DISSERTATION

REACTIVE MEMBRANE EXTRACTION IN BIOREFINERIES

Submitted by

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ABSTRACT

REACTIVE MEMBRANE EXTRACTION IN BIOREFINERIES

Separations account for 60-80% of the processing costs of most mature chemical processes. Membrane based separations offer several advantages over conventional technology such as lower energy costs and easy scale up. Here we focus on membrane extraction for removal of acetic acid, sulfuric acid, furfural, HMF and other toxic compounds from biomass hydrolysates. As membrane extraction is non-dispersive it overcomes the disadvantages of conventional extraction.

Experiments have been conducted using dilute sulfuric acid pretreated corn stover (hydrolysate). Acetic acid, in its protonated form, is extracted into an organic phase consisting of octanol/oleyl alcohol and Alamine 336, a tertiary amine, containing aliphatic chains of 8-10 carbon atoms. Co-extraction of sulfuric acid leads to an increase in hydrolyste pH. The effect of aqueous and organic phase flow rates and temperature, on the rate of extraction of acetic acid and sulfuric acid has been investigated. Changes in the rates of acetic and sulfuric acid extraction may be explained by considering the structure of the complexes formed in the organic phase.

We conducted computational modeling to elucidate the extraction process of Alamine 336 in different solvents. Extraction of carboxylic acids, Furfural and HMF in water and octanol was simulated using the Gaussian 03 package. In the past the

extraction process has been explained by the direct interaction of the carboxylic acid with the Alamine 336 to form an ion pair. More carboxylic acids could be extracted through hydrogen bonding forming a dimer or trimer complex form with the Alamine 336, stabilized by the organic solvent.

Hydrolysates treated by membrane extraction and conventional conditioning technologies were fermented using a glucose-xylose fermenting bacteria to determine the viability of membrane technology to detoxify biomass hydrolysates. Membrane extraction could be a viable hydrolysate detoxification technology because the other conditioning technologies do not remove acetic acid.

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PREFACE

The term renewable energy describes very diverse energy sectors, which can be grouped in new and old renewable energy technologies. Old technologies include hydrothermal and geothermal energy productions techniques, whereas new technologies describe energy production using wind, solar and biomass.

The first chapter provides an introduction into the biomass renewable energy sector, specially the bio-ethanol production from lignocellulosic materials. The introduction of the biorefinery concept in this chapter provides background for chapters 2, 3, and 5. These chapters are published professional papers and focus more on the membrane liquid-liquid extraction process with its performance and results. No additional knowledge is provided for the membrane extraction process in chapter 1, further an overview shows more possible applications of the membrane technology in a biorefinery. The final section in chapter 1 states my motivation for introducing liquid-liquid supported membrane extraction into the biorefinery scheme.

Chapter 5 shows the possible applications of membrane supported liquid-liquid extraction in extracting acids, HMF or glycerol. Chapters 6 tries to elucidate the chemical process during extraction presented partially in chapters 2 and 3, using quantum chemistry

calculations embedded in Gaussian 03 software. Aspen simulation is presented in the final chapter 7, comparing the cost of NREL's lignocellulosic biorefinery with and without membrane technology.

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CHAPTER 1

1 Background Introduction

1.1 Petroleum consumption in U.S. – Bio-ethanol a solution for the future?

The theory of basic human needs developed by Maslow states that human needs must be satisfied in a strict sequence starting with the most immediate needs followed by secondary needs[1]. Among these levels are characteristics defined as the need for survival and security. Energy could be defined as a part of basic human needs, specifically as a part of human fundamentals, essential for human kind. Energy is needed for manufacturing for personnel needs; it encourages development and it provides us with security. Energy is present in different forms, such as coal, nuclear energy, hydroelectric energy, natural gas, wood and petroleum. Nowadays, energy generated and consumed from petroleum exceeds by far all the other energy sources in the U.S.[2]. Petroleum is consumed in the U.S. mostly for personnel needs found in the transportation area, where fuel is used for aviation or automotive engines[3].

Figure 1 shows the total petroleum consumption in U.S., the total petroleum consumption for transportation and its fraction used by gasoline engines. As noticed, two thirds of petroleum usage is attributed due to transportation, of which over two thirds are consumed by gasoline engines. Also the steady increase of petroleum consumption using gasoline engines

correlates with the steady increase of vehicles numbers from 147.5 millions in 1988 to 191 millions in 2001, as mentioned by the U.S. Energy Information Administration (EIA)[3].

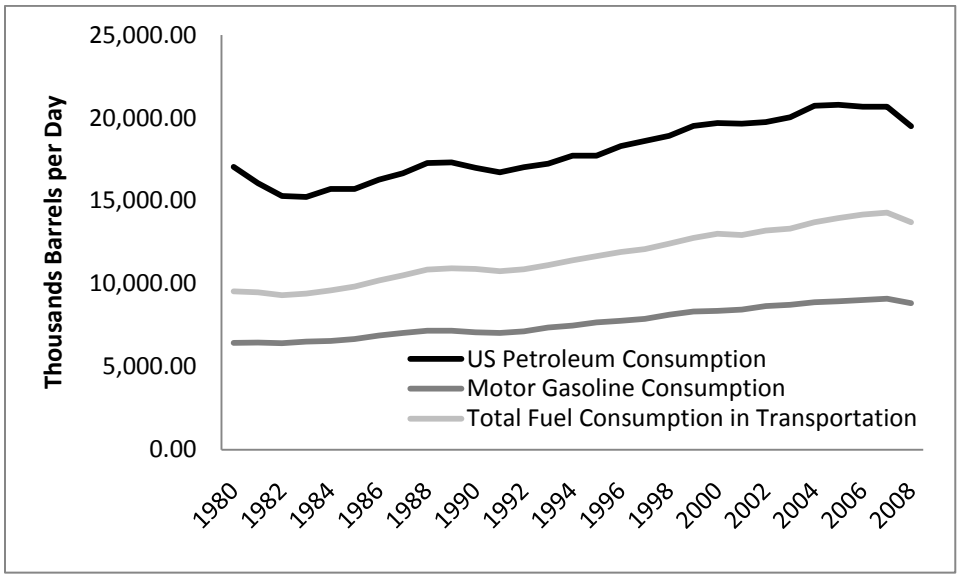

Figure 1 Comparison of petroleum, gasoline , and total transportation fuel consumption in the US from 1980 to 2008. Data reproduced from U.S. Energy Information Administration (EIA)

Today the U.S. petroleum imports reached over 11 million barrels per day, an all-time high point. It is clear we must act and change our habits. The availability of petroleum, a fossil fuel, is a limited resource and additionally there is an increase demand for this resource from such countries as China or India[4, 5].

There are several ways to decrease dependency of petroleum, including new fossil fuels resource utilization, increased energy efficiency of gasoline engines, rewards for less fossil fuel usage, and renewable energy development. Specfically bio-ethanol has gained rapid attention.

The production of ethanol needs raw materials, which are grouped in three classes; starch containing crops, sugar containing crops, and cellulosic biomass. Bio-ethanol refers to alcohol produced from lignocellulosic biomass whereas alcohol produced from food crops refers to

grain alcohol[6]. Grain alcohol is also known as first generation ethanol and is the major topic involved with the food versus fuel debate[7]. Its technology is well established and countries, such as Brazil, proved that it is possible to commercialize first generation ethanol and reduce the cost of the production significantly[8]. Due to its competition for food, countries such as China, started regulating the production of first generation ethanol[9].

Cellulosic biomass materials used for bio-ethanol production include agricultural residues, which include corn stover, bagasse, rice straw, wheat straw, woody materials, such as softwood and hardwood, wastes from the paper and pulp industry, and herbaceous materials, like switch grass.

Cellulosic biomass is considered a solution for the future. Plant biomass is available on earth in huge amounts with yearly production of 200 x 10^9 tons[10]. The cost of lignocellulosic biomass is less than gas, oil or any other energy source. Its chemical and energy input needed for production are lower than any other energy source[13,14,15]. The bio-ethanol industry will increase national energy security, create new jobs, and prevent an increase of greenhouse gases in the atmosphere. Ethanol produced from cellulosic biomass could reduce the greenhouse gas emissions by 88 %[11,12]. Biomass has a net positive energy balance, according to recent published life cycle assessments for materials such as straw, corn stover or switch grass[16,17].

According to the 'Billion ton study,' published by the Oak Ridge National Laboratory and the USDA in 2005, the United States is able to replace 30% of the petroleum consumption with 1.3 billion tons of biomass[18].

Additionally, there are several other reasons why bio-ethanol is considered as a source of choice used in the transportation sector for gasoline vehicles over other potential

considerations, such as hybrid electric vehicles, plug-in vehicles or fuel cells vehicles. For example, the production of bio-ethanol is partially based on knowledge developed in the oil refining sector. Other technologies such as fuel cell-based vehicles, are not ready for the market and need significantly more development while plug-in vehicles have started to enter the market. It is clear that their batteries, made of valuable compounds, can only be produced in certain amounts and not everyone will be able to afford the technology. Ethanol can already blend well with common gasoline up to 25 % without damaging current vehicles[19]. Therefore, it can directly close our gap of production and consumption of petroleum.

1.2 The biorefinery concept

Typical refinery uses petrochemical materials to produce a wide range of fuels (e.g, diesel, LPG, domestic fuel oil, gasoline, etc.) and high value chemicals. This wide range of products ensures a high economic efficiency of a petrochemical refinery. Thus, this type of refinery serves as a template for the future biorefinery, where one feedstock (raw material) could be the platform for a wide range of products converting biomass into high value bio-based chemicals, fuels and power.

Feedstock and technology lead to different biorefinery concepts, which are distinguished and based on different platforms, such as the thermochemical (syngas, etc), biogas and biochemical (sugar based) platform as shown in Table 1.

Table 1 Characteristics of biorefinery platforms with their products. Reproduced with few modification from 'Biorefinery technologies for biomass upgrading'[20]

Platform	*Platform material*	*Conversion Process*	*Products*
Thermochemical	Lignocellulosic biomass/ plastics. Etc.	Gasification	Syngas (also hydrogen, methanol ect.)
	Lignocellulosic biomass/ plastics	Pyrolysis	Pyrolysis oil (any chemicals, hydrogen)
Biochemical platform	Sugar and starch	Fermentation	Bioethanol
	Lignocellulosic biomass	Fermentation	Bioethanol
Biogass	Manure	Anaerobic digestion	Biogas
Oil (plants)	Plant oils	Extraction and esterification	Biodiesel

1.3 Thermochemical platform

The thermochemical biorefinery can heat up the biomass with or without oxygen, which produces syngas or pyrolysis oils. For example, pyrolysis generates sugars, esters, ketones, acids, aldehydes, phenolics and other valuable compounds, whereas syngas generates compounds, such as CO, CO2, and H2[21]. The advantage of the thermochemical process is that it does not distinguish between feed stocks; any biomass or waste can be converted. Both production methods can create products that can be further converted to high valuable chemicals or fuels.

Currently, the biogas or biomethane platform, produces mainly gas through microbiological digestion of liquid manure. This production can be economic depending on the farm size[22]. Microbiological digestion is also possible through digestion of sludge or waste products from the food industry, which increases the resources of biogas platform[23].

1.4 Biochemical platform

The biochemical platform is based on fermentation or sugars, which is performed at lower temperatures, but also has lower reaction rates. Bioethanol production is an example for a biochemical production platform.

The diversity of biomass feedstock leads to different types of biorefineries, producing biomass-ethanol. The development toward this future biorefinery is seen in the three main biomass-to-ethanol biorefinery concepts, where bio-ethanol is produced from food crops or non-food crops and where non-food crops serve not only for the production of bio-ethanol but also for simultaneous production of co-products.

For example, today biorefineries such as the corn to ethanol biorefinery, are well established and distinguished by two major processes to produce ethanol from corn by dry-grind and wet mill. The dry grind process consists of grinding, cooking, liquefaction, saccharification, fermentation and extraction of the ethanol[52]. The wet mill process extracts fibers, gluten before saccharification and fermentation is conducted[24,52]. It is a process mainly focused on by-product production with ethanol as a secondary product. Bio energy production is also the focus of a lignocellulosic biorefinery; however, it uses the whole biomaterial and increases the efficiency of a biorefinery[9,20]. The lignocellulosic biomass to ethanol concept is still under development and has not been implemented on an industrial scale. One step further, at an integrated biorefinery, biomass serves as a template for fuel production and high value chemicals from biomass is a future vision where progress is expected in the near future.

1.5 Lignocellulosic biorefinery

1.5.1 Components of Lignocellulose

The lignocellulosic biorefinery uses different feedstocks, such as crop and forest residues or grasses. From all these possible lignocellulosic materials, corn stover based lignocellulosic material seems to have the highest potential in quickly building a fuel ethanol industry in the U.S. Corn-based ethanol production increased from 1980 to 2000 from 175 million gallons to 1.63 billion gallons[24]. The corn – based ethanol industry established a solid infrastructure and logistics which could be used by corn-based lignocellulosic biorefineries. All lignocellulosic materials have a common structure built of three biopolymer units: cellulose (40-50 %), hemicelluloses (25-35%) and lignin (15-20%)[25]. In order to open the lignocellulosic biomass and produce valuable chemicals one has to change the chemistry of cellulose, hemicellulose and lignin.

1.5.2 Cellulose

Cellulose is a linear biopolymer of glucose units, which are linked by β-(1,4)-glucosidic bonds. The cellulose chain has strong tendency to form intra- and inter molecular hydrogen bonds or van der Waals bonds using the hydroxyl groups of other linear cellulose chains. These chains are 'bundled' together and form cellulose fibrils. This chemical property of the β-(1,4) linkage gives cellulose a more crystalline appearance and makes it more insoluble and resistant to depolymerization[26,28]. The crystalline appearance is widely found in biomass; however, unorganized cellulose chains can also be found. The amorphous form is more reactive to chemical or enzymatic attack.

1.5.3 Hemicellulose

Commonly xylan chain serves as the backbone for hemicellulose with its various branches of arabinose, galactose, mannose, etc. The backbone can be a homopolymer or a heteropolymer with branches being linked by β-(1.4) or β-(1.3) glycosidic bonds. Hemicellulose is more susceptible to chemical attack and builds a matrix by connecting lignin and the cellulose fibers. This feature provides more support to the lignin-cellulose-hemicellulose structure[27,28].

1.5.4 Lignin

Lignin is a phenolic polymer consisting of three monomers: coniferyl alcohol, coumaryl alcohol, and sinapyl alcohol. The amount of the units determines the characteristics of wood, where softwoods contain more coniferyl and sinapyl alcohol and hardwoods contains mostly coniferyl units. Lignin provides the plant structural support, protection against microbial attack and water resistance[27,28].

1.6 Pretreatment of lignocelluloses

1.6.1 Goal of pretreatment

The requirement for the bio-ethanol production is that chain polymers in biomass, hemicellulose and cellulose, have to be broken down (hydrolysis) into their monomer units. Cellulose releases mainly D-glucose units whereas D-xylose, D-glucose, D-rahmanose, D-mannose are mainly released from hemicellulose[29]. The National Renewable Energy Laboratory's (NREL) concept of a corn stover based lignocellulosic ethanol biorefinery is shown in Figure 2 with its essential bio-ethanol production units. Key steps in Figure 2 are the pretreatment, hydrolysis of cellulose and hemicelluloses to sugars, fermentation of the sugars to

ethanol and finally recovery of bio-ethanol (grey highlighted squares in Figure 2). Most important role is found in the pretreatment of lignocellulosic biomass, since it is an expensive unit in the bio-ethanol production scheme. Pretreatment of biomass is necessary, as seen with the yield of cellulose hydrolysis at about 20 % of the theoretical before treatment and over 90 % of theoretical after treatment[30]. During pretreatment the matrix of hemicellulose, cellulose and lignin must produce the following results: Lignin should be released from the matrix or be degraded; hemicellulose should be as much as possible hydrolyzed, and cellulose should have a reduced crystalline structure. The fraction of amorphous cellulose should be increased[28].

Additionally, pretreatment should have minimal loss of sugars, produce minimal formation of degradation products and be cost effective since it accounts for 18 % of cellulosic ethanol production[31].

These requirements lead to the development of various pretreatment methods which are of physical, thermal or chemical in nature. Only few methods seem to be promising and are discussed in the next paragraph.

