presented below in the order of DK first with three pH values from pH 3 to 7, and then DL with same pH order: 93.1%±0.3%, 85.5%±3.8%, 85.8%±1.0%, 93.7%±1.7%, 84.1%±1.6%, 81.5%±10.1%,

Since sugars have a high pKa (>12) and are in the uncharged form at the tested pH range, sugar molecules should have minimum electrostatic interactions with the membrane under these experimental conditions. Sugar retention did decrease slightly with an increase of pH. For the DK

membrane, as pH increased from 3 to 7, arabinose, galactose and glucose rejection decreased from 98.8 to 96.3%, 98.9 to 97.7%, and 99.3% to 97.4% respectively. Xylose had the most obvious decrease from 93.1 to 85.5% and ANOVA analysis demonstrated the significance of pH on xylose rejection (P value =0.00 at α =0.05). DL membrane had the same trend for each sugar, except that the decrease for each acid is slightly more than the decrease occurred in DK indicating DL membrane performance was more affected by pH. Arabinose, galactose, glucose and xylose rejection decreased from 97.5 to 90.2%, 98.2 to 93.2%, and 98.9% to 95.1%, 93.7 to 81.5% respectively. The correlation between sugar rejection and pH could be explained by the change of membrane surface charge by pH. At high pH the membrane carries more charged groups in the membrane matrix that push adjacent polymers apart, resulting in membrane swelling and an increased molecular weight cut off (Braghetta et al., 1997; Mänttäri et al., 2006), which leads to a lower rejection of uncharged sugars. At low pH, the membrane is more compact causing a higher rejection. However, this small pH effect was less than with the carboxylic acids, where higher pH and associated electrostatic interactions clearly reduced the dominance of the sieving mechanism in determining rejection rates. Interestingly, at pH 7, lactic acid rejection is even higher than, and acetic acid rejection is close to, xylose rejection even if xylose is a much larger molecule than lactic and acetic acid. This comparison emphasizes the significance of electrostatic repulsion on the separation of lactic and acetic acid by NF membrane.

In conclusion, the NF membranes chosen in this study were shown to highly reject all the major sugars present in the digestion liquor. A low pH is favored for separation, since it can achieve higher rejection of sugars and lower or similar rejection of the dominant carboxylic acids compared to separation at a more neutral pH. These findings are consistent with multiple previous studies separating acids from sugars using NF membranes (Maiti et al., 2012; Weng et al., 2009; Zhou et al., 2013a).

Membrane flux

In addition to rejection and permeation of solutes, the mass flux of the solution through the membrane is critical for evaluating membrane performance and designing separation systems. For the purposes of this study, sample flux is defined as the membrane flux during separation of digestion liquor. Figure 4-9 indicates the sample flux at various pH and pressure values. Not surprisingly, the sample flux consistently increased with increasing pressure. For the DK membrane, the sample flux was greatest at pH 5, with a descending order of pH 5> pH 3> pH 7. For the DL membrane, the sample flux order descended from pH 7> pH 3> pH 5. However these differences in sample flux as a function of pH were not as significant as the flux increases due to pressure, and the data had large error bars. Thus it is not apparent from these results that pH has a significant effect on sample flux. It has previously been reported that the permeate flux increased at high pH due to the enhanced pore size that results from enhanced electrostatic repulsion (Braghetta et al., 1997). However, other researchers found that the maximum permeate flux occurred at the isoelectric point, which is at a lower pH, with decreased electrostatic repulsion increasing pore size in some membrane systems (Childress, 2000). Additionally, some researchers also reported the flux will increase with pH only when salt is present in the organic solution (Freger et al., 2000). Further research is needed to establish a comprehensive understanding of the effect of pH on NF membrane flux, especially in complex mixed solute systems.

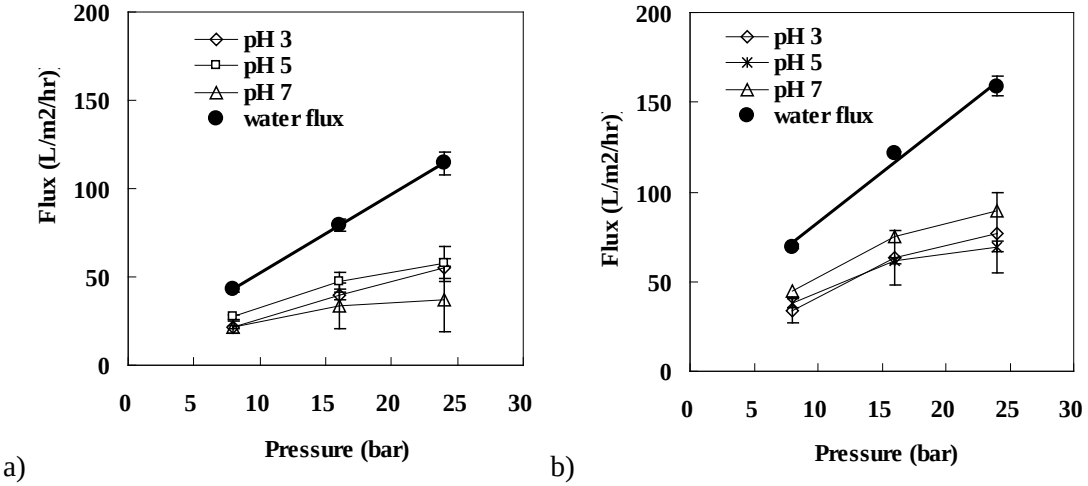


Figure 4-9. Sample flux is reported as a function of applied pressure. The control is pure water flux without pH adjustment, while digestion liquor sample flux was tested at three different pH values, all at 25°C. a) DK membrane, b) DL membrane. Error bars for the sample flux indicate the standard error for duplicate filtration experiments. Error bars of pure water flux indicate the standard error for six replicate experiments.

Effect of Pressure on membrane flux and solute rejection

NF membrane filtration is a pressure-driven process, so it is important to understand the effect of applied pressure on solute rejection and membrane flux. In this study pressure was raised during each experiment sequentially from 8 to 24 bar while using the same membrane, with the exact same feed solution, and holding pH constant.

Membrane flux

Figure 4-9 shows that the flux increased with increasing pressure, with all digestion liquor sample flux rates lower than the pure water flux under the same condition. For example, for the DK and DL membranes at 16 bar and pH 5, the observed sample flux was 47 and 62 $Lh^{-1}m^{-2}$, compared with the pure water flux of 79 and 121 $Lh^{-1}m^{-2}$ for the two membranes respectively. Since there are complex compounds in the digestion liquor generating osmotic

pressure across the membrane, the membrane flux is expected to decrease according to Equation 4. The linearity of the pressure and sample flux is less consistent than for the pure water flux. This likely results from membrane fouling and concentration polarization (CP) lowering the sample flux at higher pressure conditions. With concentration polarization, a concentrated layer of solutes is formed at the membrane surface, increasing $\Delta\pi$ and resulting in decreased flux. This effect was observed to be more serious under higher pressure, affecting the flux more than at lower pressure. This may be partly due to an artifact of the experimental design. Because the different pressure experiments for each pH were done in order from low pressure to high pressure sequentially on the same membrane, the longer the filtration experiment, the more fouling and CP effect will occur. However, it is also easier to create a CP layer on membranes at high pressures than at lower pressures.

In conclusion, the sample flux increased with increased pressure, but this increase with pressure was not as linear as the increase when filtering pure water. It is worth reiterating that the difference in flux due to pH was much less than the difference in flux due to pressure for the pH and pressure ranges tested in this study. Membrane flux increased by 30 and 45 $Lh^{-1}m^{-2}$ for DK and DL respectively when pressure is increased from 8 to 24 bar. But flux only increased by 6 and 11 $Lh^{-1}m^{-2}$ respectively for pH changes from 5 to 3 for DK and 7 to 3 for DL. Pressure thus appears to be a more important influence than pH on membrane flux in acidogenic digestion liquor separations.

Solute rejection

In addition to its impact on membrane flux, pressure can also have impacts on sugar and acid retention. At the tested condition of pH 3 and 25°C, the retention of all the measured sugars and acids rose as feed pressure was increased from 8 to 24 bar (Figures 4-10 and 4-11). Previous

research indicated that the incremental increases of solute rejection are significant from 5 bar to 20 bar, but above 20 bar solute rejection reaches plateau (Zhou et al., 2013a, b). ANOVA analysis indicated that pressure had a significant effect on rejection of xylose and all the measured acids (p values all less than 0.05). As pressure increased from 8 to 24 bars, lactic acid rejection increased the most, from 37 to 63% for the DK membrane and 49 to 70% for the DL membrane. Formic and propionic acid rejection increased by about 10 percentage points in each case. Increasing pressure caused a minimal increase in acetic and hexanoic acid rejection, reinforcing the importance of the charge effect on rejection of these two acids. Rejection of the sugars increased by less than 1 percentage point except in the case of xylose, where rejection increased from about 93 to 97% as pressure increased from 8 to 24 bars. The reason that pressure has a stronger effect on xylose rejection than the other sugar rejection is unclear, given the fact that xylose and arabinose have very similar molecular weight, Stokes diameter and pK_a (Table 4-2). The slight decrease in rejection of arabinose, galactose and glucose for the DL membrane under increasing pressure is probably due to the strong CP effect for that single experiment, where a concentrated layer of sugars formed on the surface of membrane enhanced a driving force for sugar transport through the membrane (Equation 5), leading to a decreased rejection.