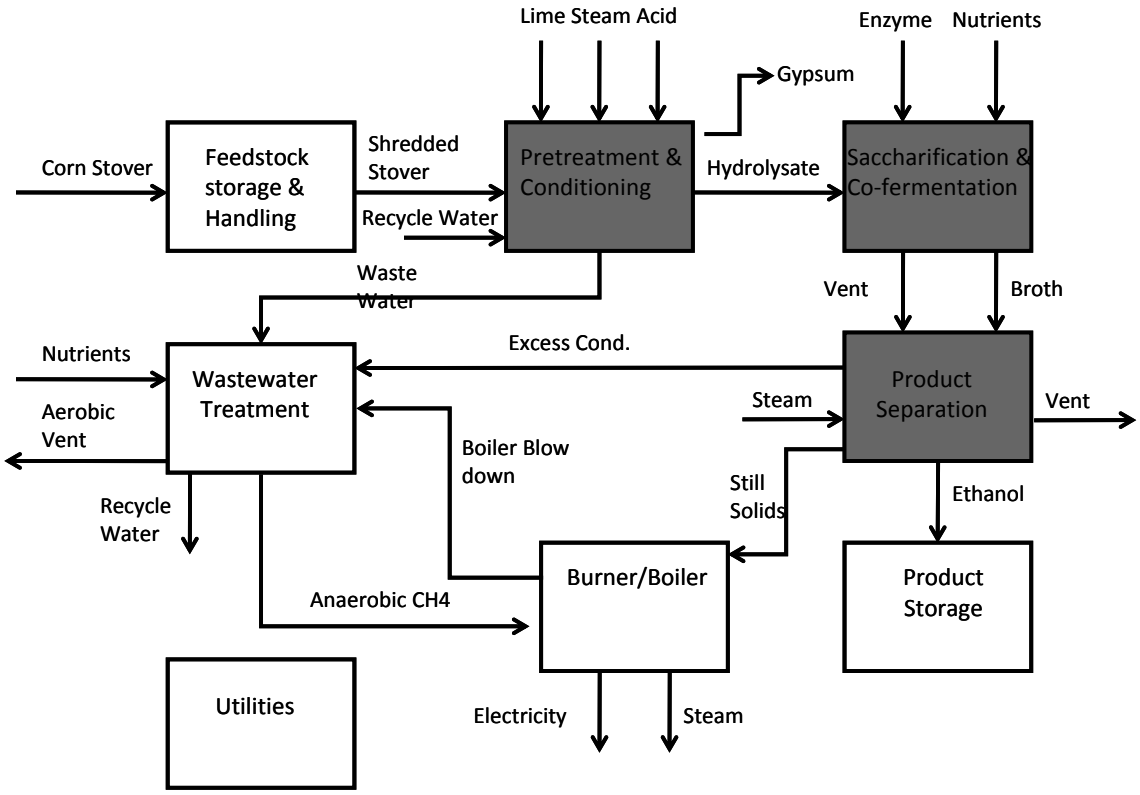

Figure 2 Reproduction of NREL cellulosic biomass to ethanol process, PFD-P110-AOOO. Technical report (NREL0TP-510-32438, June 2002[57])

1.7 Pretreatment methods

1.7.1 Physical pretreatment

Cutting lignocellulosic biomass into small particles is known as a mechanical pretreatment method. This increases the specific surface area, reduces crystallinity, and, thus, increases the total hydrolysis yield for most biomass types by 5-25%[28]. No side chemicals, such as furfural or 5-hydroxymethylfurfural (HMF) are produced. However, high energy input is required leading to an uneconomical process[32].

1.7.2 Thermal pretreatment

Heat alone is capable of hydrolyzing parts of hemicellulose and lignin. The temperature range, where hydrolysis begins varies between different biomass types due to different compositions of hemicellulose and lignin[28,33]. The hemicellulose backbone and its type of branches determine mostly the thermal stability of hemicellulose. Solubilization of hemicellulose and lignin starts to appear at temperatures above 160 °C and leads to formation of acids (e.g., acetic , formic, levulenic acids), phenolic compounds (e.g., HMF, furfural) and heterocyclic compounds(e.g., vanillin, vanillin aclcohol). Heat is used as steam or liquid hot water, and acids or alkaline can be added to increase hydrolysis yields[34].

Pressurized steam and high temperature (temperatures up to 240 °C) are applied to biomass for a few minutes to hydrolyze hemicellulose and open cellulose structure for enzymatic hydrolysis[34]. Another type of action is known as steam explosion, where a quick depressurization and cooling causes water molecules to explode in the biomass matrix. The liquid hot water method uses hot water for pretreatment, instead of steam. The ammonia fiber explosion process is based on same principle as steam explosion with liquid ammonia addition and is a type of thermal-chemical method[34].

1.7.3 Chemical treatment

Alkalis, acids, peroxide or organic solvents can be used in the chemical pretreatment area. The alkaline pretreatment mode uses chemicals, such as sodium, ammonium or calcium hydroxide, which causes swelling of the biomass, and thus, increases the digestibility as shown on hardwoods from 14% to 55 %[36]. Alkaline treatment cause more a solubilization of lignin and less solubilization of cellulose or hemicellulose[36,37]. The treatment with lime has been explored

on corn stover and removes acetyl groups and lignin leading to an enhanced digestibility of cellulose[38].

Pretreatment with acids is conducted at high acid or low acid concentrations. Both processes require equipment that is resistant to corrosion, but high acid treatment is less economic because recovery of acid is necessary[39]. Low acid treatment at concentrations below 4 wt % is a promising treatment method, but produces more degradation products because higher temperatures are used, but can produce good monomeric xylose yields of 77 % or greater when applied to corn stover[40].

Organic solvents including acetone, ethylene glycol, methanol, peracetic acid, ketones and phenols were used mainly for solubilization of hemicellulose and lignin[41]. Drawbacks of organic solvent use are the additional washing step of solids to prevent precipitation of lignin and recovery of solvents using distillation. Additional drawback may be the biocompatibility of solvents during fermentation, solvents might be inhibitory for the fermenting organisms in small amounts and need to be recovered completely. The recovery step may lead to an uneconomic process.

1.7.4 Summary

A summary of most promising pretreatment methods is given in Table 2. Up till now all of the methods possess advantages and disadvantages.

Table 3 Summary of promising pretreatment methods with their advantages and disadvantages. Data reproduced with changes from 'Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review' [39]

Pretreatment method	*Advantages*	*Disadvantages*
Milling	-Reduces crystallinity -No detoxification necessary	-High power and energy consumption
Steam explosion	-Lignin transformation and hemicellulose solubilization -No recovery of chemicals	-Partial hemicellulose degradation -Release of inhibitory compounds -High equipment and energy cost
AFEX	-Increases surface area -Low formation of inhibitors- no detoxification step necessary	-High consumption and cost of chemicals -Low lignin biomass required
Alkaline	-Low energy consumption	-Long pretreatment time - Little hemicellulose hydroysis
Concentrated acid	-High sugar release -Ambient temperatures	-Need corrosion resistant equipment -Recovery of acid necessary
Diluted acid	-Less formation of inhibitors -Few corrosion problems	-Generation of degradation Products - Need corrosion resistant equipment
Organosolv	-Hydrolysis of lignin and hemicellulose	-Recovery of solvent necessary -High cost of solvents
Wet oxidation	-Low formation of inhibitors -Good removal of lignin	-High equipment, oxygen and alkaline catalyst cost
Biological	-Low energy input -Degrades lignin and hemicellulose	-Low hydrolysis titer -Long residence times

1.8 Detoxification of biomass and NREL's detoxification status

Hydrolysis of biomass releases not only fermentable sugars but also several compounds that are considered to be toxic to the fermenting microorganism. These compounds are mainly grouped into three classes: furans, phenolics derivates and weak acids[29,35].

Figure 3 shows the source of furans, such as 5-hydroxymethylfurfural, or furfural, and weak acids, such as formic and acetic acid. Dehydration of glucose or pentose sugars like D-Rhamnose leads to 5-hydroxymethylfurfural formation. Furfural is mainly a product from xylose or arabinose dehydration. Lignin releases mainly phenolics compounds[28]. Acetic acid is due to acetylation of hemicellulose and partially from the lignin[35,43]. Acetic acid, HMF and furfural represent classes of compounds that inhibit growth and ethanol production by interacting in vitro with the microorganism[35,42,43]. The presence of one compound can enhance the inhibitions of other present compounds on microorganisms. This synergetic inhibition was proven with *S.cerevisiae* with furfural and acetic acid producing a negative effect on growth rate, cell mass production, and ethanol yield. Thus, it is essential to remove inhibitors to a minimal level[44].

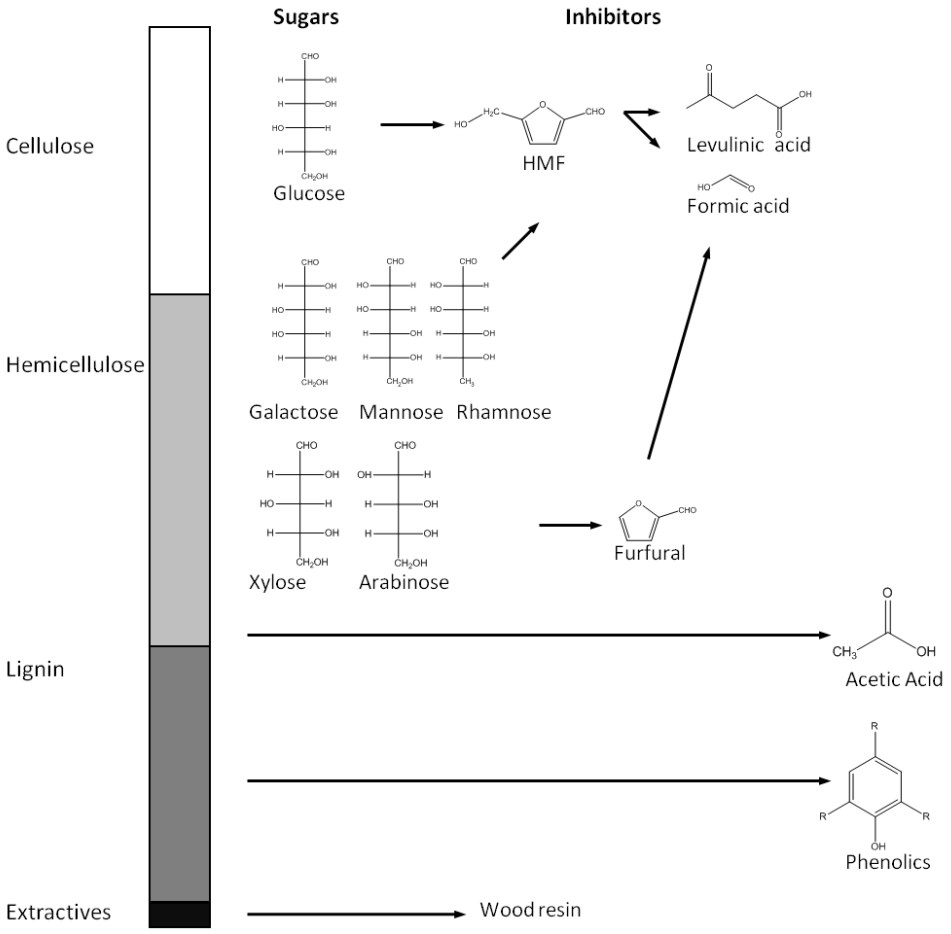

Figure 3 Average composition of lignocellulosic biomass and main derived hydrolysis products, reproduced from 'Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by *Saccharomyces cerevisiae*'[43]

Composition of toxic compounds depends on the type of lignocellulosic biomass and on the applied pretreatment method with its time, pH, temperature or pressure conditions. This diversity sparked the development of different detoxification methods, which can be biological, chemical or physical.

Biological methods use specific enzymes or microorganisms that act on phenolic compounds, such as HMF and furfural, as shown on spruce and wheat straw[45,46]. Vacuum evaporation is used as a physical method reducing the content of volatile compounds, such as

furfural or acetic acid[47]. Chemical methods include activated charcoal, diatomaceous earth, ion exchange or overliming or a combination of all mentioned methods.

A comparison of detoxification methods is difficult due to the inhibitory tolerance levels of microorganisms in the subsequent fermentation process[35].

Detoxification methods, that were applied to lignocellulosic biomass showed both positive and negative outcomes. No method is capable of removing all toxic compounds. Some methods decrease the fermentable sugar content or even dilute the hydrolysate producing lower ethanol titers[45,47]. Combination of two or more detoxification methods on same biomass hydrolysate (poplar) significantly improved ethanol titers[48]. The economic evaluation had not been performed and could show that it is impracticable to combine two detoxification methods.

Detoxification of biomass hydrolysate has not been conducted with a membrane supported liquid-liquid extraction as we proposed.

1.8.1 NREL's detoxification method

Figure 2 shows NREL's biorefinery concept from 2002, where overliming is performed on pretreated corn stover hydrolysate. The overliming process brings the hydrolysate first to pH 10 and then pH is adjusted to enzymatic hydrolysis or fermentation pH conditions of 5.0 to 6.0 using sulfuric acid. Additionally, this process produces gypsum, which is removed using hydrocyclone and rotary drum filtrations. Recent research shows that significant sugar loss occurs of 13% xylose and 12% glucose[49].

NREL's investigations on detoxification of lignocellulosic biomass are moving toward ammonium hydroxide treatment. Overliming as well as ammonium hydroxide method uses the same treatment procedures. As with the overliming process, the goal of ammonium hydroxide

treatment is to produce a less toxic biomass for further fermentation steps. This new process is implemented in the current, but not yet published 2010 NREL process model. We will compare our membrane process to the ammonium hydroxide process.

1.9 Possible usage of membranes in the biorefinery

The introduction of membranes into the production stream of a bioethanol biorefinery with second generation biofuels could reduce the cost of separation and purification significantly. Current separation processes account for up to 80% of the processing costs of most chemical processes[50]. It is expected that this will be similar in a lignocellulosic biorefinery. Membranes could be introduced at different stages in the process scheme of a second generation biofuel biorefinery to enhance the quality of existing bio-products and reduce high production costs. Conceptually, this has been investigated for biorefineries of first and second generation biofuels[51.52]. For example, at the beginning of a second generation biorefinery process, valuable compounds can be directly recovered using membranes. Fractionation of black liquor has been demonstrated using ultrafiltration membranes. The 5 and 10 kDa lignin fractions are possible adhesives, which are considered to be valuable by-products[53]. Other valuable biomass by-products such as 5-Hydroxymethylfurfural and furfural could also be recovered using membranes processes. This method has not yet been explored. Traditionally, recovery of bioethanol from fermentation broth is conducted via distillation. This last step in a biorefinery could be conducted with the help of pervaporation. Pervaporation with silicalite membranes of bioethanol-fermentation broth has been demonstrated by several research studies[54,55]. More research must be conducted to reduce the potential threat of acids to pervaporation

membranes. However, significant cost reduction may be realizedby using membranes for biomass hydrolysate treatment.

Biomass hydrolysate treated with ion exchange membranes showed a higher acetic acid capacity with ion exchange membranes than ion exchange resins. Sugar losses were noticed, but were lower than using ion exchange resins[56].

1.10 Motivation

Another possible application for membranes is for detoxification of biomass hydrolysate using extractive liquid-liquid membrane configurations. Membranes using a hollow fiber configuration are contacted with fluids on both sides on the membrane, where extraction is conducted on the interface of each membrane pore. Diffusion of toxic compounds from the aqueous phase into the organic phase leads to a less toxic hydrolysate, thus higher pontentially increasing ethanol yields. This system has several advantages over conventional chemical processes, such as easy scale up, no emulsion formation and higher efficiency.

Our goal is an economic replacement of current detoxification methods with extractive membrane supported liquid-liquid extraction method.

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CHAPTER 2

MEMBRANE EXTRACTION FOR REMOVAL OF ACETIC ACID FROM BIOMASS HYDROLYSATES

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Abstract

Production of bioethanol from lignocellulosic biomass requires pretreatment of the biomass in order to improve the susceptibility of the cellulose to enzymatic hydrolysis to glucose. When dilute acid is used to perform this process, the hemicellulose is also hydrolyzed to its component sugars while simultaneously releasing acetyl groups attached to the hemicellulose backbone. Other compounds from the lignin and sugar degradation products are also produced that inhibit subsequent bioconversion of the solubilized sugars to the desired

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products. In this work we focused on removal of acetic acid from a dilute sulphuric acid pretreated corn stover hydrolysate.

Acetic acid has been extracted into an organic phase at pH values below its pK_a. The organic phase diluent consisted of octanol. Alamine 336, a tertiary amine and Aliquat 336 a quaternary amine were used as the aliphatic amine extractants. Our results indicate more than 60% removal of acetic acid using Alamine 336. Extraction rates were much slower for Aliquat 336 probably due to the higher viscosity of the Aliquat 336/octanol phase.

The presence of sulphate anions, as a result of dilute sulphuric acid pretreatment, results in the co-extraction of bisulphate anion. Bisulphate anion is preferentially extracted at pH values below its pK_a. Consequently the pH of the hydrolysate increases from between 1 and 2 to above 4.0 during extraction. In addition, extraction of low molecular weight lignins and phenolics is also observed. Thus the membrane extraction process developed here may be used not only for removal of acetic acid but also to adjust the pH of the hydrolysate to values that are more compatible for fermentation and to remove other inhibitory compounds.

Key words: Aliphatic amine extractant, acetic acid, membrane extraction, hydrolysate, sulphuric acid

2.1 Introduction

Biomass represents a renewable resource for the production of biobased products and biofuels. However a major obstacle to the large-scale industrial production of these materials is the lack of cost-effective separation methods for product isolation and purification[1], as separations currently account for 60-80% of the processing costs of most mature chemical processes.

Lignocellulosic biomass consists of three main polymers: cellulose, hemicellulose and lignin. Before fermentation, the biomass has to be hydrolyzed to release sugars from the polymeric matrix. Different processes have been developed to hydrolyze hemicellulosic sugars from lignocellulosic materials. Dilute sulphuric acid pretreatment is commonly used because it is effective at producing a xylose-rich hemicellulose hydrolysate liquor while enhancing cellulose enzymatic digestibility[2].

During pretreatment, toxic compounds are produced that inhibit subsequent bioconversion of the solubilized sugars to the desired products. For example, acetic acid is produced as a result of hydrolysis of acetyl groups present in the hemicellulose. Further depending on the severity of the pretreatment conditions the pentose-based sugars that are produced from the hydrolysis of hemicellulose may be further hydrolyzed to furfural. Recently Bower et al[3] have shown that corn stover contains appreciable amounts of sucrose. Depending on the severity of the pretreatment, this sucrose may be hydrolyzed to 5-hydroxymethylfurfural, which could be further hydrolyzed to levulinic and formic acid. Removal of these toxic compounds is essential in order to maximize sugar yields. Here we focus on acetic acid removal.

In its protonated form, acetic acid can diffuse through the cytoplasmic membrane of cells and detrimentally affect cell metabolism[4,5]. Undissociated acetic acid can diffuse through the cell cytoplasm, where it lowers the intracellular pH, resulting in impaired transport of various ions and increased energy requirements[6]. The sensitivity of the microorganism to acetic acid depends on the operating conditions[7]. Acetic acid concentrations in lignocellulosic hydrolysates can be up to several grams per litre. However, Maiorella et al[8] note that concentrations as low as 0.25 g L^{-1} can affect growth and reduce the rate of ethanol production, indicating the importance of acetic acid removal. This is particularly important, as the economic viability of a cellulosic ethanol plant will depend on the efficiency of ethanol production. The amount of acetic acid produced depends on the lignocellulosic biomass. In general, herbaceous biomass produces less acetic acid than an equivalent amount of woody biomass. Thus, in the past removal of acetic acid from herbaceous biomass after pretreatment has not been considered necessary. However, given the trend towards higher solids loadings (more than 30% during pretreatment), the acetic acid concentration in hydrolysate liquors is expected to rise.

Recovery of carboxylic acids such as acetic acid is important in a number of industries. While acetic acid is a toxic by product during the production of cellulosic bioethanol, carboxylic acids such as lactic acid are frequently produced by fermentation[9]. In addition removal of caroboxylic acids is also important from aqueous waste streams in the petrochemical, chemical and pulp and paper industries[10]. Classical methods for recovering low-volatility carboxylic acids involve formation of the insoluble calcium carboxylate salt[11]. Besides being energy intensive and consuming 1 mol of a mineral acid such as sulphuric acid and calcium base per mol of carboxylic acid produced, calcium acetate is a soluble salt. Here we have investigated the use of Alamine 336 a long chain water insoluble tri-octyl/decyl amine and Aliquat 336 a water insoluble

quaternary ammonium salt made by the methylation of mixed tri-octyl/decyl amine. These amine extractants were dissolved in octanol.

Long chain aliphatic amines may be used for the extraction of carboxylic acids from dilute aqueous solutions[12,13,14,15,16,17,18,19,20,21]. An amine extractant is dissolved in an organic solvent known as the diluent. Choice of the diluent is important as it can help stabilize the amine-carboxylic acid complex thus leading to greater extraction of the carboxylic acid. The diluent also helps to control the viscosity and density of the organic phase.

In this work we focused on extraction of acetic acid from corn stover hydrolysates produced by dilute sulphuric acid pretreatment. After pretreatment, the pH of the hydrolysate is between 1 and 2. Thus important variables that will affect extraction of acetic acid are: co-extraction of species such as H_2SO_4, HSO_4^- and SO_4^{2-} and the diluent and amine extractant used. In the next section we summarize the considerable body of literature relevant to extraction of acetic acid in the presence of sulphate and bisulphate anions using Alamine 336 and Aliquat 336 dissolved in octanol.

For this work we developed a hollow fibre membrane-based liquid extraction system for extraction of acetic acid. Schlosser et al.[22] present a summary of published studies on the use of membrane extraction for the recovery of various carboxylic acids. Membrane extraction offers a number of advantages over conventional liquid extraction. Traditionally, liquid extraction is conducted using a number of mixer-settlers in series or a continuous, counter-current extraction column. However, irrespective of the equipment used, all conventional extraction processes suffer from a number of disadvantages. These include the dispersion of one phase in the other,

which requires subsequent coalescence of the dispersed phase after extraction; emulsification problems; flooding and loading concerns; and difficulty of scale up.

Microporous hollow fibres can overcome all of these disadvantages. In our experiments, the organic octanol phase is pumped outside polypropylene hollow fibres while the aqueous hydrolysate is pumped inside the fibres. The organic phase/aqueous phase interface located at the inside surface of the fibres (as the fibres are hydrophobic) is stabilized by maintaining the pressure of the aqueous phase at a value equal to or greater than the organic phase pressure[23]. The hollow fibre membrane immobilizes the organic phase/aqueous phase interface. Importantly, extraction is achieved without dispersion of one phase in the other, so no coalescence step is needed. The flow rates of the aqueous and organic phases can be varied independently of each other over a wide range of flow rates. Finally, the interfacial area for mass transfer can be very large, leading to rapid extraction[24,25,26,27].

The theory of hollow fibre-based liquid extraction is well developed[28]. It is assumed that the overall mass transfer coefficient depends on three individual mass transfer coefficients, which describe mass transfer across a concentration boundary layer in each of the phases and through the membrane. Numerous correlations are available in the literature for predicting the mass transfer coefficient inside the fibres, outside the fibres and in the membrane pores[29].

2.2 Theory

The use of primary, secondary, tertiary and quaternary amines for extraction of carboxylic acids has been described in the literature[11]. For effective extraction of acetic acid it is essential that the aliphatic amine has minimal solubility in the hydrolysate. However, longer carbon chains results in an increased organic phase viscosity. Aliphatic amines with less than six

carbon atoms per chain are poor extractants due to their water solubility[12]. Kertes and King[12] note that the extraction power of aliphatic amines depends on the basicity of the amine. In general this is greatest for tertiary amines though the organic phase diluent has a marked effect on the proton association constant. In addition primary and secondary amines often form gels. Unlike primary, secondary and tertiary amines, the capacity of quaternary amines does not drop off at pH values approaching the pK_a of acetic acid (4.76). In this work, we use Alamine 336 and Aliquat 336, a commercially available tertiary and quaternary amine. Both amines have been frequently used in previous studies. They offer a good compromise between low water solubility and organic phase viscosity.

An ideal organic phase diluent is immiscible with water and has a low viscosity. Octanol offers a good compromise between low water miscibility and viscosity. Further octanol stabilizes the acid amine complex by hydrogen bonding. Tamada et al.[13] indicate that overloading, i.e., loading greater than 1 of the amine is possible. Loading (Z) is defined as the concentration of acetic acid in the organic phase due to complexation with amine divided by the amine concentration. Depending on the percentage of amine in the organic phase and the volume of the organic phase relative to the aqueous phase, it may be necessary to account for the solubility of acetic acid in octanol. Here we determine the total amount of acetic acid that transfers to the organic phase from the change in the aqueous phase concentration. We then subtract the amount of acetic acid that would have transferred into the same volume of octanol in the absence of amine in order to determine the amount of acetic acid that complexes with the amine. Overloading indicates that complexes with more than one acetic acid molecule per amine have been formed.

A unique feature of the hydrolysates being treated here is the presence of both a mineral (sulphuric) and carboxylic acid. The first and second dissociation constants for sulphuric acid are 10^{3} and 10^{-2}. Consequently at the pH values after pretreatment of 1-2, sulphuric acid will be present almost entirely as HSO_4^- and SO_4^{2-}. Eyal and Canari[21] describe four major mechanisms for acid extraction by amine-based extractants. Ion pair formation occurs when the amine is basic enough (and the acid strong enough) to bind a proton to form the ammonium cation (for Alamine 336). Given that sulphuric acid is a strong acid, it is likely that this mechanism will lead to significant removal of sulphuric acid from the hydrolysate according to the following expressions:

$$R_3N_{(organic)} + H^+_{(aq)} \rightarrow R_3NH^+_{(organic)}$$

$$R_3NH^+_{(organic)} + HSO_4^-{}_{(aq)} \rightarrow R_3NH^+HSO_4^-{}_{(organic)}$$

At pH values well below the pK_a of acetic acid protonated acetic acid will be extracted. Barrow and Yerger[30] indicate that the acetic acid molecule reacts with the amine to form an ion pair. Further, overloading of the amine is possible by hydrogen bonding of a second acid molecule to the carbonyl oxygen of the first acid molecule. Octanol is an 'active' diluent that is able to hydrogen bond to the acid amine complex thus stabilizing it.

Since Aliquat 336 is a quaternary amine, extraction of sulphuric acid will occur by ion exchange thus maintaining charge neutrality in the organic phase. At pH values well below the pK_a of acetic acid the protonated form of acetic acid will be extracted. Importantly unlike Alamine 336, as the pH approaches and becomes greater than the pK_a of acetic acid, acetate

anion may be extracted by ion exchange. Reisinger and King[20] indicate that acetic acid may be extracted by Aliquat 336 at pH values above 12.

2.3 Experimental

Initial batch extraction experiments were conduced using acetic acid in DI water in order to determine the level of overloading if any of the amine. Acetic acid (cat. Nr. 279307), Aliquat 336 (cat. Nr. 205613), and octanol (cat. Nr. 297887) were obtained from Sigma-Aldrich (Sigma-Aldrich Corp. St. Louis, MO). Alamine 336 was obtained from Cognis (Cincinnati, OH). In each experiment, 4 mL of an aqueous phase containing acetic acid was contacted with 4 mL of the octanol/amine organic phase in a 15-ml centrifuge tube. Table 1 gives the actual compositions of the aqueous and organic phases. The solution was shaken for few seconds using Fisher Scientific Vortex Genie shaker (Thermo Fisher Scientific, Inc. Waltham, MA), after which it was allowed to settle for 10 minutes. Next it was centrifuged at 300-500 rpm for 10 min using Beckman benchtop centrifuge (Beckman Coulter, Inc. Fullerton, CA). The lower aqueous phase was removed by pipeting and the residual acetic acid concentration was determined using a 1050 HP HPLC equipped with a Biorad Aminex HPX-87 H column and a HP refractive index detector (Quantum Analytics, Inc. Foster City, CA) . For the mobile phase, a 10 N sulphuric acid solution was diluted to 0.01 N with HPLC grade water and prefiltered using a 0.2 µm filter. A series of calibration standards and calibration verification standards (CVS) were obtained from Absolute Standards Inc., Hamden, CT. The flow rate was set at 0.6 mL/min at a column temperature of 55 °C.

Table 1 Compositions of the aqueous and organic phases for batch extraction of acetic acid

Acetic acid concentration (wt%)	*Alamine: octanol (mL)*	**Aliquat :octanol (mL)**
1, 5, 10	1:3, 2:2, 3:1	1:3, 2:2, 3:1

Membrane extraction experiments were conducted using two different LiquiCel Membrane Contactors (Membrana, Charlotte, NC). Details of these two modules are given in Table 2.

Table 2 Specifications of the two hollow fibre modules used in the extraction experiments

Module	*MiniModule 1 x 5.5*	2.5 x 8 Extra-Flow
Configuration	Parallel flow	Extra-flow, centre baffle
Membrane	Polypropylene	Polypropylene
Porosity	40%	40%
Fibre OD/ID (μm)	300/220	300/220
Potting material	Polyurethane	Polypropylene
Active surface area (m^2)	0.18	1.4
Priming volume lumen/shell (mL)	16/25	150/400

The experimental set-up for both modules is given in Figure 1. Corn stover hydrolysate, pretreated using 0.1 wt% sulphuric acid was provided by the National Renewable Energy Laboratory and is termed here as the aqueous phase. The organic phase consisted of octanol and Alamine 336 or octanol and Aliquat 336. The various operating conditions used in the experiments are given in Table 3.

Flexible chemical resistant Masterflex tubing, precision silicone tubing 6410-18 and Tygon 2075 tubing were used to connect the two peristaltic pumps, Master Flex HV-77410-10, (all from Cole-Parmer, Vernon Hills, IL) to the LiquiCel module. The system was started by first turning on the aqueous phase pump followed by organic phase pump. The pump speed was

adjusted till the desired flow rate was reached. At all times the aqueous phase pressure was about 0.07 bar above the organic phase pressure. The aqueous phase pH was recorded using a pH meter (Thermo Orion 520) equipped with a Metler Toledo pH probe (Cole-Parmer). At frequent intervals 1 mL samples of aqueous phase from the return tube to the aqueous phase reservoir were removed for HPLC analysis.

Table 3 Operating conditions for the hollow fibre extraction experiments

Module	*Aqueous phase*	**Organic Phase**
MiniModule	1000g hydrolysate flow rate 7.5 L h^{-1}	500g octanol and 500 g Alamine 336 flow rate 3.7 L h^{-1}
MiniModule	1000g hydrolysate flow rate 7.5 L h^{-1}	500g octanol and 500 g Aliquat 336 flow rate 3.7 L h^{-1}
Extra-Flow	1000g hydrolysate flow rate 53 L h^{-1}	500g octanol and 500 g Alamine 336 flow rate 3.7 L h^{-1}
Extra-Flow	1000g hydrolysate Flow rate 165 L h^{-1}	500g octanol and 500 g Alamine 336 flow rate 3.7 L h^{-1}
Extra-Flow	1000g hydrolysate flow rate 165 L h^{-1}	500g octanol and 500 g Alamine 336 flow rate 15.1 L h^{-1}
Extra-Flow	1000g hydrolysate flow rate 165 L h^{-1}	500g octanol and 500 g Alamine 336 flow rate 33 L h^{-1}
Extra-Flow	1000g hydrolysate flow rate 165 L h^{-1}	750 g octanol and 250 g Alamine 336 flow rate 33 L h^{-1}
Extra-Flow	1000g of DI water containing 12 g L^{-1} acetic acid flow rate 165 L h^{-1}	500g octanol and 500 g Alamine 336 flow rate 31 L h^{-1}
Extra-Flow	1000g of DI water containing 12 g L^{-1} acetic acid and 0.1 wt% sulphuric acid flow rate 165 L h^{-1}	500g octanol and 500 g Alamine 336 flow rate 30 L h^{-1}

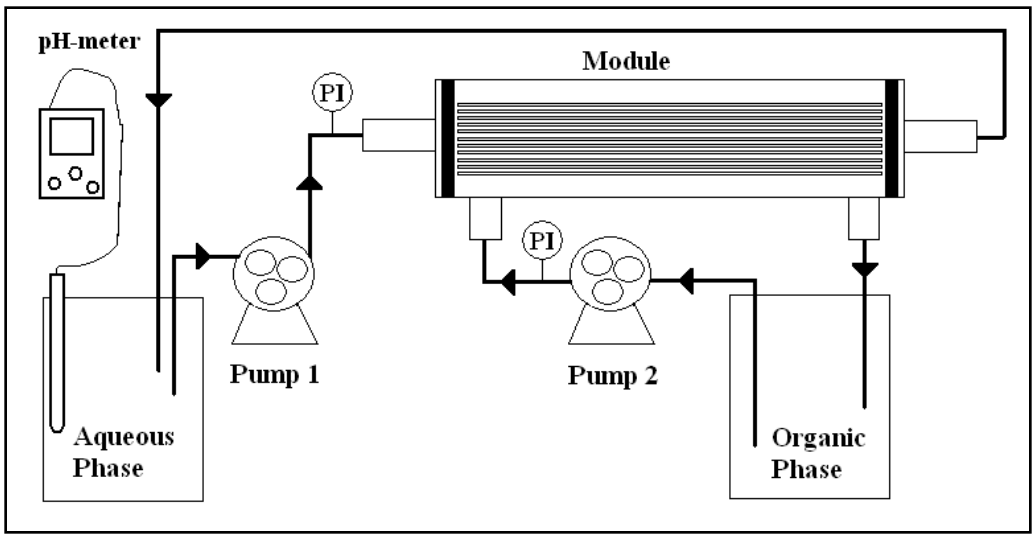

Figure 1 Experimental set-up

Hydrolysate samples before and after extraction were analyzed using a UV/Vis spectrophotometer with an 8 cell sample holder (Agilent model 8453, Santa Clara CA). Agilant UV/Vis Chemstation 845x software was used.

2.4 Results

The variation of acetic acid loading Alamine 336 and Aliquat 336 with percent amine in the organic phase is given in Figure 2. In all experiments the pH of the aqueous phase remained approximately constant at around 3.5. Thus even after extraction, there was sufficient acetic acid present in the aqueous phase to ensure no shift in the pH. At the highest acetic concentration, 10 wt%, overloading of Alamine 336 is observed. However for the range of conditions tested, overloading of Aliquat 336 is not observed. Previous results by Tamada et al.[13] also indicate overloading of Alamine 336 by succinic acid in an aqueous phase consisting of octanol and chloroform.