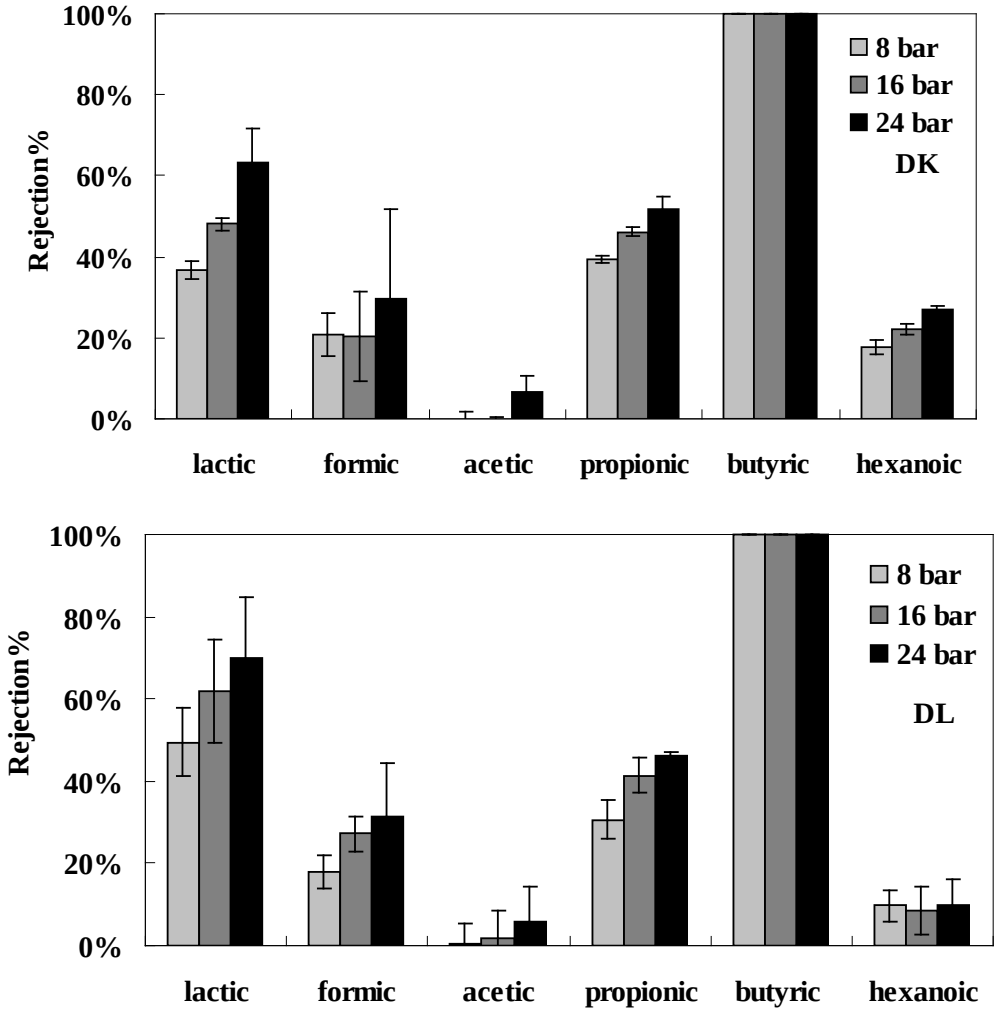


Figure 4-10. Carboxylic acid rejection at three different pressures at pH 3 and 25 °C for two NF membranes, DK and DL. Error bars for carboxylic acid rejection indicate standard error for duplicate sets of filtration experiments.

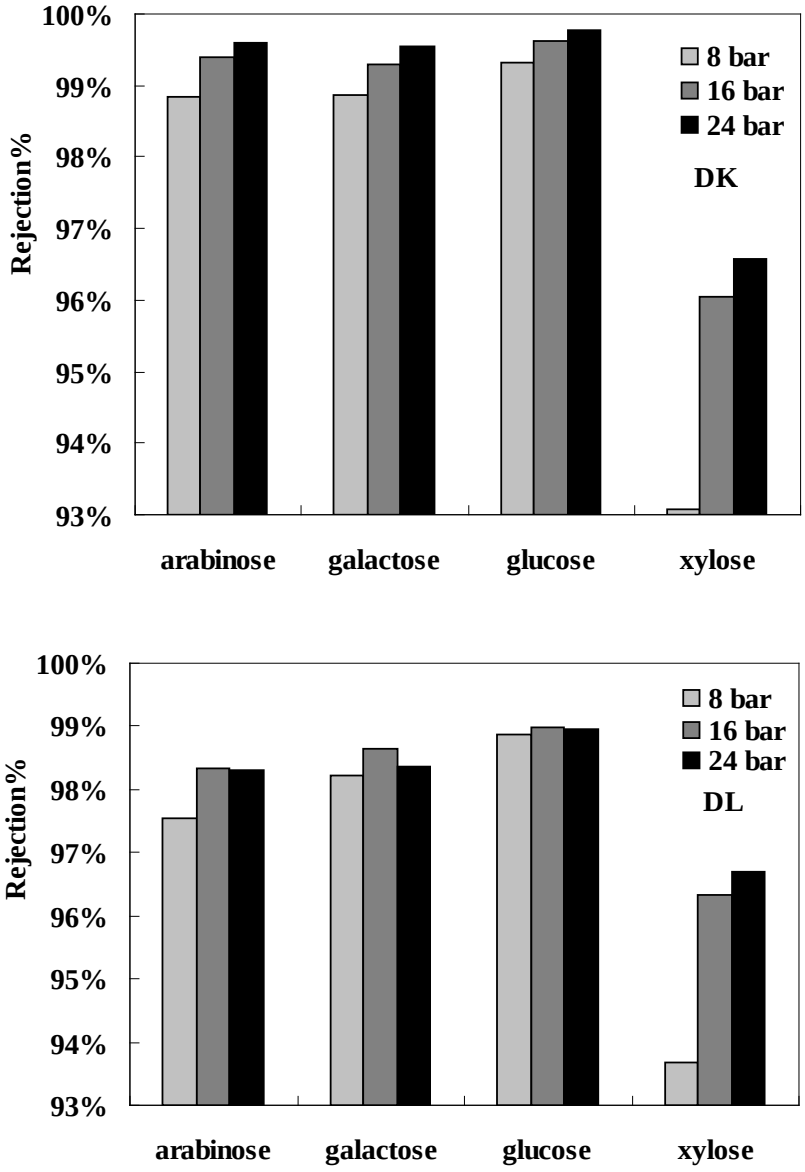


Figure 4-11. Sugar rejection at three different pressures at pH 3 and 25°C for two NF membranes, DK and DL. Arabinose, galactose and glucose data were from one single set of experiments. Xylose data was from duplicate experiments. Data with standard error of the xylose are presented below in the order of DK first with three pressures from 6 to 8 bars, and then DL with same pressure order: 93.1%±0.3%, 96.1%±0.4%, 96.6%±0.4%, 93.7%±1.8%, 96.3%±1.7%, 96.7±2.0%.

One way of explaining this pressure effect is by comparing the driving forces for solvent (water) and solute transport. Water flux is driven by the applied pressure and reduced by osmotic

pressure according to Equation (4). Equation (5) describes the diffusion of the solute through the membrane.

$$J_s = B(C_f - C_p) \quad (5)$$

In equation (5) J_w is the solute flux ($Lh^{-1}m^{-2}$), and B is the solute permeability constant ($Lh^{-1}m^{-2}\ bar^{-1}$), while C_f and C_p are solute concentrations in the feed and permeate respectively. As equation 5 illustrates, solute flux is governed by the difference between solute concentrations in the feed and the permeate, and is independent of the applied pressure. While increased pressure enhances the water flux, solute transport remains at a relatively constant rate. Hence with increased pressure more solvent (in this case water) passes through the membrane and arrives in permeate than occurs for the solute, resulting in a higher rejection of the solute (Cho, 2012; Geankoplis, 2003). Another way to interpret this result is by a solution-diffusion mechanism. As pressure increases, water has a stronger mass transfer to the membrane than the solute molecules, thus more water passes through the membrane and dilutes the permeate, resulting in a higher rejection of solute as observed (Zhou et al., 2013a).