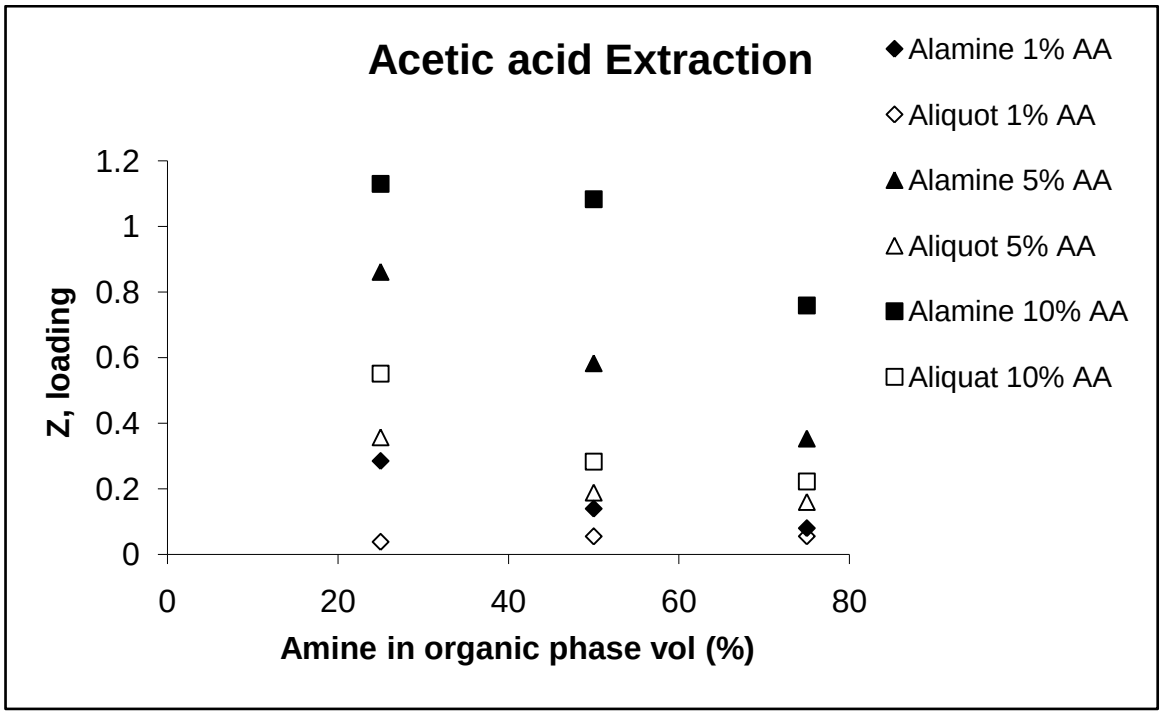

Figure 2 Batch extraction results at various acetic acid (AA) concentrations

Figure 3 gives results for the MiniModule. The left hand side y-axis gives the variation of acetic acid concentration (squares) while the right hand side y-axis gives the variation of pH (triangles). Filled symbols are Alamine 336 while open symbols are for Aliquat 336. At the same aqueous and organic phase flow rates, after 200 minutes of operation Alamine 336 removes about 40% of the acetic acid present while Aliquat 336 removes only about 15%. The pH of the aqueous phase increases significantly for extraction with Alamine 336. Between pH values of 1.5 and 2.5 the pH increases very rapidly. In contrast the pH of the aqueous phase remains almost constant during extraction with Aliquat 336. Given the much more rapid removal of acetic acid using Alamine 336, all experiments with the Extra-Flow module were conducted Alamine 336 only.

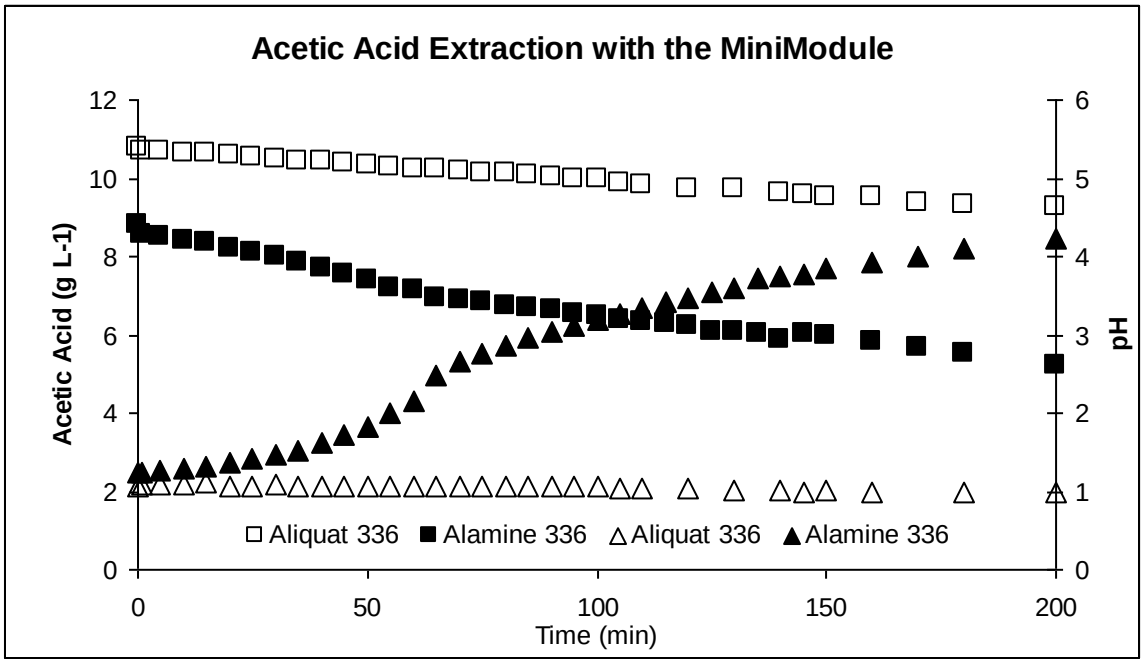

Figure 3 Acetic acid concentration (squares) and pH (triangles) of hydrolysate during extraction with the MiniModule

Figures 4-6 give results for extraction of acetic acid from hydrolyste using the Extra-Flow module. In these three figures the variation of acetic acid concentration with time in the hydrolysate (filled symbols) is read using the left hand side y-axis. The variation of the aqueous phase pH (open symbols) is given by the right hand side y-axis. Figure 4 gives results at two different hydrolysate flow rates that differ by a factor of three. As can be seen there is little change in the variation of acetic acid concentration as a function of time.

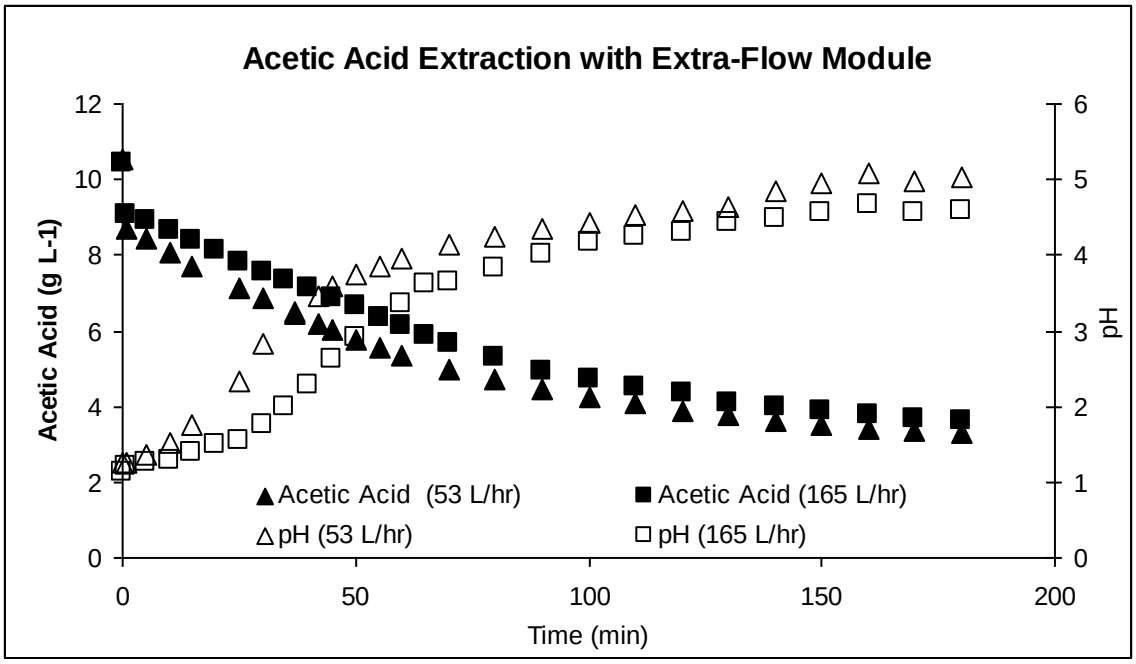

Figure 4 Acetic acid extraction using the Extra-Flow module at hydrolysate flow rates of 53 L/hr^{-1} (triangles) and 165 L/ hr^{-1} (squares)

Figure 5 gives results for three organic phase flow rates. As can be seen, changing the organic phase flow rate by a factor of ten has no effect on the rate of acetic acid removal.

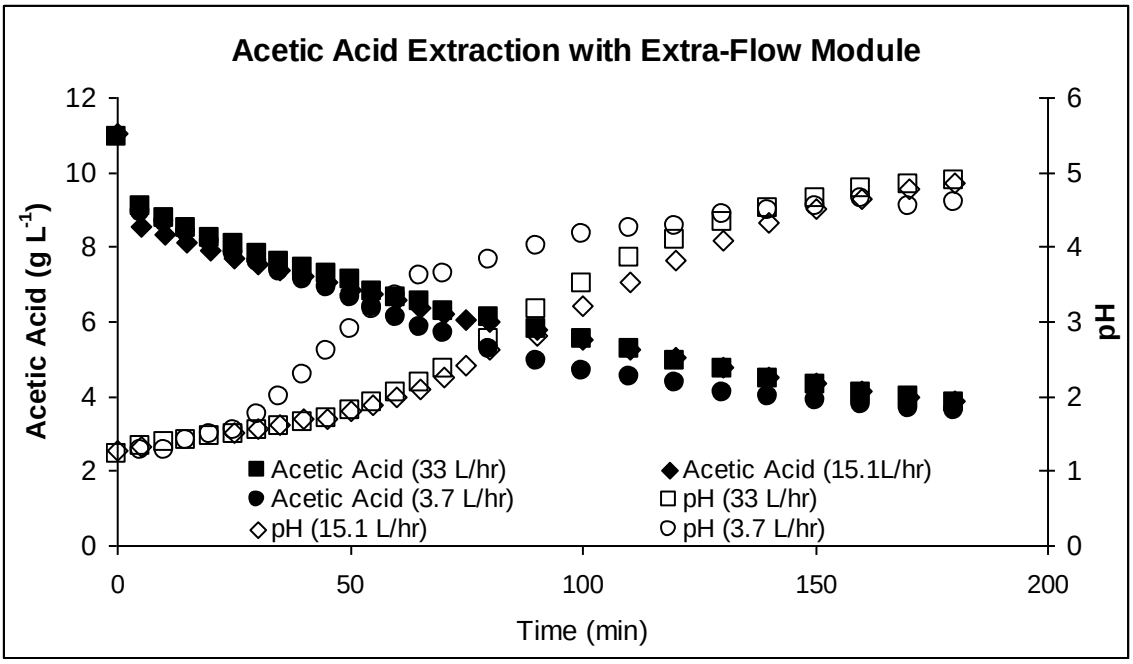

Figure 5 Extraction using the Extra-Flow module at different organic phase flow rates with 3.7 L/h r^{-1} (circles), 15.1 L/hr^{-1} (diamonds), and 33 L/hr^{-1} (squares)

Finally Figure 6 indicates that halving the Alamine 336 concentration in the organic phase has little effect on the rate of acetic acid removal.

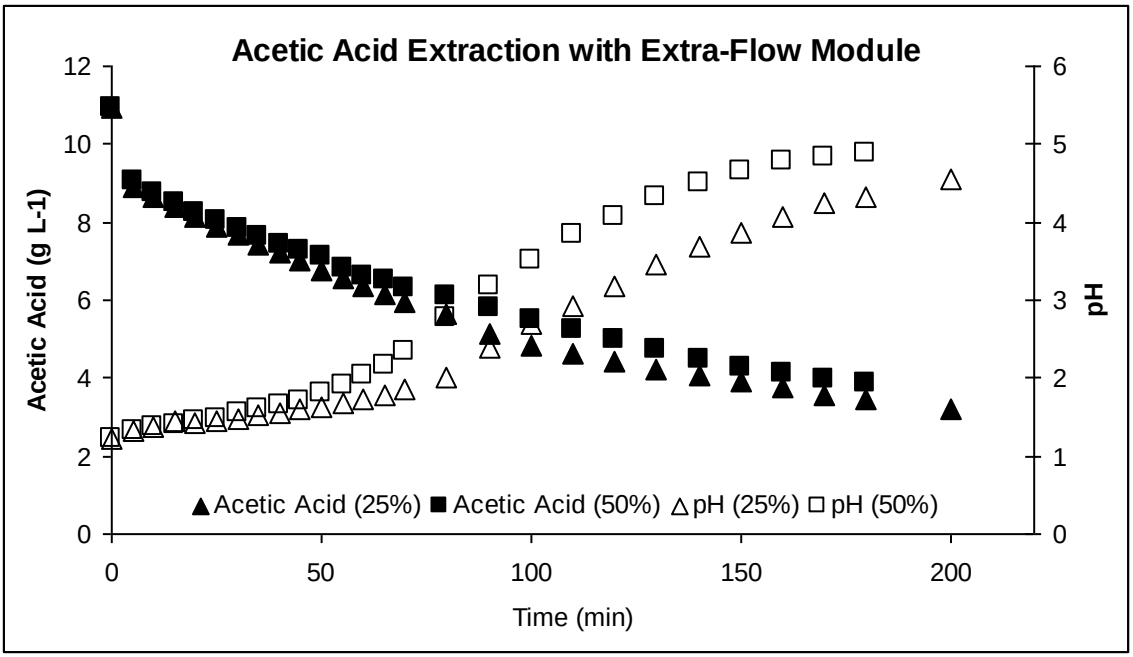

Figure 6 Extraction using Extra-Flow module at two different Alamine 336 concentrations of 25% (triangles) and 50 % (squares)

However, Figs. 4-6 indicate that the variation of the aqueous phase pH is much more sensitive to the operating conditions.

Figure 7 gives the variation of acetic acid concentration and pH with time for acetic acid in DI water and sulphuric acid and acetic acid in DI water. Results for acetic acid in DI water show a steady decrease in acetic acid concentration and an increase in pH with time. Results for acetic acid and sulphuric acid in DI water are similar to the results obtained for hydrolysates. There is a rapid increase in pH near the pKa of the bisulphate anion. Further the rate of acetic acid removal increases after the pH rises above the pKa of the bisulphate anion.

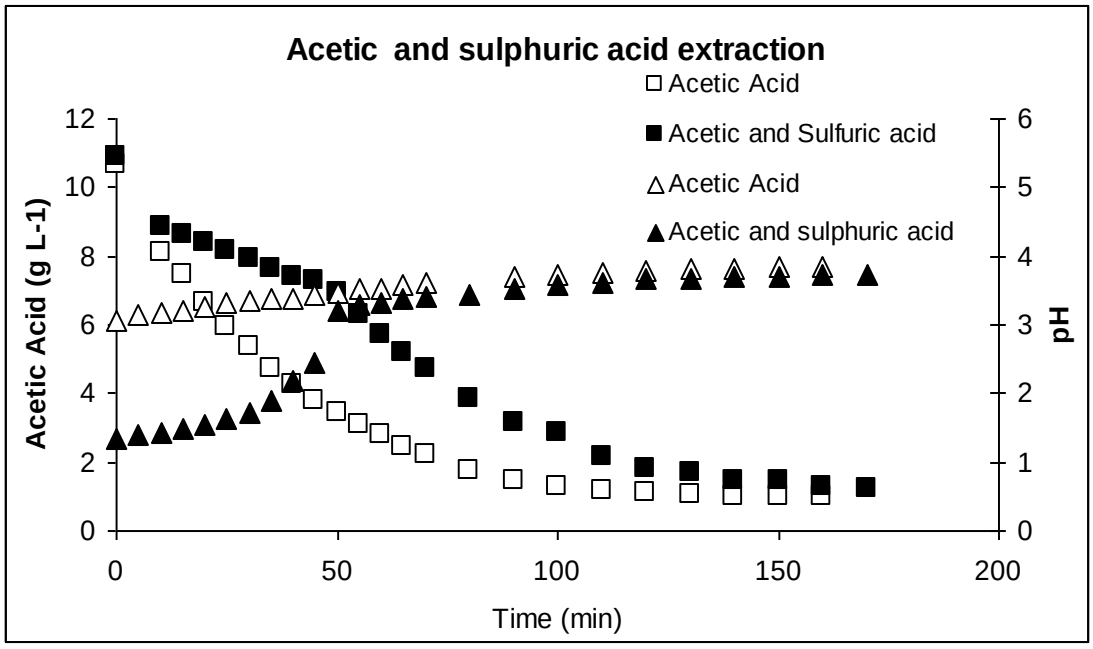

Figure 7 Extraction of 12 g L^{-1} acetic acid and 12 g L^{-1} acetic acid and 0.1 wt% sulphuric acid in DI water

Finally Figure 8 gives absorption spectra for hydrolysate and hydrolysate after extraction with the Extra-Flow module. As can be seen, the broad peak at 280 nm is significantly reduced after extraction. Further the shoulder at 320 nm is also reduced.

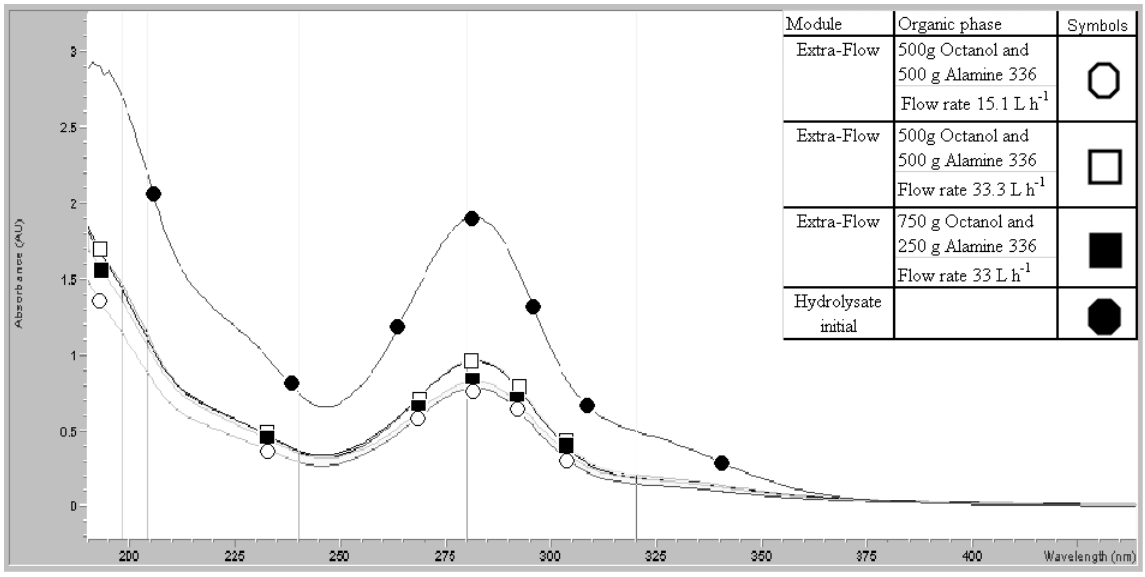

Figure 8 Absorbance spectra for hydrolysate

2.5 Discussion

Increasing the acetic acid concentration in the aqueous phase leads to overloading of Alamine 336 at lower volume percent Alamine 336. The degree of overloading depends on the acetic acid concentration in the aqueous phase as well as the octanol:Alamine 336 ratio in the organic phase. For the experimental conditions considered here, no overloading of Aliquat 336 was observed (see Figure 2). A similar result was reported by Reisinger and King[20].

The overall mass transfer coefficient based on the organic phase for transfer of acetic acid or sulphuric acid from the aqueous to the organic phase is given by:

$$\frac{1}{K} = \frac{m}{k_h} + \frac{1}{k_m} + \frac{1}{k_o} \qquad (1)$$

where m is the distribution coefficient of the acetic acid or sulphuric acid species that transfers to the organic phase, K is the overall mass transfer coefficient and k_h, k_m and k_o are the hydrolysate, membrane and organic phase mass transfer coefficients, respectively. In these studies the acetic and sulphuric acid species that transfer to the organic phase react with the amine present. Reaction with the amine will increase the rate of mass transfer due to an enhanced mass transfer coefficient and increased concentration driving force[28,31,32]. Due to the very low solubility of acetic acid and sulphuric acid in octanol, the reaction front is likely to be inside the membrane pores.

Figure 3 indicates that the rate of acetic acid removal when Aliquat 336 is used is much less than for Alamine 336. However the viscosity Aliquat 336 is 1450 cP at 30 °C while that of Alamine 336 is about 20 cP. Thus the organic phase containing Alamine 336 is about 70 times less viscous than the organic phase containing Aliquat 336. Since the organic phase mass

transfer coefficient depends on viscosity it is not surprising that the rate of mass transfer is much slower when Aliquat 336 is used as the organic phase.

Using a model aqueous phase consisting of acetic and sulphuric acid at the same concentration that is found in the hydrolysate, we see that the bisulphate anion is preferably extracted (Figure 7). Only when the pH of the aqueous phase reaches the pK_a of the bisulphate anion, 2.0, is there significant extraction of the protonated form of acetic acid. This is in agreement with previous results and highlights the fact that sulphuric acid is a much stronger acid than acetic acid, thus extraction of a proton by the amine followed by extraction of the bisulphate anion occurs in preference to extraction of acetic acid.

The increase in the rate of acetic acid removal once the pH is above the pK_a of the bisulphate anion is clearly seen in Figure 7 for the model aqueous feed. However for real hydrolysates (Figs. 3-6) this increase is not so distinctive. We suspect this is due to the presence of other organic compounds such as furfural, phenolics and low molecular weight lignins. Further since changing the aqueous and organic phase flow rates has little effect on the rate of removal of acetic acid, it appears the rate of acetic acid removal is limited by the rate of reaction in the organic phase and not the feed or aqueous phase mass transfer coefficients. Further Figures 4-6 indicate that the change in pH is much more sensitive to operating conditions, probably due to the fact that pH changes rapidly near the pK_a value of the bisulphate anion leading to larger measurement errors.

We have explored the removal of other toxic compounds such as low molecular weight lignin, phenolics and furfural. Figure 8 gives the absorption spectra of hydrolysate as well as hydrolysates that have been treated by membrane extraction. The untreated hydrolysate

shows a broad peak around 280 nm. This peak represents furfural and hydroxymethyl furfural [33]. The treated samples indicate that a significant amount of furfural and hydroxymethyl have been extracted into the octanol. The absorbance in the region less than 225 nm is also reduced in the treated samples. Since absorbance in this region may be attributed to acid soluble lignin it appears a significant amount of lignin is also being extracted into octanol. Finally the shoulder around 320 nm is often attributed to phenolic compounds. As can be seen these compounds are also extracted into octanol.

Thus qualitatively extraction with octanol is successful in removing other toxic compounds.

Currently after hydrolysis, the hydrolysate is conditioned for simultaneous saccharification and fermentation by adding calcium hydroxide in order to remove toxic compounds and adjust the pH to the optimal value for fermentation. Our results indicate that hollow fibre extraction is capable of not only extracting acetic acid but also adjusting the pH and extracting toxic compounds. Thus it could be a viable process for conditioning the hydrolysate after pretreatment. While the solubility of octanol and Alamine 336 is very low in water, Tamada et al[13] have shown that commercially available Alamine 336 contains small amounts of lower molecular weight and more water-soluble amines. Due to their toxicity, transfer of these lower amines to the hydrolysate could lead to decreased ethanol yield during fermentation. From a practical perspective it will be necessary to conduct fermentation studies using hydrolysates that have been detoxified using membrane extraction. The ethanol yields should be compared to yields obtained using current detoxification methods such as addition of calcium hydroxide (overliming).

Development of a practical extraction process for removal of acetic acid will also depend on the economical regeneration of the amine extractant. In addition recovery of the acid (sulphuric and carboxylic in this work) for reuse would be very beneficial. Currently the hydrolysate is frequently detoxified by addition of calcium hydroxide. This leads to precipitation of gypsum (calcium sulphate) as well as higher molecular weight lignin. In addition the pH of the hydrolysate is increased to around 5-6 in order to conduct fermentation. In keeping with current practice, the organic phase could be back extracted with calcium hydroxide leading to the formation of gypsum. Alternatively Huang et al.[35] describe a back extraction process using NaOH. Other methods for regeneration of the amine described in the literature include diluent-swing regeneration[34] and gas antisolvent-induced regeneration[35]. We are currently exploring various methods for regeneration of the amine and possible recovery of acetic acid.

2.6 Conclusions

Hollow fibre-based liquid extraction has been used to remove acetic acid from lignocellulosic hydrolysates after pretreatment with dilute sulphuric acid. Our results indicate that using a 50:50 mixture of Alamine 336 in octanol up to 60% of the acetic acid can be removed. Further we remove sulphuric acid thus increasing the pH to levels that are better suited for fermentation. In addition extraction of other toxic organic compounds is observed.

2.7 Acknowledgements

Funding for this work was provided by the U.S. Department of Energy's Office of the Biomass Program and funding for Colorado State University was provided by a subcontract with the National Renewable Energy Laboratory (KXDJ-0-30622-02).

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Chapter 3

DETOXIFICATION OF BIOMASS HYDROLYSATE BY REACTIVE MEMBRANE EXTRACTION

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Abstract

Economical conversion of lignocellulosic biomass into biofuels is essential to reduce the world's dependence on fossil fuels. The typical biochemical process for biomass conversion includes a thermochemical pretreatment step to improve enzymatic cellulose hydrolysis and to release hemicellulosic sugars from the polymer matrix. However compounds that are toxic to microorganisms in subsequent fermentation steps may also be released. This work investigates the use of membrane extraction to detoxify or remove these toxic compounds from corn stover hydrolysates pretreated using dilute sulphuric acid.

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Extraction of sulphuric, acetic, formic and levulinic acid as well as 5-hydroxymethylfurfural and furfural has been investigated. Octanol and oleyl alcohol were used as organic phase solvents. Alamine 336 was used as the aliphatic amine extractant. Reactive extraction of sulphuric, acetic, formic and levulinc acid was observed while 5-hydroxymethylfurfural and furfural were extracted due to their distribution in the organic solvent. Significant removal of all toxic compounds investigated was obtained as well an increase in pH from 1.0 to 5.0. As small quantities of the organic phase transferred into the hydrolysate during extraction, the toxicity of the organic phase must be considered. As it is likely that detoxification will require the use of another unit operation in combination with membrane extraction, the economical viability of the combined process must be considered.

Key words: Aliphatic amine extractant, detoxification, hydrolysate, lignocellulosic biomass, membrane extraction

3.1 Introduction

Development of efficient unit operations for conversion of lignocellulosic biomass into biofuels will be essential in order to replace up to 30% of the petroleum-based transportation fuels with biofuels by 2030[1]. The economic viability of producing biofuels from biomass relies significantly on obtaining high yields of sugar from lignocellulosic biomass at low cost[2,3]. Here the focus is on the production of bioethanol from corn stover. Corn stover is a likely near-term feedstock because it is readily available in large quantities. The main steps involved in the conversion of corn stover into bioethanol are: pretreatment, hydrolysate detoxification, enzymatic cellulose hydrolysis and co-fermentation of the sugars, and product separation and purification[4].

Lignocellulosic biomass consists of three main polymers: cellulose, hemicellulose and lignin. In the pretreatment step, biomass is treated to improve the susceptibility of the cellulose to enzymatic hydrolysis. Many different mechanical and thermochemical methods have been proposed for biomass pretreatment[5]. Dilute sulphuric acid was used to produce the material tested in this study. Dilute sulphuric acid has been shown to be effective at producing a xylose-rich hemicellulose hydrolysate liquor while enhancing cellulose enzymatic digestibility[6]. Effective dilute sulphuric acid pretreatment not only releases xylose and acetic acid, but also results in the formation of sugar degradation compounds such as 5-hydroxymethylfurfural (HMF), furfural, levulinic and formic acid and phenolic-based lignin fragments that inhibit subsequent bioconversion of the solubilized sugars into ethanol[7,8].

The quantity of toxic compounds produced depends on the severity of the reaction (temperature, concentration and time of dilute sulphuric acid pretreatment)[9,10]. While lower severity leads to lower concentrations of toxic compounds, it also leads to lower sugar yields.

Thus optimized pretreatment and subsequent detoxification steps, to remove toxic compounds that are produced, are essential to maximize sugar yields and hence increase ethanol yields in the subsequent fermentation step.

In earlier work7 it was shown that acetic acid may be removed by reactive hollow fiber based membrane extraction. Schlosser et al.[11] present a summary of published studies on the use of membrane extraction for the recovery of various carboxylic acids. Membrane extraction has a number of advantages over conventional extraction. Most importantly for the detoxification of biomass hydrolysates, membrane extraction avoids the need to disperse one phase in the other thus minimizing the likelihood of entrainment of small amounts of organic phase in the aqueous hydrolysate phase. Given that the organic phase is likely to be toxic to the microorganisms used in the subsequent fermentation step, minimizing transfer of organic phase into the aqueous phase will be critical.

Alamine 336, a long chain aliphatic amine was used as the extractant while octanol was the organic solvent. Alamine 336 chemically complexes with the acetic acid present. Further, sulphuric acid present in the hydrolysate is also removed. Eyal and Canari[12] have described four major mechanisms for reactive extraction of acids by amines. Since sulphuric acid is a strong acid it is likely that it forms an ion pair with the amine in the organic phase. At pH values below the pK_a of acetic acid, the protonated form of acetic acid is extracted. Further overloading of the amine is possible by hydrogen bonding[13]. Membrane extraction was shown not only to remove acetic acid but also to increase the pH of the hydrolysate (by removal of sulphuric acid). After dilute sulphuric acid pretreatment, the pH of the hydrolysate is about 1. Increasing the pH to 5-6 is essential in order to conduct the subsequent enzymatic cellulose hydrolysis and fermentation steps.

The hydrolysate contains other toxic compounds such as HMF, furfural, formic and levulinic acid. The economic viability of a membrane extraction process may depend on the extent to which these other toxic compounds are also removed. Removal of HMF, furfural, formic and levulinic acid as well as acetic and sulphuric acid have been investigated. While formic and levulinic acid will form chemical complexes with Alamine 336, it is likely that HMF and furfural will be removed by a non-reactive mechanism. In addition, as the reactive extractant and organic phase solvent could be toxic to the microorganisms used in the subsequent fermentation step, it is essential to determine the concentration of these compounds in the hydrolysate after extraction. The results indicate that the efficiency of extraction of the different toxic compounds present in the hydrolysate varies greatly. Further, some transfer of the organic phase into the hydrolysate always occurs.

3.2 Experimental

Figure 1 is a schematic representation of the hollow fibre extraction set up. A LiquiCell Extra-Flow 2.5 x 8 membrane contactor (Membrane, Charlotte, NC) was used. The module contains polypropylene hollow fibers, 300 μm OD, 220 μm ID, pore size 0.04 μm, 40% porosity and surface area 1.4 m^2. The module also contains a central baffle to enhance mixing of the shell side fluid. Two gear pumps (NCI 00198KE, flow rate 0-7.5 L min^{-1} and NCI00198KD, flow rate 0-15 L min^{-1}), two controllers (70021-10) and four pressure transducers (EW 07356-53) were obtained from Cole Parmer, Vernon Hills, Il. Two flow meters (8051K13, flow rate 0.75-7.5 L min^{-1} and 80514K14, flow rate 2.0-20 L min^{-1}) were obtained from McMaster-Carr Supply Company, Elmhurst, IL. Two 316 stainless steel spring loaded piston check valves (50 psi) Swagelock Solon, OH, (see Figure 1) were included to prevent overpressurization. Needle valves

(SS-18RS8-BKP) from Swagelock were installed on each inlet and outlet of the module to ensure optimal pressure control. Half-inch (1.27 cm) stainless steel piping was used throughout the setup.

The aqueous phase consisted of 2 L of corn stover hydrolysate liquor provided by the National Renewable Energy Laboratory (NREL). Two organic phase solvents were used: octanol (297887) and oleyl alcohol (O7600), both from Sigma-Aldrich, St Louis, MO. Alamine 336 (Sigma-Aldrich) was used as the reactive extractant. Table 1 summarizes the compositions and flow rates used for the organic phase.

The effect of aqueous and organic phase flow rates on the rate of extraction was determined for an organic phase consisting of 25% Alamine 336 in octanol by varying the aqueous phase flow rate from 3.8 to 8.6 L min^{-1} at an organic phase flow rate of 2.0 L min^{-1} and varying the organic phase flow from 2.0 to 5.8 L min^{-1} at an aqueous phase flow rate of 3.8 L min^{-1}. Additional experiments were conducted with an organic phase of 15% Alamine 336 in octanol with aqueous and organic phase flow rates of 5.6 and 2.2 L min^{-1}, respectively. Finally experiments were conducted using 15% Alamine 336 in oleyl alcohol at the same flow rates. All testing was conducted at 22 °C except extraction into oleyl alcohol which was conducted at 42 °C. The viscosity of oleyl alcohol is about 1.5×10^{-2} Pa s while octanol is 7.2×10^{-3} Pa s at 25 °C. To compensate for the lower mass transfer coefficient due to the higher viscosity of oleyl alcohol, all experiment using oleyl alcohol as the organic solvent were run at 42 °C instead of 22 °C. A temperature of 42 °C was maintained by placing the organic phase reservoir in a water bath while 22 °C represents room temperature. All experiments were run for 360 minutes.

Since the membrane is hydrophobic, the organic phase will fill the membrane pores. It is essential to minimize transfer of the organic phase into the aqueous phase. Thus, the system was started by first turning on the aqueous phase, followed by the organic phase pump. The organic phase was pumped on the shell side. At all times the inlet and outlet aqueous side pressures were 7,000 to 14,000 Pa above the organic side pressure to minimize loss of the organic phase in the aqueous phase. The pH of the hydrolysate was measured using a Thermo Orion 520 (Thermo Fisher Scientific, Waltham, MA) pH meter equipped with a Mettler Toledo pH probe (Cole-Parmer). At frequent time intervals, 8 mL samples of the aqueous phase were removed from the return tube for compositional analysis.

The concentration of acetic, formic and levulinic acid as well as HMF, furfural, glucose, xylose and arabinose in the aqueous phase was determined using a 1100 HP HPLC (Agilent, Santa Clara, CA) equipped with a Biorad Aminex HPX-87 H (Bio-Rad Hercules, CA) column and a HP refractive index detector. The mobile phase consisted of 10 N sulphuric acid solution diluted to 0.01 N with HPLC grade water and prefiltered using a 0.2 µm filter. A series of calibration standards and calibration verification standards (CVS) were obtained from Absolute Standards Inc., Hamden CT. The flow rate was set at 0.6 mL min^{-1} at a column temperature of 55 °C and injection volume of 6 µL. All measurements were taken at least three times and average results are reported.