The increased rejection of sugars due to pressure is much less than the increased rejection of acids because the sugars are bigger molecules than acids. Although high rejection of sugar is favored for the purpose of this study, so is high permeation of the acids. At higher pressures it appears that the benefit from increased sugar rejection is less than benefit from decreased acid rejection, although this would need to be confirmed with techno-economic analysis of a detailed system design. In this context, a lower pressure is likely to be preferred for carboxylic acid separation by NF membranes.

The rejection of xylose from the DK and DL membranes does not vary significantly according to the ANOVA results, which gave an adjusted R-squared value of 46.85% with p-value = 0.301 (at α =0.05). The DK membrane consistently had a slightly higher rejection of

arabinose, glucose and galactose than the DL membrane, according to Figure 4-11. However, the rejection increase between the two membranes is small, and it would not be appropriate to select one or the other of the membranes on the bases of such small and uncertain differences.

Membrane fouling evaluation of cross-flow filtration

Each filtration experiment on the cross-flow system using digestion liquor took at least 75 minutes, and was then followed by 2-3 hour DI water wash. Table 4-3 presented the water permeability constant of the virgin membrane and the membrane after each experiment plus wash. The water permeability constant after the filtration experiments had a very minimum change compared to the virgin membrane, indicating the cleaning procedure adopted in the study with DI water wash was effective to recover the flux, and that no severe fouling happened during the filtration experiment.

Table 4-3. Water permeability constant of virgin membrane and membrane washed for two hours after sample run. Membrane was filtered on cross-flow system for at least 75 minutes.

	DK: water permeability constant ($Lh^{-1}m^{-2}bar^{-1}$)					
Experiment #	1	2	3	4	5	6
Virgin membrane	5.42	4.22	3.39	3.86	5.39	4.48
After sample run membrane	4.26	3.56	3.50	3.51	4.82	4.52

	DL: water permeability constant ($Lh^{-1}m^{-2}bar^{-1}$)					
Experiment #	1	2	3	4	5	6
Virgin membrane	5.61	4.05	5.01	6.40	6.28	6.27
After sample run membrane	4.47	4.14	4.81	5.92	6.36	6.79

Phase 3: Integration of acidogenic digestion with nanofiltration

In this phase the acidogenic digestion process was coupled with nanofiltration to simulate *in-situ* acid removal. Acidogenic digestion of pretreated willow biomass generated a digestion liquor that was treated for acid removal by dead-end filtration with a DL membrane. Permeate was collected as the acid product while retentate, which was also the concentrated feed, was recycled back into the bioreactor. After each filtration event with acid product removal, water was added to maintain constant liquid volume so as not to increase the volumetric solids loading rate. During Batch 1 (P3b2), acid was removed by NF every five days. For Batch 2 (P3b2), acid was removed by NF every day from day 2 to day 7, followed by acid removal every third day until the end of the experiment. The DL membrane was selected because of its significantly higher permeability than the DK membrane, although the DL membrane did not have an obvious advantage in acid removal over DK membrane. Given the fact that low pH achieved high rejection of sugars and low rejection of lactic and acetic acid, filtration was conducted at the original pH of the digestion liquor, which was pH 3.5. Low pressure is favored for low rejection of acids, so the dead-end filtration was conducted at 5 bar in order to further maximize acid recovery in the permeate. Batch one (P3b1) included four filtration experiments, and was used to evaluate dead-end filtration performance metrics including permeate flux, solute rejection and acid recovery. Later, data from both Batch 1 and Batch 2 data (P3b1 and P3b2) was used to demonstrate the ability of this experimental integrated acid removal set-up to reduce acid concentrations in digestion liquor. Total acid yield in this integrated system was compared to that of a control digestion without acid removal.

Water flux and permeate flux

The water permeability constant of DL was calculated using pressure –flux data obtained by changing the pressure from 1.7 to 3.4 to 5 bars. Under these pressure conditions *A* varied from 5.8 to 7.8 $Lh^{-1}m^{-2}bar^{-1}$ (Table 4-6), which was higher than what was observed on the cross-flow system. The reason for this higher *A* value may be because the membrane on the cross-flow system was compacted at 16 bars prior to the experiment, while on the dead-end filtration system the membrane was compacted at a lower pressure of 5 bars. During compaction the flux decreased over time and eventually stabilized. The purpose of compaction is to allow the membrane to reach a stable flux in order to avoid confounding flux decline due to compaction from flux decline due to fouling or concentration polarization caused by filtration of the digestion liquor.

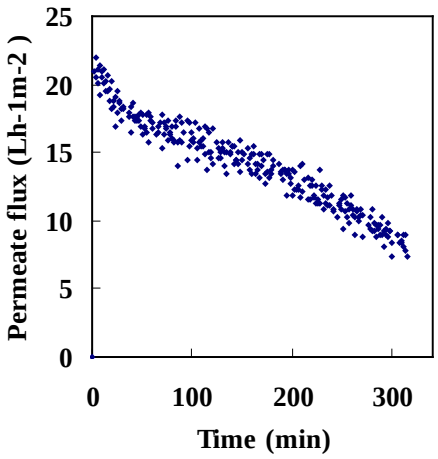


Figure 4-12. Permeate flux change with time during a single filtration event from Batch 1. Filtration condition: 5 bar, pH 3.49. Feed solution is the digestion liquor from day 15 containing 2.8 g/L lactic, 0.05 g/L formic, 0.46 g/L acetic, 2.7 g/L propionic, 2.2 g/L butyric and 23.3g/L hexanoic acid.

Figure 4-12 shows the permeate flux decline during sample filtration. The flux decreased 63.6%, from 22 to 8 $Lh^{-1}m^{-2}$, over the course of a 5.2 hour filtration experiment. Because of the volume reduction during dead-end filtration, highly rejected solute can be accumulated in the feed, elevating the osmotic pressure and weakening trans-membrane pressure. Other causes for flux decline can be concentration polarization, adsorption, and plugging of membrane pores (Weng et al., 2010). Other researchers have also observed significant flux decline during NF of biomass hydrolyzate (Murthy et al., 2005; Weng et al., 2010).

Acid and sugar concentration in permeate

Figure 4-13, 4-14 and 4-15 shows the change of acid and sugar concentration in the permeate as a function of permeate to feed volume ratio. As dead-end filtration proceeded, V_p/ V_f increased more slowly because of flux decline (data not shown), and acids with different rates of rejection had distinct changes in permeate concentration. Lactic and propionic acid concentration increased very gradually until V_p/ V_f reached around 0.7, when a sharp increase was observed. Lactic and propionic acid had the highest rejection, 27.2 and 29.9% respectively (Table 4-4), which allowed the accumulation of these two acids in the feed solution as V_p/ V_f increased. Higher concentration in the feed leads to higher concentration in permeate, and by generating a CP layer on the membrane surface further elevate acid concentration in the permeate. A corollary of this same process caused a corresponding sharp increase in sugar concentration, as shown in Figure 4-15, which is due to the higher rejection of sugars (>90%) relative to acid rejection. Hence the sugar was much more concentrated in the feed during filtration than lactic and propionic acid. Acetic, formic and hexanoic (Figure 4-13 and 4-14) acid concentrations remained relatively steady during filtration indicating the low rejection of these acids. Continuing filtration to reach a higher V_p/ V_f can result in more acid recovery, but considering the rapidly increasing

sugar loss at the same time, each filtration event should probably stop when V_p/ V_f is around 0.7. The optimum V_p/ V_f at which to stop filtration in a real system will be a function of the costs of feedstock (and lost sugar) as well as downstream acid concentration and/or conversion costs.

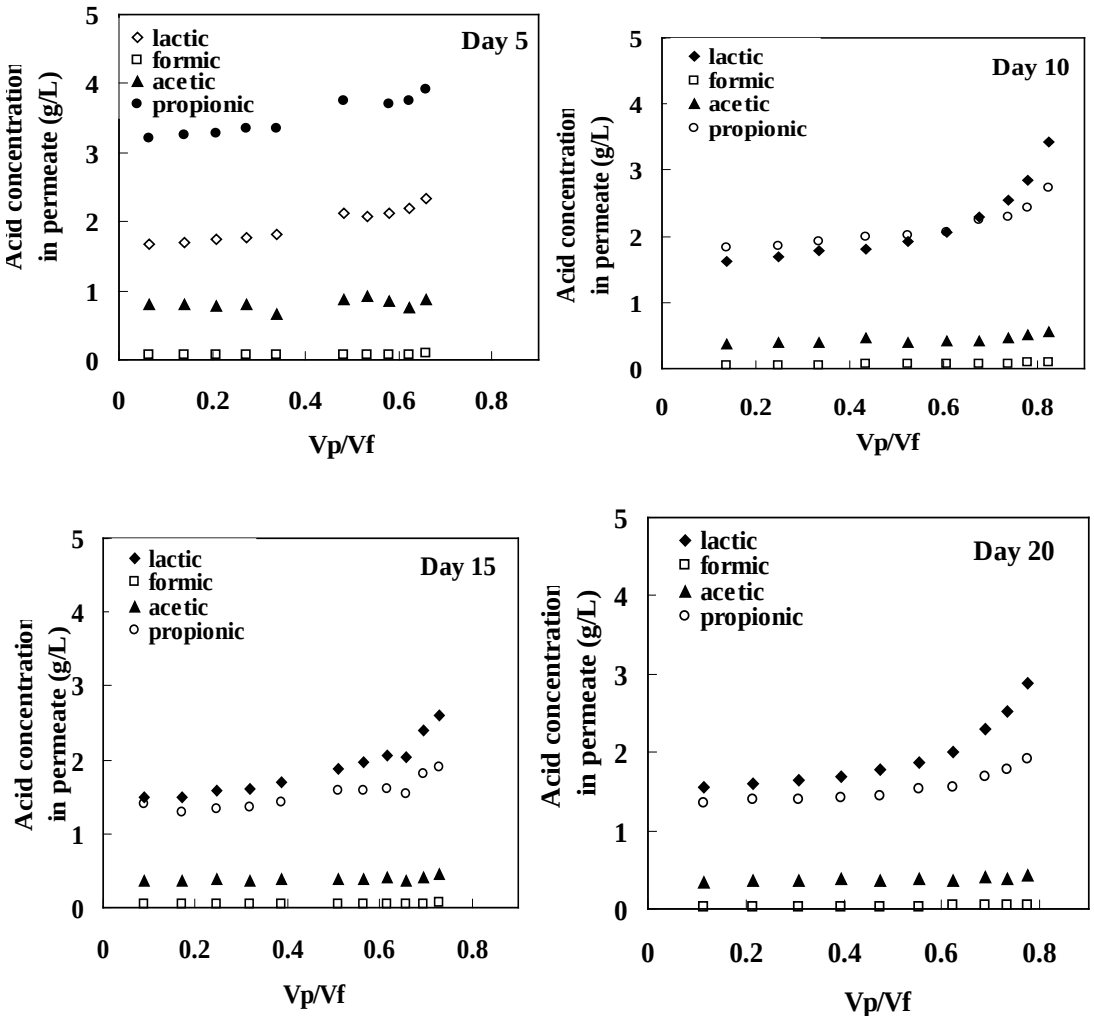


Figure 4-13. Changes in lactic, formic acetic and propionic acid concentrations in the permeate during filtration on days 5, 10, 15 and 20. V_p/V_f represents the ratio of the volumes of permeate and feed.

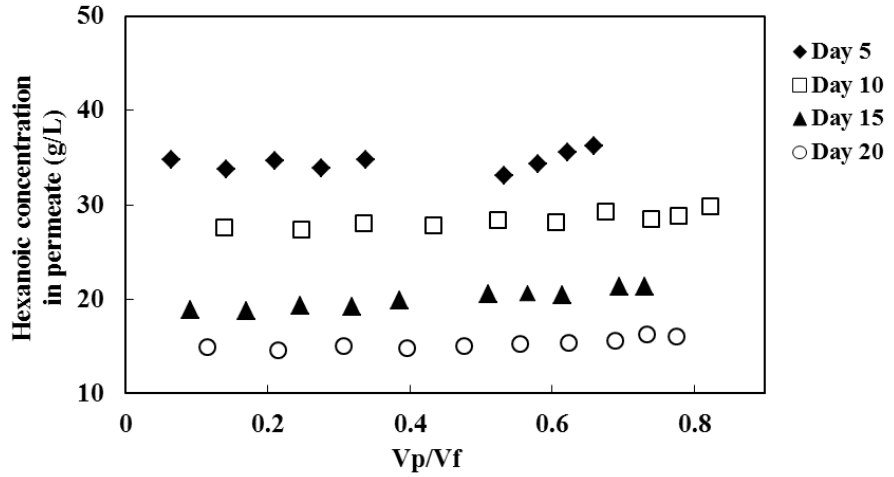


Figure 4-14. Hexanoic acid concentration change in the permeate during filtration on days 5, 10, 15 and 20.

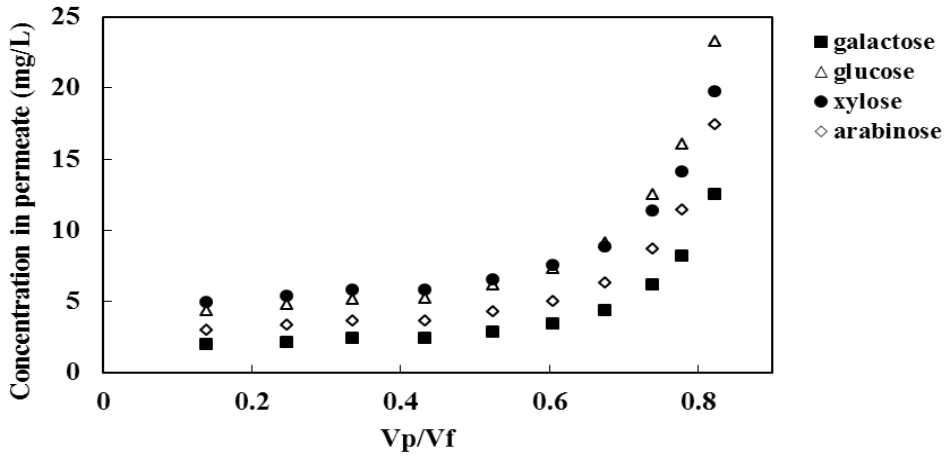


Figure 4-15. Sugar concentration changes in the permeate during filtration on day 10. Filtration condition: 5 bar, pH 3.49. The day 10 digestion liquor contained 29.7 mg/L arabinose, 26.8 mg/L galactose, 42.8 mg/L glucose, 20.9 mg/L xylose.

Acid and sugar rejection by dead-end filtration

Table 4-4 indicates the average rejection value from four filtration experiments. Each rejection was calculated using the feed in the beginning and accumulated permeate in the end.

Because the permeate concentrations increase during filtration, these rejection values were underestimated compared to the rejection reported in the previous cross-flow system filtration experiments at 8 bar pressure and pH 3, where permeate and retentate were both recycled back into the feed solution during filtration.

Table 4-4. Average solute rejection of four dead-end filtration experiments with DL.

Arabinose	Galactose	Glucose	Xylose	Lactic	Formic	Acetic	Propionic	Butyric	Hexanoic
89.1% ± 2.2%	92.3% ± 1.8%	92.0% ±3.3%	79.8% ±5.1%	27.2% ±5.5%	3.8% ± 5.5%	3.8% ±2.5%	29.9% ± 5.9%	100%	4.8% ±3.9%

Acid mass recovery and sugar mass loss during separation

Average total acid recovery is 22.3%±0.8% and sugar loss is only 3.1%±0.6% during each cycle of filtration. The total acid mass removed in the end was 57.1% of the total acid produced for Batch 1 (P3b1), and 86.8% for Batch 2 (P3b2). In each case additional acid could have been removed with additional filtration cycles.

Table 4-5 presents the individual acid recovery and individual sugar losses for each of the constituents measured. Individual acid mass recovery during each cycle of filtration ranged from 19.3 to 29.9% while sugar loss was only 2 to 5%. These values reflected the low acid rejection and high sugar rejection that was a primary objective of this integrated system design. It is worth mentioning that the mass recovery is also largely affected by the volume ratio of digestion liquor removed every time for acid removal versus the total amount of liquor in the digestor. The method adopted in this study only removed 26 to 30% of the total reactor volume during each filtration event. Acid recovery could potentially be significantly enhanced by a continuous filtration set-up with access to all of the digester liquor.

Table 4-5. Average sugar loss and acid recovery percentage by dead-end filtration with DL.

Arabinose	Galactose	Glucose	Xylose	Lactic	Formic	Acetic	Propionic	Butyric	Hexanoic
2.8%±0.5%	2.0%±0.4%	2.0%± 0.8%	5.2%± 1.1%	20.4% ±1.4%	29.9% ±4.8%	23.5% ±0.9%	19.3%± 2.2%	0%	25.0%± 0.9%

Membrane fouling evaluation of Dead-end filtration

Each experiment on the dead-end filtration system filtered digestion liquor for 5 to 7 hours, and was then followed by 2-3 hour DI water wash. Table 4-6 presents the water permeability constant of the virgin membrane and the membrane after the experimental cycle including the water wash was complete. The water permeability constant after the filtration experiment experienced only minimal changes compared to the virgin membrane, indicating the cleaning procedure with DI water wash was effective to recover the flux, and no severe or long-term fouling resulted from the filtration experiment.