Sulphuric acid was measured in the aqueous phase using Lab X pro titration machine (Mettler-Toledo Inc., Columbus, OH). Three pH values: 4, 7, and 10 were used as standards with a running time of 5 min/sample. Measurements were made in triplicate and average results were reported.

Alamine 336 and octanol were analyzed in the aqueous phase using GCMS (Agilent 5973 Network Mass Selective Detector, Agilent 6890N Network GC System) equipped with a fused silica capillary column (DB-Wax-Liquid phase, 0.5 µm film thickness 30 m x 0.249 mm). The run time was 17.5 min using a continuous temperature gradient starting from 135 to 240 °C. Standards were prepared by serial dilution using Alamine 336, 100 to 1 ppm, and octanol, 1 to 3 mg/ml, in hydrolysate.

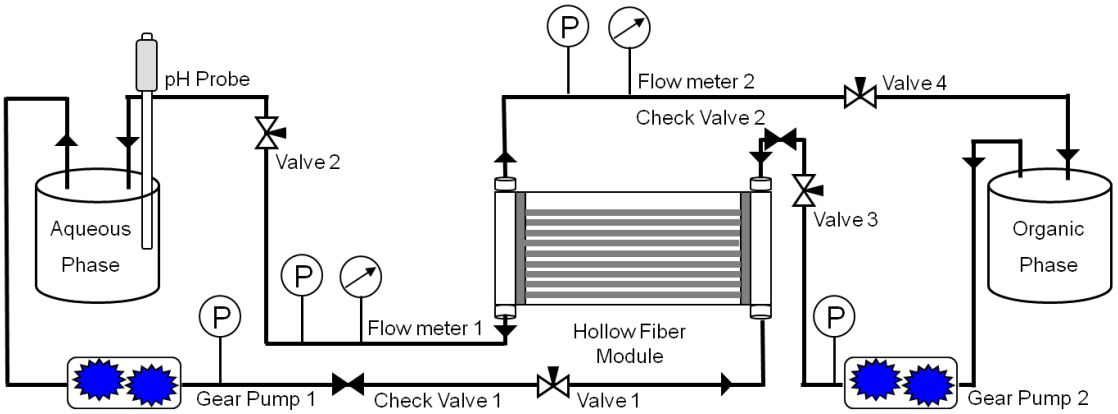

Figure 1 Schematic representation of experimental set up

Table 1 Summary of organic and aqueous phases and flow rates used

Organic solvent	*Mass of organic solvent (g)*	*Alamine (g)*	Aqueous/organic phase flow rates tested ($L\ min^{-1}/L\ min^{-1}$)
Octanol	1125	375	3.8/2.0; 5.6/2.0; 8.6/2.0;
Octanol	1250	250	3.8/4.0; 3.8/5.8
Oleyl alcohol	1250	250	5.6/2.2
			5.6/2.2

3.3 Results

Figures 2-6 give extraction data for acetic acid, formic and levulinic acid, sulphuric acid, HMF and furfural, respectively. With the exception of formic and levulinic acid, results are given for all the experimental conditions investigated (see Table 1). Since no significant difference in the rate of extraction of any of the compounds was observed for an organic phase of 25% Alamine 336 in octanol for the range of aqueous and organic phase flow rates investigated, these results are show with the same symbol. Generally, the batch to batch variation in the concentration of a given compound is small, the exception being for HMF. As can be seen in Figure 5, the initial HMF concentration in the hydrolysate treated with oleyl alcohol as the organic solvent was much lower. The design of a commercial process must account for the small natural variation in hydrolyste pH and toxic compound concentration. Since the initial concentration of formic and levulinic acid is low, extraction of these two acids will not be limiting. Consequently, only the result for 15 % Alamine 336 in octanol and oleyl alcohol are shown to highlight that these acids are successfully extracted.

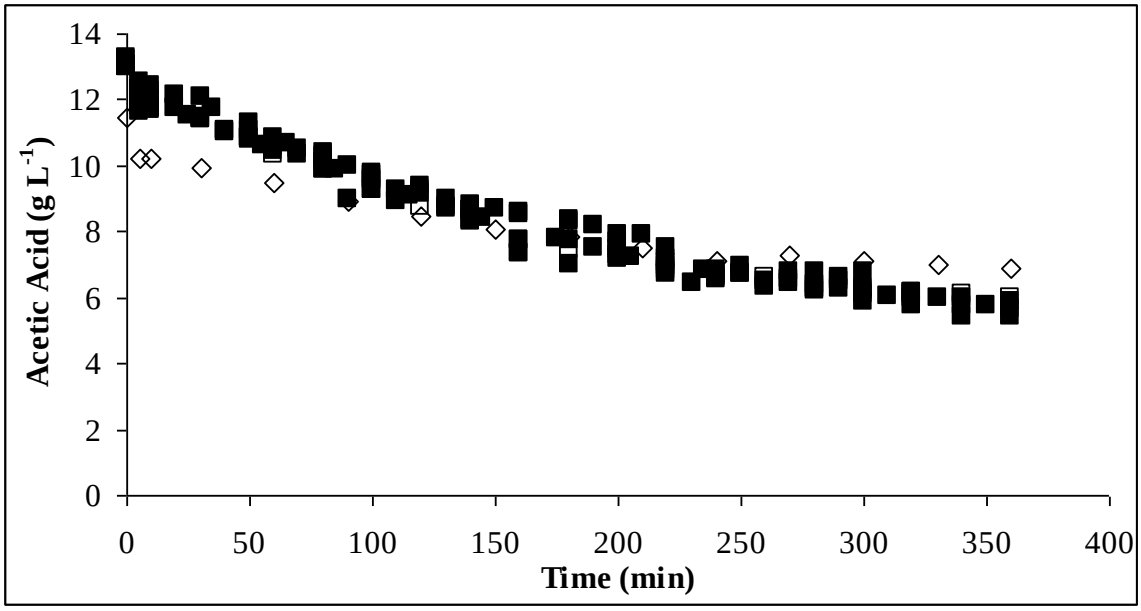

Figure 2 Acetic acid extraction results. ■ are for 25% Alamine 336 in octanol at different aqueous and organic phase flow rates (see Table 1). □ are for 15% Alamine 336 in octanol while ◊ are for 15% Alamine 336 in oleyl alcohol

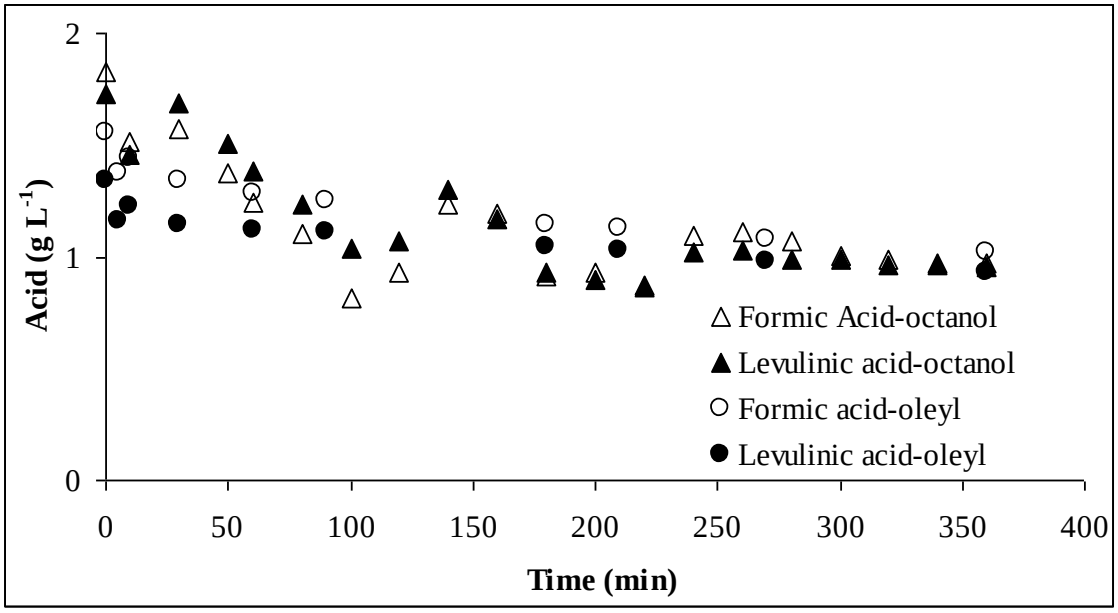

Figure 3 Formic and levulinic acid extraction results for hydrolysate and organic phase flow rates of 5.6 and 2.2 L min^{-1}. Δ are for 15 % Alamine 336 in octanol and ○ are for 15 % Alamine 336 in oleyl alcohol

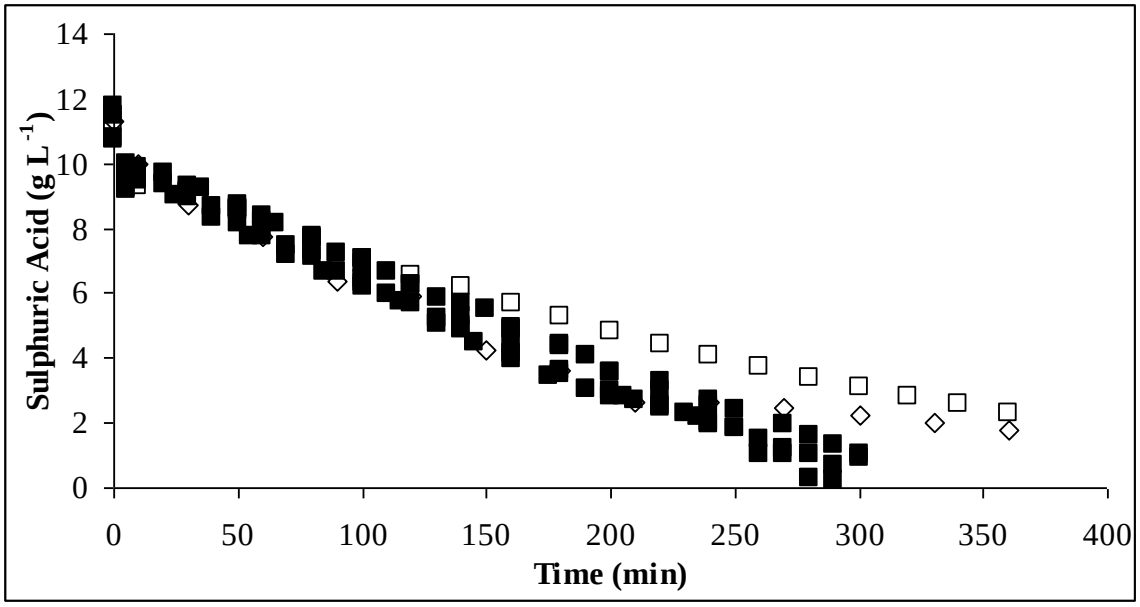

Figure 4 Sulphuric acid extraction results. ■ are for 25% Alamine 336 in octanol at different aqueous and organic phase flow rates (see Table 1). □ are for 15% Alamine 336 in octanol while ◊ are for 15% Alamine 336 in oleyl alcohol

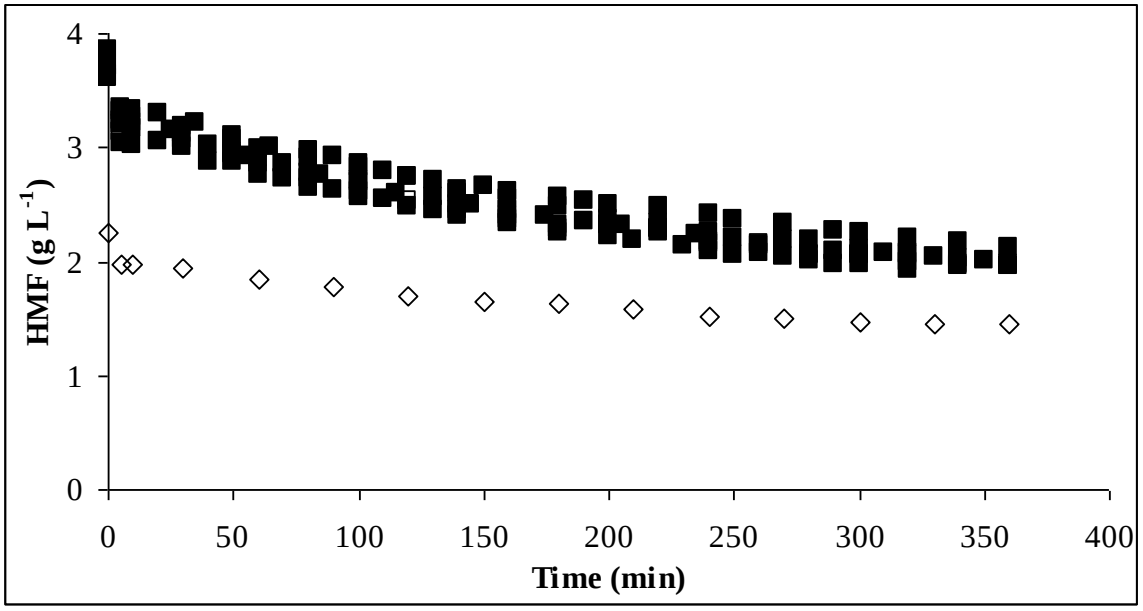

Figure 5 HMF extraction results. ■ are for 25% Alamine 336 in octanol at different aqueous and organic phase flow rates (see Table 1). □ are for 15% Alamine 336 in octanol while ◊ are for 15% Alamine 336 in oleyl alcohol

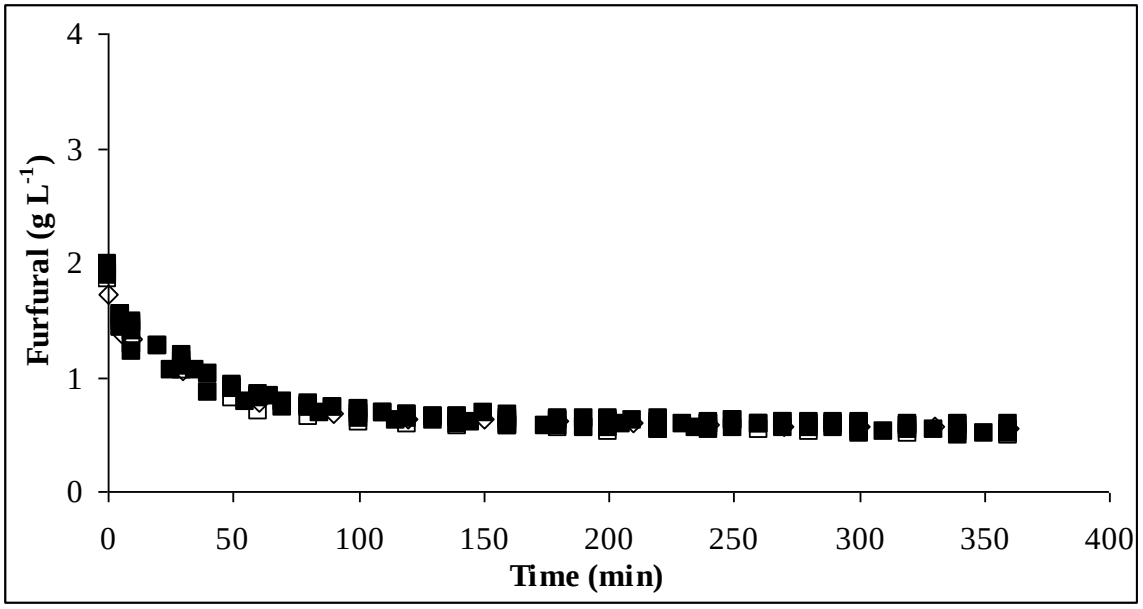

Figure 6 Furfural extraction results. ■ are for 25% Alamine 336 in octanol at different aqueous and organic phase flow rates (see Table 1). □ are for 15% Alamine 336 in octanol while ◊ are for 15% Alamine 336 in oleyl alcohol

Figures 7-10 give the natural logarithm of the initial concentration of acetic acid, sulphuric acid, HMF and furfural divided by the concentration at time t versus time. Since results for 25% Alamine 336 in octanol for the range of aqueous and organic phase flow rates investigated here fall on the same straight line for each compound, these results are shown with the same symbol. Semi-logarithmic plots for formic and levulinic acid are not included as design of an extraction system will not be limited by removal of these acids.