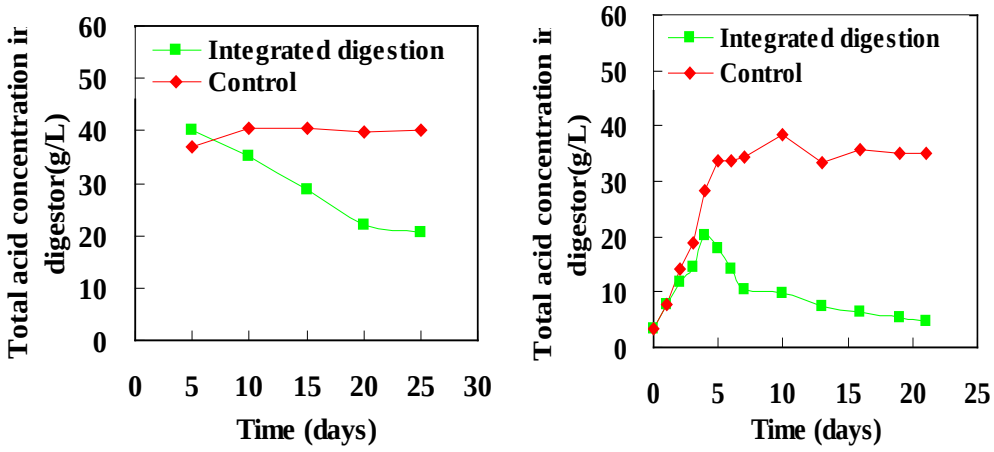
Table 4-6. Water permeability constant of virgin membrane and membrane washed for two hours after sample run. Dead-end filtration with the membrane lasted for 6 to 7 hours.

	Water permeability constant (L/m2/hr/bar)			
Experiment #	1	2	3	4
Virgin membrane	5.8	7.5	7.8	7.2
Membrane after sample run	6.7	6.8	7.4	7.2

Acid concentration during the integrated digestion trials

Figure 4-16 illustrates the total acid concentration change in the digestor. The control data from Batch 2 (P3b2) implied that acid was produced at a rate of roughly 5 $gL^{-1}day^{-1}$ the first three days, and reached the maximum productivity at day 4 with approximately 9 $gL^{-1}day^{-1}$ and then returned back to 5 $gL^{-1}day^{-1}$. The productivity values were roughly estimated by the acid concentration difference measured each day. After day 5, the acid concentration remained steady until day 21. The batch 1 (P3b1) control data repeated the minimum concentration change in acid concentration from day 5 until day 25. The concentration decline from day 5 for Batch 1 (P3b1) and from day 4 for Batch 2 (P3b2) indicated that acid production was lower than acid removal

after days 5 and 4 respectively. This acid production rate is similar to the value previously reported using bagasse and rumen bacteria as feedstock and inoculum (Matei and Playne, 1984).



Batch 1 (P3b1) Batch 2 (P3b2)

Figure 4-16. Total acid concentration changes with time in the integrated digestion and separation system. The concentration of reported for batch 1 (P3b1) is the concentration measured every time before acid removal and that from batch 2 (P3b2) is the concentration measured after acid removal.

Integrated digestion achieved a significantly lower acid concentration than the control once acid removal was initiated. For Batch 1 (P3b1), the acid concentration in the integrated digestion system was 13 to 49% lower than the acid concentration in the control, after four times acid removal using NF, which started at day 5. For Batch 2 (P3b2), the difference in acid concentration between integrated digestion with separation and the control started to occur at day 2 (17% acid concentration difference), increased as acid removal was being conducted every day, and reached 47% at day 5. Eventually the integrated digestion system had an 87% lower acid concentration than the control after acid removal occurred 11 times by NF. From day 5 to day 21, the acid concentration in the integrated digestion decreased from 17 to 4 g/L. At day 21, the acid concentration in the integrated system was 4.6 g/L compared to 35.1 g/L in the control digestion.

This demonstrated the capacity and effectiveness of NF for reducing acid concentration in this integrated acidogenic digestion process.

Total acid Yield comparison

As shown in Figure 4-17, Phase 3 Batch 1 and Batch 2 yielded 0.64 and 0.53 g total acid/ g dry biomass by the end of the integrated digestion process. The control yields were 0.54 and 0.49 g acid/ g dry biomass for Batch 1 and Batch 2 respectively. These yield values were similar or higher compared to other studies utilizing lignocellulosic biomass with undefined mixed cultures (Agbogbo and Holtzapple, 2007; Domke et al., 2004; Matei and Playne, 1984; Thanakoses et al., 2003). The theoretical yield was simplified but assuming glucose as the sole substrate, and the stoichiometric yield ranges from 0.48 to 3.06 g acids/g glucose for different acid products (Appendix, Table A-1). An estimation of total acid theoretical yield can be calculated by the sum of individual theoretical yield multiplying the percentage of each acid out of total acids. Different acid profiles results in glucose-acid theoretical yield in a range of 0.56-0.76 g acids/g glucose, which is higher than glucose-ethanol theoretical yield (Table A-1). Multiplying the carbohydrate fraction (50-55%) in the biomass (Appendix, Figure A-1) by theoretical yield with glucose as a substrate gives theoretical yield in a range of 0.28-0.38 g acids/ g biomass, which is lower than the measured yield reported in this study and previous study. This supported the statement that some of the other non-lignin parts in the biomass, such as pectins, proteins and lipids, can be biologically converted to acids (Datta, 1981; Holtzapple and Granda, 2009). If these other feedstock constituents were included in the calculations the theoretical yield of acids would be higher. Additional stoichiometric equations can be developed to predict the acid yield more precisely.

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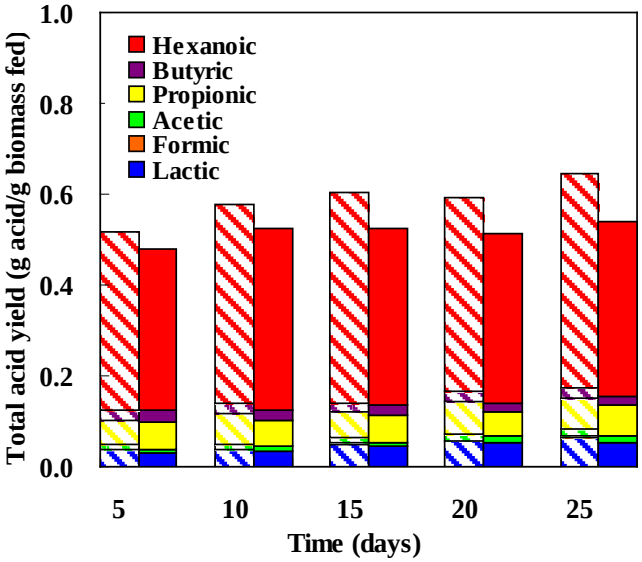
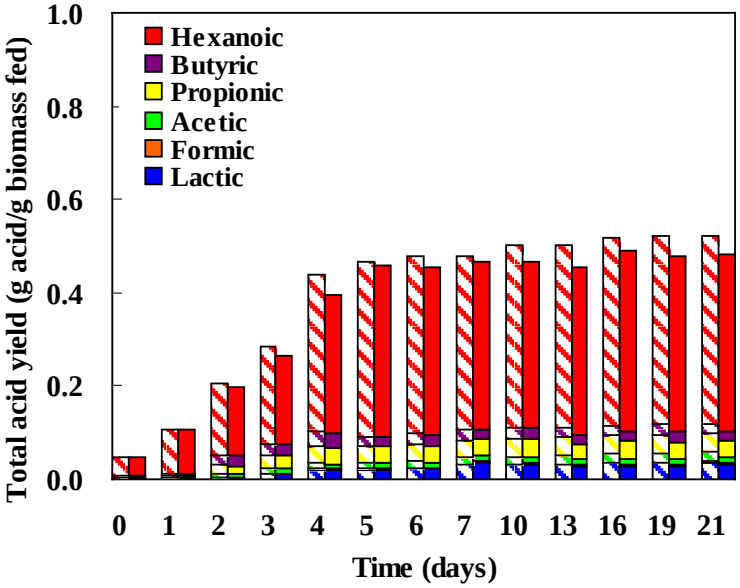


a)



b)

Figure 4-17. Total acid yield (g total acid/ g biomass fed) of integrated digestion and control. a) Batch 1 (P3b1), b) Batch 2 (P3b2). The columns with stripes indicate integrated digestion yield, and the solid columns indicate control yield. The colors presented in the legend for individual acids apply to both integrated digestion and control data. The total acid of integrated digestion is the sum of the acid recovered by filtration and the final acid in the digestor in the end.

In both batches, the acid yield of integrated digestion was higher than the yield of the control after acid removal was initiated, but not always by a significant amount. Batch 1 had 8 to 19% higher yields and Batch 2 had less than a 10% yield increase, with the yield difference fluctuating with time. Two independent sample t tests indicated the yield difference in Batch 2 was insignificant, giving a P value=0.744 (at α=0.05), while yield difference in Batch 1 was significant with a P value=0.021 (at α=0.05). If the reduced acid productivity starting from day 5 was because of severe inhibition of microbial activity caused by 35 to 40 g/L acid, reducing the acid concentration to less than 10 g/L in Batch 2 should presumably have reduced the inhibition and enhanced the yield more than occurred in Batch 1, where acid concentration was only reduced to a range of 30 to 20 g/L. Therefore, the acid yield enhancements observed in Batch 1 after day 5 appear not to have resulted from the acid concentration reduction. The difference in yield enhancement and final yield between Batch 1 and Batch 2 might be due to the variation between batches.

The fact that reducing the acid concentration by a factor of three did not result in enhanced yield strongly suggests that the carboxylic acids accumulated after day 5 did not have an inhibitory effect on acid production. Multiple studies have reported volatile fatty acid production leveled off around day 5 (Choi et al., 2013; Maiti et al., 2012; Roddick and Britz, 1997). One of the reasons that acid production can slow at day 5 is a lack of easily utilized substrate. Figure 4-18 indicates the dissolved sugar concentration changes with time. Total sugar concentration was highest at day zero, resulting from sugar release during hot water pretreatment, and decreased dramatically in the first four days from 1.2 g/L to 0.1 g/L and then leveled off at < 0.05 g/L after day 5. The change of sugar concentration was related to the change of acid concentration. However, as explained in the beginning of the discussion of the Phase 2 experiment, sugar concentration is an indirect indication of the availability and utilization of total

food substrates. Considering all these results together suggests that the cause of this slowing of acid production could be a lack of sugar hydrolyzed from biomass.

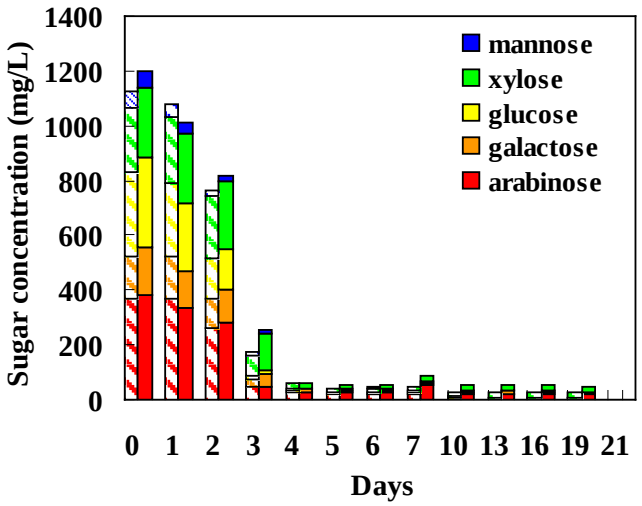


Figure 4-18. Sugar concentration change with time in integrated digestion and control for Batch 2 data. The column with strips data was from integrated digestion, and the solid column data was from control. The colors presented in the legend for individual sugars applied to both integrated digestion and control data.

Another possible reason the yield did not increase could be the low pH of the digestion, which remained low even with significant acid removal. This seems plausible since pH was consistently below 4, and if so this low pH could be the largest challenge in increasing acid yield. Other studies have suggested optimum reactor conditions should be > pH 5.5 for production of different organic acids (Matei and Playne, 1984; Roddick and Britz, 1997; Thanakoses et al., 2003). As shown in Figure 4-19, the hot water pretreated willow had a pH of 3.91 that stayed very close to constant. Inoculum had an initial pH of 5.66 and that decreased to 3.9 after two weeks due to acetic and butyric acid production. Acidic biomass was partially neutralized by inoculation which resulted in a starting pH of 4 for digestion. The pH of Phase 3 controls and integrated digestion treatments decreased from 4 to 3.5 and 3.7 respectively, and stayed steady.

The change of pH was correlated with acid production, although the integrated digestion treatment pH was only 0.2 units higher than the pH of the control experiment. Most of the acid removed in the permeate in the integrated digestion treatment was hexanoic acid (see figures 4-13 and 4-14). If the observed 0.2 unit elevation in pH is assumed to be due to hexanoic acid removal, then the associated reduction in hydrogen ions in the integrated digestion treatment only accounted for 0.016 g/L hexanoic acid. Smaller molecular weight carboxylic acids would have a higher pH to g acid ratio, but even the smallest, acetic acid, would only double the pH increase per gram of acid removed. With concentrations of carboxylic acids in the digester dropping from roughly 20 to 10 g/L (figure 4-16), that 10 g/L it appears that something other than carboxylic acid production is contributing to this low pH. Carboxylic acid removal can moderate pH, as was demonstrated by Choi *et al.* (2013) using *in situ* solvent extractive fermentation to maintain the pH in an aqueous fermentation broth around pH 6.5 with an initial pH 6.8, while the pH of the culture without extraction dropped to pH 5.5. It may be possible that the willow digestion liquor had a strong buffering capacity around pH 3.5 to 4, preventing pH from increasing. Further investigation needs to be done to identify and confirm the cause of acidity of hot water pretreated biomass. Neutralizing this acidity may make it possible to further enhance carboxylic acid yield once the pH can be maintained at a higher level.

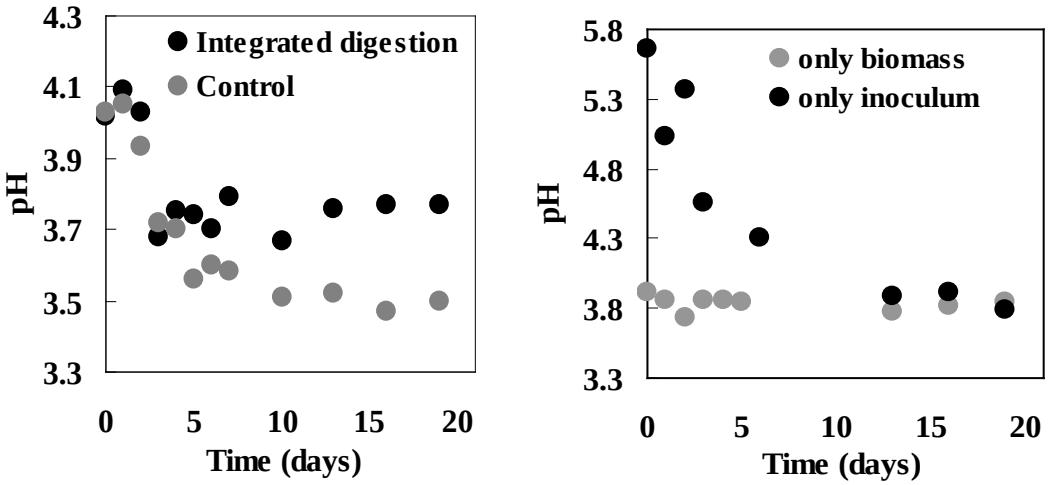


Figure 4-19. pH of integrated digestion, control, only biomass with the same solid loading rate without inoculation, and only inoculum with the same solid loading rate without biomass.

Whatever process is causing the inhibition, it appears to become active within the first five days of acidogenic digestion. The acid concentration differences between the integrated digestion and the control treatments were not significant during the first three days, but at day 4 the difference of acid concentration between these two treatments was 28% and reached 47% at day 5. Some prior researchers initiated *in situ* product removal after 18-24 hours of fermentation (Choi et al., 2013; Roddick and Britz, 1997), improving hexanoic acid yield. Increasing acid removal rate in early stages of an integrated process has potential to further enhance the acid yield.

In addition to the acid products, other lignocellulosic biomass derived inhibitors released during pretreatment may also be inhibitory to microbial growth. Based on their functional groups, inhibitors can be generally categorized into three classes. Aldehyde inhibitors, such as fufural and HMF, are toxic because of the presence of furan rings and aldehyde groups, which can inhibit cell growth, enzymatic activities, DNA /RNA synthesis and metabolic conversion (Liu and Blaschek, 2010). Fufural and HMF can decrease the ethanol productivity (Larsson et al., 1999) but they

were not detected in this study with 75 g/L pretreated willow loading rate. Organic acid inhibitors, such as levulinic, syringic, ferulic and vanillic acid, are toxic for the same reasons as carboxylic acids, as discussed in Chapter 2, while the higher hydrophobicity of these acids also causes further inhibition of cell growth by increasing cell membrane leakage (Zaldivar and Ingram, 1999). Phenolic inhibitors, derived from lignin, changes the membrane fluidity and permeability (Liu and Blaschek, 2010). Although most previous research investigating causes of inhibition focused on yeast or ethanol-producing bacteria, the results can be used to speculate potential toxicity on acidogenic bacteria. However, the actual effects of any specific chemical inhibition can vary largely among different strains. Syringaldehyde, ferulic, and r-coumaric acids were strong inhibitors to *Clostridium beijerinckii* growth and acetone-butanol-ethanol production at low concentration (0.3g/L), while HMF and fufural at up to 3 g/L did not affect microbial growth (Ezeji et al., 2007). In addition to the biomass derived compounds, ammonia can be introduced by the use of rumen fluid in inoculum. Ammonia has been reported to have inhibitory effect on different acidogenesis pathways in a concentration range of 2 to 16 g N/L (Lu, 2008) . Thus there are many possible inhibitorory compounds that should be investigated and explored.