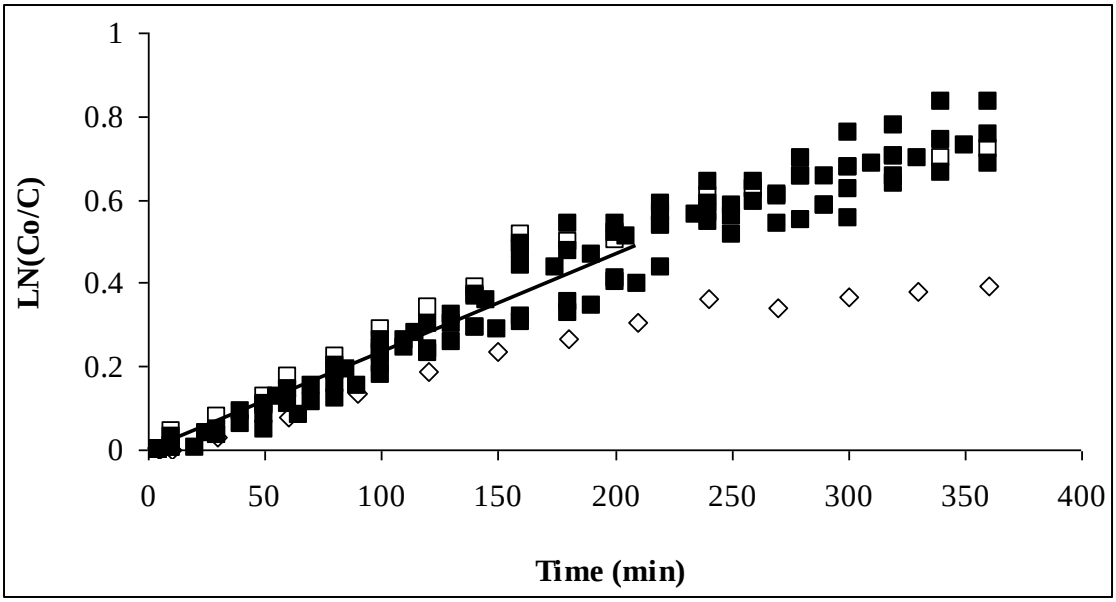

Figure 7 Determination of overall mass transfer coefficient for removal of acetic acid. ■ are for 25% Alamine 336 in octanol at different aqueous and organic phase flow rates (see Table 1). □ are for 15% Alamine 336 in octanol while ◊ are for 15% Alamine 336 in oleyl alcohol. The mass transfer coefficient was determined from the slope of the straight line

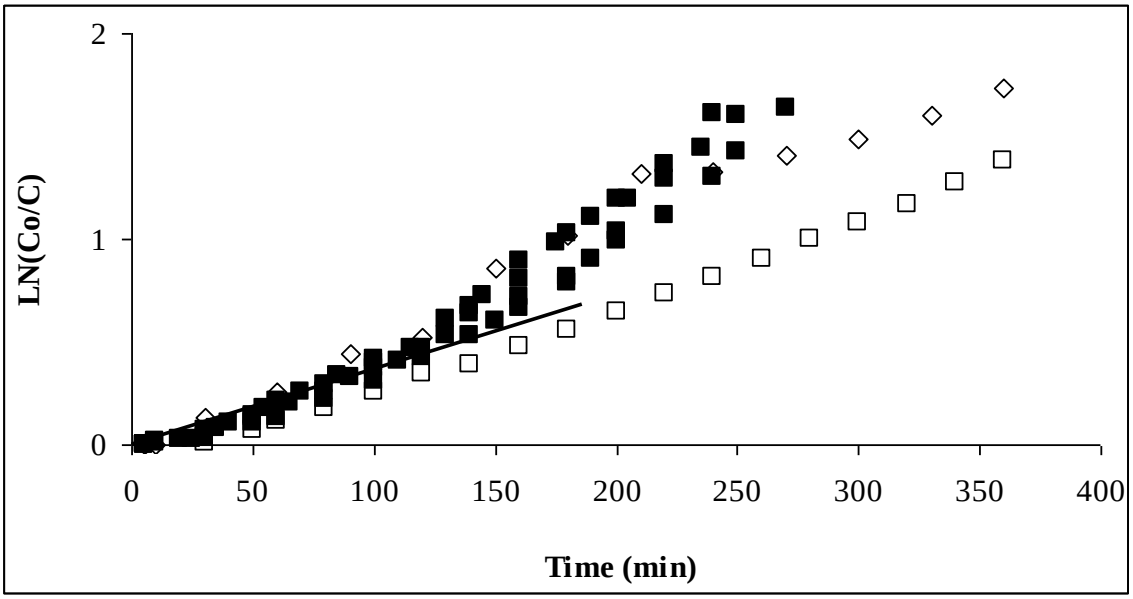

Figure 8 Determination of overall mass transfer coefficient for removal of sulphuric acid. ■ are for 25% Alamine 336 in octanol at different aqueous and organic phase flow rates (see Table 1). □ are for 15% Alamine 336 in octanol while ◊ are for 15% Alamine 336 in oleyl alcohol. The mass transfer coefficient was determined from the slope of the straight line

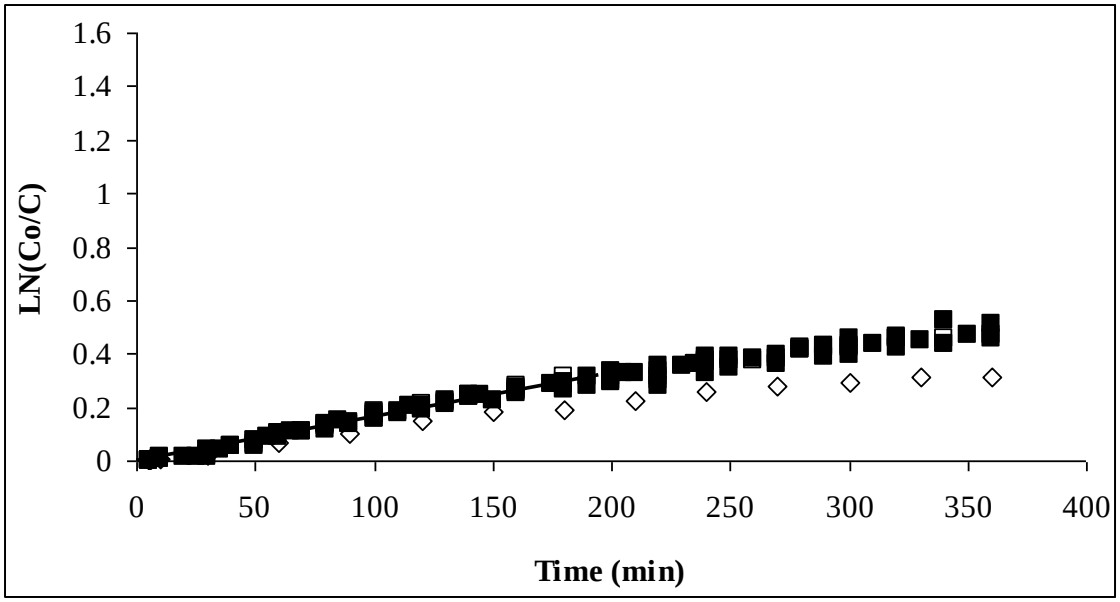

Figure 9 Determination of overall mass transfer coefficient for removal of HMF. ■ are for 25% Alamine 336 in octanol at different aqueous and organic phase flow rates (see Table 1). □ are for 15% Alamine 336 in octanol while ◊ are for 15% Alamine 336 in oleyl alcohol. The mass transfer coefficient was determined from the slope of the straight line

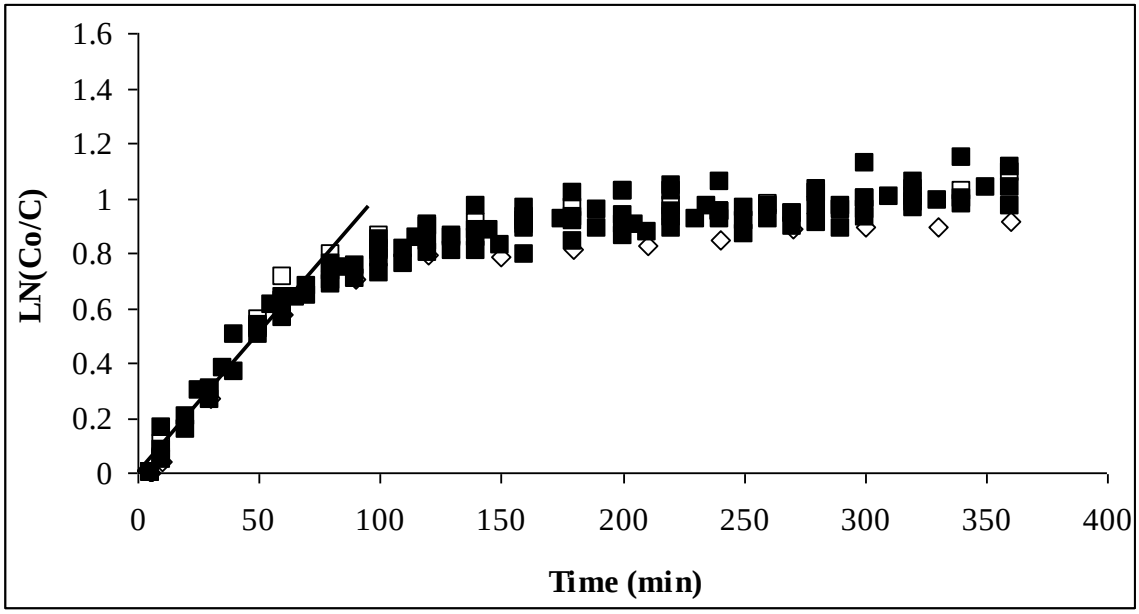

Figure 10 Determination of overall mass transfer coefficient for removal of furfural. ■ are for 25% Alamine 336 in octanol at different aqueous and organic phase flow rates (see Table 1). □ are for 15% Alamine 336 in octanol while ◊ are for 15% Alamine 366 in oleyl alcohol. The mass transfer coefficient was determined from the slope of the straight line

The concentration of octanol and Alamine 336 in the hydrolysate during extraction is given in Figure 11 for 15% Alamine 336 in octanol. The concentration of Alamine 336 and octanol in the aqueous phase was similar for all conditions tested when octanol was used as the organic solvent. As can be seen a small but quantifiable amount of octanol and Alamine 336 transfer into the hydrolysate during extraction. It is possible that these organic compounds may have an impact on fermentation performance, which could be important in determining the viability of membrane extraction for detoxification of biomass hydrolysates.

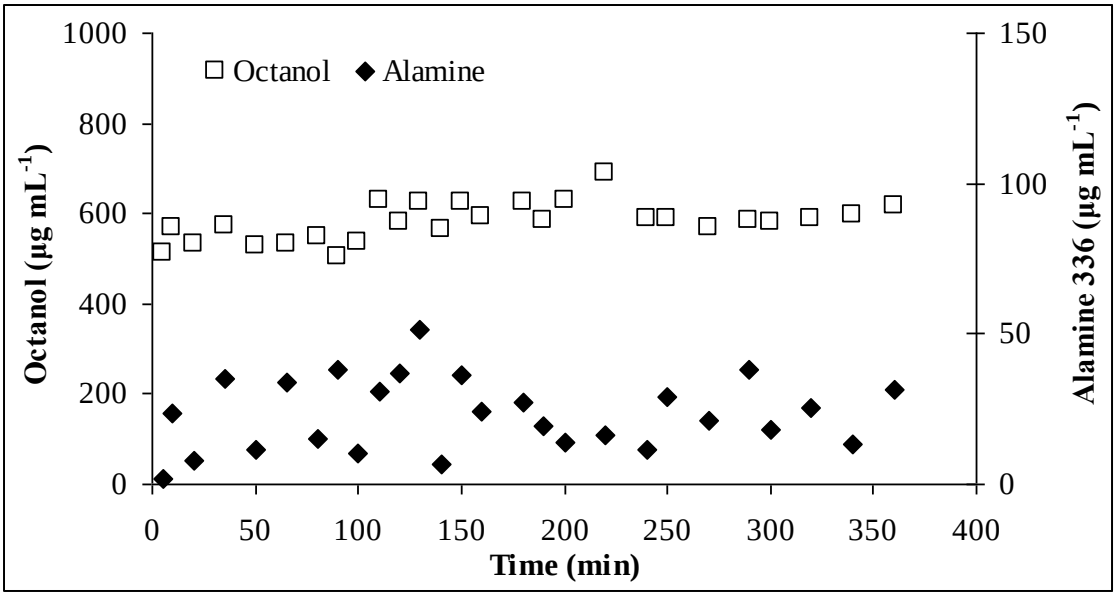

Figure 11 Variation of octanol and Alamine 336 in the hydrolysate during extraction for hydrolysate and organic phase flow rates of 5.6 and 2.2 min^{-1}. The organic phase contained 15 % Alamine 336

Figure 12 gives the concentration of three sugars; glucose, xylose and arabinose in the hydrolysate during extraction. Results are shown for 15% Alamine 336 in the organic phase. As can be seen there is no detectable loss of any of these sugars from the hydrolysate during extraction. Similar results were obtained for all of the experimental conditions investigated. Maximizing sugar yields is critical in the design of economically viable biorefineries, thus highlighting the potential benefit of membrane extraction for hydrolysate detoxification.

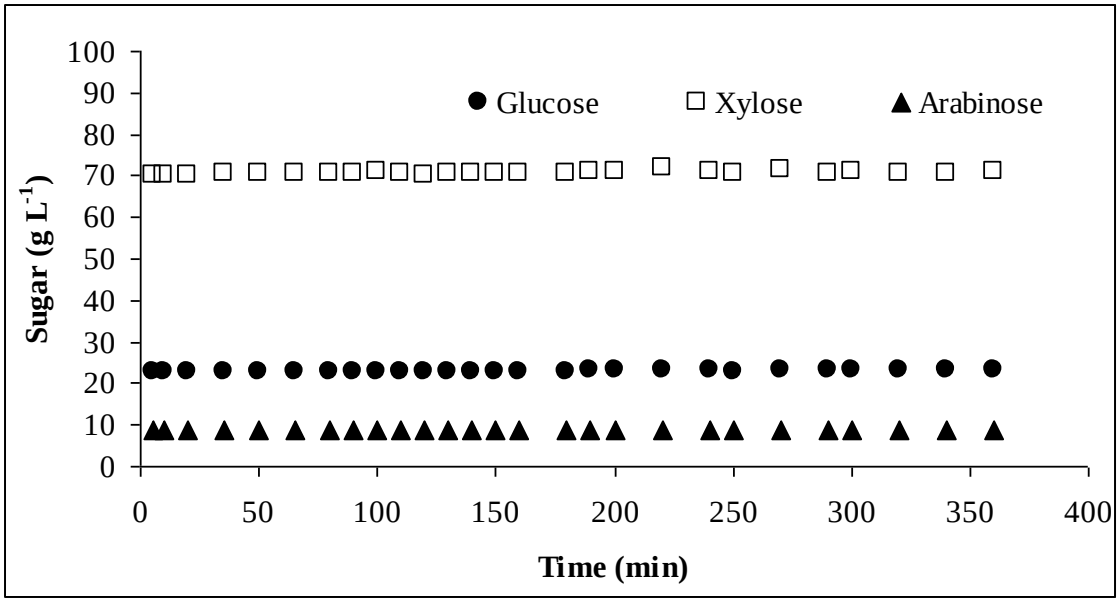

Figure 12 Variation of glucose, xylose and arabinose in the hydrolysate during extraction for hydrolysate and organic phase flow rates of 5.6 and 2.2 min^{-1}. The organic phase contained 15 % Alamine 336

3.4 Discussion

Hydrolysate contains a mixture of acetic, formic and levulinic acids with pK_as of 4.80, 3.74, 4.62, respectively. The first and second dissociation constants of sulphuric acid are 10^3, and 10^{-2}, respectively. Since sulphuric acid is a much stronger acid than the three organic acids it will be preferentially extracted. In fact in earlier studies it was shown that for mixtures of acetic and sulphuric acid in DI water, the rate of acetic acid removal increases rapidly once the pH increases above 2, the pK_a of the bisulphate anion7.

After pretreatment the pH of the hydrolysate is between 1 and 2. After membrane extraction for 360 minutes the pH of the hydrolysate is close to 5.0. Previous investigators have shown that the extraction capacity of tertiary amines decreases rapidly as the pH approaches the pK_a of the acid[14,15,16,17,18]. Thus based on the pK_a values for acetic, formic and levulinic acid, the rate of formic acid extraction will decrease as the pH increases above 3.74.

Figures 2 and 3 indicate that membrane extraction leads to at least a 50% decrease in the concentration of all three acids in the hydrolysate. However given the concentration of acetic acid in the hydrolysate is almost an order of magnitude greater than formic and levulinic acid, design of a membrane extraction process for hydrolysate detoxification will focus on acetic acid removal. Figure 3 indicates much greater than 50% removal of sulphuric acid, as expected, since it is a much stronger acid.

A mass balance around the feed reservoir for a given compound (acetic acid, sulphuric acid, HMF, furfural) gives

$$V\frac{dC}{dt} = -KA\left(C - C^*\right) \qquad (1)$$

where V is the volume of hydrolysate (2 L), K is the overall mass transfer coefficient based on the aqueous phase, A is the membrane surface area (1.4 m^2), C is the concentration of the compound in the aqueous phase and C^* is the concentration of the compound in the aqueous phase that would be in equilibrium with the concentration in the organic phase. Since fresh organic phase is used for each experiment initially C^* may be assumed to be zero. A number of standard assumptions are implicit: the feed reservoir is fully mixed, the rate of change of acetic concentration in the feed per pass through the module is small.