Chapter 5

Conclusion and future work

Conclusion

In Phase 1 using poplar wood as feedstock, increasing solid loading rate in the range of 10 to 69 g DM/L was observed to have a negative effect on acid yield. The inoculum loading rate did not have a significant effect on acid production when using switchgrass as feedstock, but the data had large variation. Methane concentrations were consistently under the detection limit during the course of a 42 day digestion.

In Phase 2, digestion liquor derived from a high solid loading rate digestion of pretreated willow was utilized to conduct nanofiltration experiments. The feasibility of NF membrane to achieve low acid rejection and high sugar rejection using actual digestion liquor was successfully demonstrated, with one important exception. Under the pH 3 and 8 bar condition, we observed rejection of hexanoic, formic and acetic acid at less than 20%, while propionic and lactic acid had rejections of 35 and 40% respectively. Acetic and lactic acid rejection was significantly decreased at low pH while the other acids were not significantly affected by pH. Butyric acid was highly rejected by NF membrane, possibly due to the molecular interference by the digestion liquor matrix. Low pressure yielded low rejection of acids, although the pressure effect was less significant at low pH, especially for acetic acid, implying electrostatic repulsion was an important mechanism for this NF membrane separation. Sugars had high rejection, > 90%, with the exception of xylose rejection, which decreased to 82% at pH 7. There was no severe membrane fouling observed and membrane flux fully recovered after a simple DI water wash. DL and DK membranes had slightly different acid rejection and similar sugar rejection performance. The DL

membrane did have a significantly higher membrane flux than the DK membrane, indicating that the DL membrane could be a better candidate for acid recovery. It was concluded that nanofiltration membrane technology can be an effective separation process for carboxylic acid recovery from lignocellulosic biomass digestion liquor.

In Phase 3, a lab-scale batch digestion coupled with nanofiltration separation was set up to simulate *in-situ* acid removal. After settling, 28% of the digestion liquor was pre-processed by centrifuge and microfiltration prior to NF filtration at low applied pressure at the original pH. In two replicated trials and 11 acid removal cycles, on average 22% of the acid was recovered and only 3% of the sugar was lost during each cycle of filtration. In total, 86.8% of total acids were recovered after 11 NF acid removal cycles, lowering acid concentration in the digestor to half at day 5 and 87% in the end of digestion, compared to digestion without acid removal. The acid yield was slightly improved by acid removal process, indicating the need for a deeper understanding of the challenge of inhibition on the digestion side, and for further improving acid removal processes during the early stages of digestion.

Future work

This study provided preliminary engineering design data for product separation in a membrane bioreactor setup that can promote the establishment of carboxylate platform of lignocellulosic biofuel production. This particular strategy and combination of technologies was newly proposed for this study, and there is still a great deal of research needed to build an industrial scale production plant that is economically viable.

On the digestion side, first there is a strong need to know the optimal pH for the digestion with current feedstock and inoculum. If the acid yield can be increased by partially neutralizing

the acidity from pretreated biomass, it is worth investigating the cause of the low pH and buffering capacity observed in our trials with hot water pretreated willow. It appears that raising the pH of digestion initially could enhance the overall system performance. The high pH seems not to affect the hexanoic and propionic acid recovery but does increase the lactic and acetic acid rejection by the negatively charged NF membrane. Thus it would be useful to evaluate the acid removal capacity by NF integrated with digestion system with better buffers at a more neutral pH. Sodium hydroxide will be ideal solution to adjust the pH, as the NF with around 150 Dalton MWCO will allow sodium to permeate the membrane, thus there will be no osmotic pressure build up on the feed side.

If as expected the acid removal capacity decreases in a neutral pH environment, and this also decreases product yield, at least two strategies can be considered to enhance acid recovery. The addition of sodium chloride (common table salt) was previously reported to reduce acid rejection because the high ion strength reduces the double layer of the charge on the membrane, hence increasing solute passage and reducing rejection (Braghetta et al., 1997). Figure 5-1 showed that the zeta potential of membrane was clearly closer to zero in the 40mM NaCl solution than in a 1mM NaCl solution under pH values from 3 to 10. Observations are consistent with increased ion strength weakening the charged effect on the membrane at high pH (>3). With a less negatively charged membrane, it is likely that the acid rejection can be further reduced. Previous studies have reported that the presence of NaCl reduces lactate rejection (Umpuch et al., 2010) as well as formic acid rejection (Choi et al., 2008).

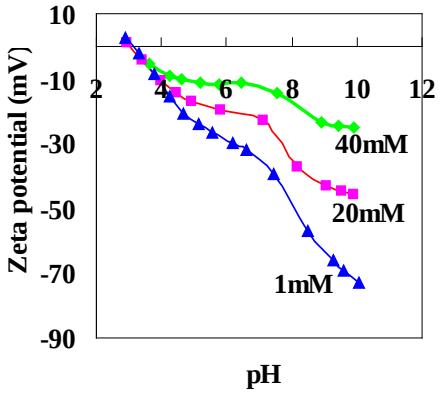


Figure 5-1. Zeta potential of DL membrane under 3-10 pH conditions with 1mM, 20 mM and 40mM NaCl solution as background electrolyte.

Another idea is to develop and manufacture a membrane that has fewer negative charge groups on the membrane (at high pH) and more positive charge groups. For example, by decreasing the frequency of the carboxyl group on the membrane, the dissociated acid will not be as easily rejected by the membrane at high pH due to electrostatic repulsion.

A central hypothesis of this investigation is that early stage separation with higher acid removal rate could further enhance the yield during acidogenic digestion. Building a continuous acid removal system, coupling in situ microfiltration with ex situ nanofiltration, would provide a platform to significantly enhance acid removal rates at early stages of digestion. Once a continuous system is established, it should be tested with a higher solid loading rate such as 100 to 150 g/L, since the yield of higher solid loading rate system might be more sensitive to lower acid concentration in the system achieved by acid removal. A higher solid loading rate also reduces the process water, saving capital and energy investment. Furthermore, given the evidence from multiple indicators that biomass digestibility and availability diminished at day 5, fed-batch or continuous digestion system might enhance the acid yield and production.

Despite the progress of this investigation, at present very little is known about the mechanisms or concentrations by which inhibition from biomass-derived feedstocks and processes affect acidogenic bacteria growth and acid production. A wise start would be to intensively investigate the type, fate and concentration of inhibitors in pretreated willow digestion liquor in order to screen the potential strong inhibition players in the digestion liquor. Following this, spike samples of different acids and concentrations could be applied to evaluate inhibitory effects. Studying the fate of inhibitors during NF filtration could further identify the effective inhibitors and explore the feasibility of using NF to remove the inhibitory compounds.

Finally, it is also important to test the carboxylate platform integrated with NF with a wide range of feedstocks and alternative separation strategies. Such feedstocks could include other bioenergy crops, solid food, and municipal and industrial wastes, any of which could provide sufficient feedstock in our region to extend the carboxylic acid platform from research to commercialization. A comprehensive evaluation of separation options would further promote the carboxylate platform, developing a model to predict acid yield, productivity and composition in response to various feedstocks, as well as a model to predict acid separation efficiency by NF with different feed strengths and composition under different pH and pressure conditions. Conducting a techno-economic analysis can offer more comprehensive information to adjust the separation technology and potentially industrialize and commercialize the platform.

Appendix

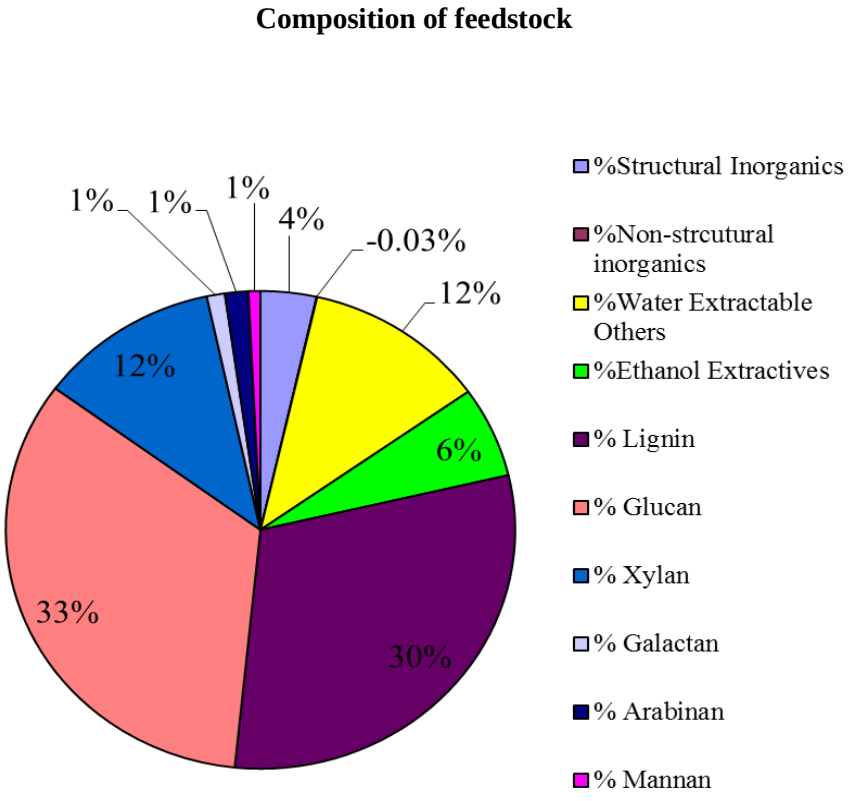


Figure A-1. Chemical composition of unpretreated willow wood.

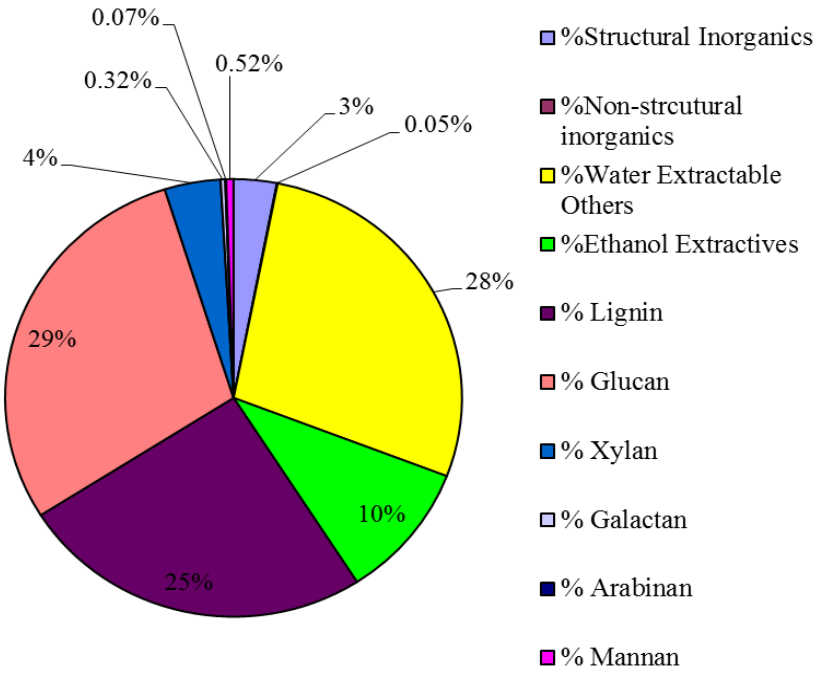


Figure A-2. Chemical composition of hot water pretreated willow wood. Pretreatment condition: 190°**C**, 5 minutes on AdvanceBio hydrolyzer.

Stoichiometry of carboxylic acid and ethanol as product

In order to develop the stoichiometry of common reactions associated with biomass conversion to carboxylic acid products, the half-reaction approach was adopted (Rittamann, 2001). The half- reactions of glucose, ethanol, formate, acetate, lactate and propionate were obtained from Rittmann and McCarthy (2001). Because the aqueous environment is acidic, bicarbonates in the half-reactions are cancelled by the carbon dioxide and water molecules on the right side of the equation.

Other half-reactions were derived based on the principles of charge and molecule number balance and under the assumption that the species involved are the same as the known half-reactions. An example of derivation of the valerate half-reaction is given below.

1. Acetate has a known half-reaction from reference:

$$\frac{1}{8}CO_2+\frac{1}{8}HCO_3^-+H^++e^-\rightarrow\frac{1}{8}CH_3HCOO^-+\frac{3}{8}H_2O \quad (6)$$

2. Start with an equation containing the similar species as known half-reactions.

$$CO_2+HCO_3^-+H^++e^-\rightarrow CH_3(CH_2)_3COO^-+H_2O \quad (7)$$

3. Assume the coefficient of valerate is 1/x, and then in order to balance the charge, the coefficient of bicarbonate has to be the same as the coefficient of valerate, 1/x.

$$CO_2+\frac{1}{x}HCO_3^-+H^++e^-\rightarrow\frac{1}{x}CH_3(CH_2)_3COO^-+H_2O \quad (8)$$

4. Balance the carbon molecule numbers in the equation to yield the coefficient of carbon dioxide on the left side of the equation.

$$\frac{4}{x}CO_2+\frac{1}{x}HCO_3^-+H^++e^-\rightarrow\frac{1}{x}CH_3(CH_2)_3COO^-+H_2O \quad (9)$$

5. Balance hydrogen molecule numbers of the equation to yield the coefficient of water on the right side of the equation. The coefficients of proton and electron are assumed to be 1 according to the known half-reactions.

$$\frac{4}{x}CO_2+\frac{1}{x}HCO_3^-+H^++e^-\rightarrow\frac{1}{x}CH_3(CH_2)_3COO^-+(\frac{1+x}{x}-\frac{9}{x})H_2O \quad (10)$$

6. x can be solved by balancing oxygen numbers,. And final half-reaction can be determined.

$$\frac{2}{13}CO_2+\frac{1}{26}HCO_3^-+H^++e^-\rightarrow\frac{1}{26}CH_3(CH_2)_3COO^-+\frac{9}{26}H_2O \quad (11)$$

The mass yield values in the Table A-1 were calculated by the equation below:

*Theoretical mass yield= (molar coefficient of product * molecular weight of the molecule)/ (molar coefficient of glucose* molecular weight of glucose)*

Table A-1. Stoichiometric equations of glucose conversion to ethanol and carboxylic acids.

Product	Stoichiometric equation	Theoretical mass yield (g product/g substrate)
Ethanol	$\frac{1}{2}\,C_6H_{12}O_6 \rightarrow CH_3CH_2OH + CO_2$	0.51
Formic acid	$\frac{1}{12}C_6H_{12}O_6 + \frac{1}{2}CO_2 + \frac{1}{2}H_2O \rightarrow HCOOH$	3.07
Acetic acid	$\frac{1}{3}C_6H_{12}O_6 \rightarrow CH_3OOH$	1.00
Lactic acid	$\frac{1}{2}C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH$	1.00
Propionic acid	$\frac{7}{12}C_6H_{12}O_6 \rightarrow CH_3CH_2COOH + \frac{1}{2}CO_2 + \frac{1}{2}H_2O$	0.71
Butyric acid	$\frac{5}{6}C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + \frac{1}{2}CO_2 + \frac{1}{2}H_2O$	0.59
Valeric acid	$\frac{13}{12}C_6H_{12}O_6 \rightarrow CH_3(CH_2)_3COOH + \frac{3}{2}CO_2 + \frac{3}{2}H_2O$	0.52
Hexanoic acid	$\frac{4}{3}C_6H_{12}O_6 \rightarrow CH_3(CH_2)_4COOH + 2CO_2 + 2H_2O$	0.48

Carboxylic acid product profile of using switchgrass as feedstock

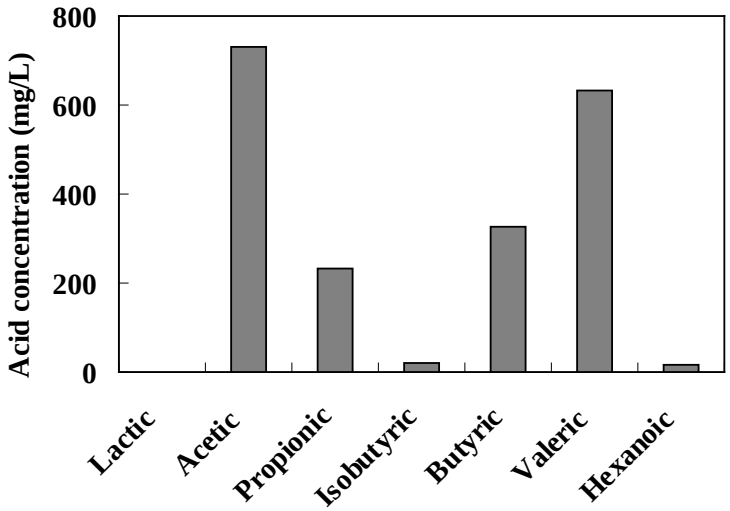


Figure A-3. Carboxylic acid concentration in 42 day old digestion liquor of pretreated switchgrass with 10 g DM/L solid loading rate.

ANOVA analysis results

Table A-0-2. The summary of P-value of all the ANOVA analysis results at significance level of α=0.05.

Solute rejection	pH	Pressure	Membrane type	pH*pressure	Pressure*Membrane	Membrane*pH
Lactic	0.000	0.020	0.000	0.971	0.137	0.000
Formic	0.002	0.000	0.740	0.171	0.815	0.030
Acetic	0.000	0.001	0.029	0.487	0.970	0.290
Propionic	0.094	0.002	0.048	0.981	0.957	0.015
Hexanoic	0.277	0.03	0.437	0.926	0.914	0.005
Xylose	0.000	0.034	0.301	0.990	0.999	0.390

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