Integration of Equation (1) leads to

$$Ln\left(\frac{C_0}{C}\right) = \frac{KAt}{V} \qquad (2)$$

Plotting the left hand side of Equation (2) against run time, t should lead to a straight line the slope of which is the equal to the overall mass transfer coefficient. The overall mass transfer

coefficient based on the aqueous phase is made up of three individual mass transfer coefficients,

$$\frac{1}{K} = \frac{1}{k_h} + \frac{m}{k_m} + \frac{m}{k_o} \qquad (3)$$

where k_h, k_m, and k_o are the hydrolysate, membrane and organic phase mass transfer coefficients and m is the distribution coefficient of the toxic compound between the two phases. The hydrolysate and organic phase mass transfer coefficients depend on the hydrolysate and organic phase flow rates[19]. Numerous empirical correlations have been derived in order to predict these mass transfer coefficients[20,21,22,23]. The membrane mass transfer coefficient on the other hand, does not depend on either of the phase flow rates. It is given by

$$k_m = \frac{D\varepsilon}{\tau l} \qquad (4)$$

where D is the diffusion coefficient of the solute of interest in the membrane pores, ε is the membrane porosity (40%), τ is the membrane tortuoisty and *l* is the wall thickness of the hollow fibres (40 μm). Reaction of acetic and sulphuric acid with Alamine 336 in the organic phase will lead to an enhanced organic phase mass transfer coefficient. Due to the very low solubility of acetic and sulphuric acid in octanol, the reaction front is likely to be inside the membrane pores. If the membrane mass transfer coefficient controls the rate of mass transfer, the overall mass transfer coefficient will appear to be independent of either phase flow rate. Thus a plot of $Ln(C_0/C)$ against t should result in the same straight line for a range of aqueous and organic phase flow rates. In Figures 7 and 8 the variation of $Ln(C_0/C)$ for acetic and sulphuric acid versus t for a range of aqueous and organic phase flow rates for 25% Alamine 336 in octanol is shown with the same symbol. The results fall on the same curve indicating that the membrane mass

transfer coefficient is the limiting mass transfer resistance. These results are in agreement with previous studies[20]. Further for run times less than 100 minutes results for 25% and 15% Alamine 336 in octanol and 15% Alamine 336 in oleyl alcohol fall on the same curve.

For acetic acid (Figure 7), HMF (Figure 9) and furfural (Figure 10) the mass transfer coefficient appears to decrease at long run times indicating that the assumption of a non-zero concentration in the organic phase may not be applicable. In the case of sulphuric acid (Figure 8) the data at longer run times are much more scattered. This is most likely due to the very low measured concentration in the hydrolysate (Figure 4).

Figures 2 and 7 indicate that while the initial flux of acetic acid was the same for all experiments, at higher run times, the rate of extraction of acetic acid is the same for 15 and 25% Alamine 336 in octanol but is significantly lower for 15% Alamine 336 in oleyl alcohol. Previous investigators have shown that 'overloading' of the amine, where more than one acetic acid molecule is extracted per amine molecule is possible by hydrogen bonding a second acetic acid molecule to the carbonyl oxygen of the first molecule[24]. Octanol is an active solvent that can hydrogen bond to the acid amine complex thus stabilizing it and promoting overloading. Given the presence of a double bond and the folded non-linear structure of oleyl alcohol steric hindrance effects are likely to limit its ability of form hydrogen bonds with the acetic acid amine complex thus limiting overloading. This explains the lower extraction capacity observed in Figures 2 and 7.

Figures 4 and 8 indicate that at longer run times the rate of sulphuric acid extraction decreases for 15% Alamine in octanol and oelyl alcohol. Since sulphuric acid is a strong acid it is extracted by ion pair formation. As overloading by hydrogen bond formation is not possible, at

the lower Alamine 336 concentration it is likely that the rate of extraction decreases due to saturation of the extractant. This result supports the fact that use of octanol as a solvent is beneficial as it can promote overloading of the amine thus increasing the organic phase acetic acid capacity.

Extraction of HMF and furfural (Figures 5 and 6) will be driven by the distribution of these compounds in the organic solvent rather than by reaction with the Alamine 336. Consequently using 15% and 25% Alamine 336 has no effect on the extraction of these two compounds. The initial HMF concentration in the hydrolysate, when oleyl alcohol was used as the organic solvent was much lower than for the runs with octanol as the organic solvent as shown in Figure 5. However Figure 10 indicates that the mass transfer coefficient is similar for all runs as is expected if the mass transfer coefficient is independent of concentration[25]. Extraction results for HMF and furfural indicate that at large run times the concentration of HMF and furfural in both phases approach an equilibrium value.

Table 2 gives estimated and calculated overall mass transfer coefficients based on the aqueous phase using octanol as the organic phase. To calculate the mass transfer coefficient it is necessary to estimate the membrane tortuosity and the diffusion coefficient of each solute species in octanol. Tortuoisity factors between 2 and 12 have been reported[26]. Here an average value of 3 is used as suggested by Cussler[25]. The solute diffusion coefficient in $cm^2\ s^{-1}$ was estimated using the Wilke Chang equation[27,28]

$$D = \frac{7.4\times10^{-8}\left(\phi M_2\right)^{0.5}T}{\mu V^{0.6}} \qquad (4)$$

where ϕ is the association parameter assumed to be 1.5 for alcohols, M_2 is the molecular weight of octanol (130 g mol^{-1}), T is the temperature (295 K), μ is the viscosity of the solvent and V is the molar volume of the solute. The presence of Alamine 336 is ignored and the viscosity of octanol at 22 °C is used (7.2×10^{-3} Pa s). The molar volume of each compound is calculated from its density and molecular weight (see Table 2)[29].

Experimental mass transfer coefficients were determined from Figures 7-10. A straight line was fitted to the initial data as shown in the Figures 7-10. The slope of this straight line represents the quantity (KA)/V. The calculated and experimental mass transfer coefficients are in reasonable agreement. There are however a number of simplifications that have been made in estimating the mass transfer coefficient. Errors in diffusion coefficients predicted by the Wilke-Chang Equation are much higher for non-aqueous solutions[28]. Further the viscosity of octanol was assumed.

The experimentally determined mass transfer coefficient is based on aqueous phase concentrations. However the membrane pores are filled with the organic phase. Thus a distribution coefficient for each solute between the two phases should be included in the calculated mass transfer coefficient[26]. Assuming pure octanol as the organic phase, the octanol water partition coefficients for HMF and furfural are log P = -0.45 and 0.41, respectively, where P is the ratio of the concentration of the undissociated solute in octanol to water. Thus the calculated mass transfer coefficients for HMF and furfural will change by a factor of between 2 and 3. Estimation of the distribution coefficient for acetic and sulphuric acid is more complicated as the concentration of the dissociated species in the aqueous phase as well as acid bound to amine in the organic phase must be included, resulting in the distribution coefficient being a function of pH. Consequently the effect of the distribution coefficient on the mass

transfer coefficient has been ignored. Nevertheless the results indicate that reactive membrane extraction is successful in removing the major toxic compounds present in biomass hydrolysates.

Table 2 Calculated and experimentally determined mass transfer coefficients

Compound	*Molecular Weight* ($g\ mol^{-1}$)	*Density* ($g\ cm^{-3}$)	*D* ($m^2\ s^{-1}$)	k_m *calculated* ($m\ s^{-1}$)	k_m experimental ($m\ s^{-1}$)
Acetic Acid	60	1.049	3.8×10^{-6}	1.2×10^{-6}	3.2×10^{-6}
Sulphuric acid	98	1.840	3.9×10^{-6}	1.3×10^{-6}	5.2×10^{-6}
HMF	126	1.206	2.6×10^{-6}	8.8×10^{-7}	2.2×10^{-6}
Furfural	96	1.160	3.0×10^{-6}	1.0×10^{-6}	1.4×10^{-5}

Figure 11 indicates that there will always be a small amount of organic solvent and extractant transferred into the aqueous phase, which could be toxic to the microorganisms used in the subsequent fermentation step. Choice of the organic solvent will be critical in the design of a viable membrane extraction process. The results indicate choosing an 'active' solvent that promotes overloading of the amine is beneficial as it increases the acetic acid capacity of the organic phase.

Choosing a higher molecular weight alcohol will reduce its solubility in the organic phase though it could be more toxic. Further the viscosity of the organic phase is likely to be higher leading to increased pumping costs. Previous investigators have shown that the toxicity of oleyl alcohol is much less than octanol[30,31]. The results indicate that oleyl alcohol may be an alternative organic solvent to octanol. While the acetic acid capacity is slightly reduced, and the viscosity of the organic phase is higher, efficient extraction of toxic compounds was still

observed. However oleyl alcohol is more expensive than octanol, indicating that octanol may be a more economically viable solvent.

Numerous fermentation strategies have been proposed to overcome the effects of organic solvent toxicity[32,33]. The actual toxicity of a given compound depends on the microorganisms present and interaction between other chemical species present in the growth medium[34]. Bar and Gainer[30] indicate that a distinction must be made between toxicity due to dissolved solvent molecules and that due to a separate solvent phase. Future work will focus on determining ethanol yields after fermentation using hydrolysates detoxified by membrane extraction with octanol and oleyl alcohol as the organic solvent. A major advantage of membrane extraction using octanol or oleyl alcohol as the organic solvent (Figure 12) is that sugar losses are minimized. Maximizing sugar yields will be critical when designing a commercially viable biorefinery.

The economic viability of membrane extraction has to be determined as part of an integrated process. Efficient methods must also be developed to regenerate the amine extractant7. In addition, recovery of toxic compounds such as acetic acid and HMF could be a major advantage over current detoxification methods. All of these issues must be considered when determining the viability of membrane extraction for biomass detoxification.

3.5 Conclusions

Reactive membrane extraction has been used to detoxify corn stover hydrolysates after pretreatment with dilute sulphuric acid. Unlike many earlier studies the extraction of several toxic compounds: acetic, formic, levulinic and sulphuric acid, HMF and furfural from a real hydrolysate have been quantified. Efficient extraction of acetic, formic, levulinic and sulphuric

acid as well as HMF and furfural was obtained. The pH of the detoxified hydrolysate was about 5.0. Octanol and oleyl alcohol were used as the organic solvent while Alamine 336 was the reactive amine extractant. Losses of the organic phase in the hydrolysate have been quantified. The membrane resistance which represents the major resistance to mass transfer, may be predicted thus enabling design of larger scale systems.

Loss of small quantities of the organic phase into the hydrolysate was observed. Choosing an organic phase solvent that maximizes the capacity of the organic phase for removal of toxic compounds and minimization of toxic effects on the microorganisms will be essential. The amount of acetic acid removed for a given quantity of amine may be increased by selecting a solvent that promotes overloading of the amine. The economic viability of membrane extraction for biomass detoxification must be determined as part of an integrated process.

3.6 Acknowledgements

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Chapter 4

FERMENTAION OF REACTIVE MEMBRANE EXTRACTED AND AMMONIUM HYDROXIDE CONDITIONED DILUTE ACID PRETREATED CORN STOVER

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Abstract

Acid-pretreated biomass contains various compounds (e.g., acetic acid, furfural, etc.) that are inhibitory to fermentative microorganisms. Removal or inactivation of these compounds using detoxification methods such as overliming or ammonium hydroxide conditioning (AHC) improves sugar-to-ethanol yields. In this study, the liquor fraction of dilute-acid pretreated corn stover was treated using AHC and a new reactive membrane extraction

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technique, both separately and in combination, and then the sugars in the treated liquors were fermented to ethanol with the glucose-xylose fermenting bacterium, *Zymomonas mobilis* 8b. Reactive extraction was performed with mixtures of octanol/Alamine 336 or oleyl alcohol/Alamine 336. The best ethanol yields and rates were achieved for oleyl alcohol-extracted hydrolysates followed by AHC hydrolysates, while octanol-extracted hydrolysates were unfermentable because highly toxic octanol was found in the hydrolysate. The addition of olive oil significantly improved yields for octanol-extracted hydrolysate. Additional work is underway to determine if this technology is a cost-effective alternative to traditional hydrolysate conditioning processes.

Keywords: Pretreatment, Bioethanol, Reactive membrane extraction, Ammonium hydroxide conditioning, Fermentation

4.1 Introduction

The dependence of the United States on foreign energy sources is currently a major area of concern. Both first (starch) and likely second (lignocellulosic) generation feedstocks could produce enough bioethanol to decrease the United State's dependence on imported energy supplies. Bioethanol also reduces particulate, carbon monoxide and mono-nitrogen oxides emissions and lowers net carbon dioxide production compared to conventional fuels (Sivakumar et al., 2010, Steffes et al., 1996, Luo et al., 2009). Additionally, large amounts of lignocellulosic biomass are potentially available according to a study by Perlack et al. (2005). But low cost production of bioethanol from lignocellulosic biomass must still be demonstrated.

Lignocellulosic biomass contains cellulose and hemicellulose, which can be hydrolyzed to the fermentable sugars, glucose and xylose. A biochemical-based process for converting biomass to ethanol requires a thermochemical pretreatment step to enhance the susceptibility of cellulose to enzymatic hydrolysis. The goals of pretreatment are to improve enzymatic digestibility of biomass while limiting production of inhibitory sugar degradation products and other compounds (Gamage et al., 2010). Typical byproducts produced by dilute-sulfuric-acid pretreatment include acetic, formic and levulinic acids; furfural and 5-hydroxymethylfurfural (HMF). These compounds are known to reduce ethanol yields from fermentation of biomass-derived sugars (Agblevor et al., 2004; Almeida et al., 2009; Ranatunga et al., 1997; Yang et al., 2010). Researchers have developed several methods of treating pretreatment hydrolysates to improve fermentation performance, such as overliming, fungal treatment, steam treatment, solvent extraction and ion exchange treatment. Most of these methods remove some of the inhibitory compounds, but not all of them. One of the most effective and low cost methods, overliming, produces glucose and xylose losses of 12% and 13%, respectively (Mohagheghi et al.,

2006). An NH_4OH detoxification method, as proposed by Alrikson et al. (2005), is an alternative to overliming (Alriksson et al., 2005, 2006). Jennings and Schell (2011) showed that this method produced a 7% improvement in ethanol yields compared to the overliming method.

Several recent publications reviewed use of membrane processes, such as microfiltration, ultrafiltration and nanofiltration for applicability to lignocellulosic biorefining. Purification and separation of compounds in multi-component, biomass-derived feed streams are usual application of membranes. For example, in starch-based ethanol production, microfiltration as well as ultrafiltration could be used to remove enzymes and starch residues from the glucose stream prior to fermentation (Lipinzki, 2010). Nanofiltration was used to fractionate hot water extractants from woody biomass, such as sugars, acetic acid, sugar degradation products and phenolic compounds (Amidon and Liu, 2009; Huang et al., 2008). In another example, Schlesinger et al. (2006) showed retention of 90 % of the hemicellulose in alkaline-treated process liquor obtained from a viscose fiber production process using nanofiltration membranes. Finally, Sjoman et al. (2008) increased xylose purity in a hydrolysate by 1.4-1.7 fold using nanofiltration membranes.

In this study, a dilute-acid pretreated, corn stover hydrolysate (liquor fraction) was conditioned using NH_4OH and a membrane-facilitated, liquid-liquid extraction process using octanol or oleyl alcohol solvents mixed with an extractant, Alamine 336. Acetic acid and other compounds in the hydrolysate are transported across a hydrophobic membrane into the solvent phase. Biomass sugars remain in the aqueous phase (hydrolysate) and are not degraded. However, previous investigations showed that a small amount of the solvent transfers across the membrane and is found in the hydrolysate at very low concentrations in the µg/mL range (Grzenia et al., 2008, 2010).

The purpose of this study was to test the ability to ferment sugars in membrane supported liquid-liquid extracted and ammonium hydroxide conditioned (AHC) hydrolysate. Untreated hydrolysate is highly toxic to many fermentative microorganisms and a detoxification process is necessary to achieve good conversion of sugars to ethanol. Sugars in variously-treated hydrolysates were fermented to ethanol by the recombinant glucose-xylose fermenting bacterium, *Zymomonas mobilis* 8b. The pure solvents, octanol and oleyl alcohol, were tested alone and in a 1:1 mixture. Additionally, olive oil was added to some of the liquid-liquid extracted hydrolysates to test its reported ability to scavenge solvents and improve ethanol yields (Yabannavar et al., 2001).

4.2 Material and Methods

4.2.1 Hydrolysate

Dilute-acid pretreated, corn stover hydrolysate was produced at the National Renewable Energy Laboratory (Golden, CO) in a 900 dry kg/d pilot-scale continuous reactor. Pretreatment operating conditions were 25 % (w/w) total solids, 190°C, 55 mg acid/g dry biomass and an approximate residence time of 1 min. The liquor fraction was recovered using a Q-120 Quadramatic basket centrifuge (Western States Machine Company, Hamilton, OH).

4.2.2 Ammonium hydroxide conditioning (AHC)

Ammonium hydroxide (29.8%, J.T Baker, Phillipsburg, NJ, USA) was added to hydrolysate liquor until the pH reached 8.5. The temperature increased from 19°C to 30°C during this process. After holding the solution for 30 min, it was filtered through a 0.22 µm polyethersulfone top filter (Nalgene, Rochester, NY). The pH was then adjusted to the value required for fermentation (pH 5.7) with 10 N sulfuric acid (Mallinckrodt Baker, Inc. Phillipsburg,

NJ, USA). The solution was then filtered a second time using a 0.22 µm top filter and stored at 4°C.

4.2.3 Liquid-liquid extraction

Liquid-liquid extraction was performed in a LiquiCell Extra-Flow 2.5 x 8 membrane contactor (Membrana, Charlotte, NC) as described in previous papers (Grzenia et al., 2008, 2010). Briefly, 2 L of hydrolysate liquor was contacted across the membrane with 1500 g of an organic solvent for 300 min. The solvents were octanol (297887) and oleyl alcohol (O7600) both acquired from Sigma-Aldrich (St Louis, MO). Alamine 336 (Sigma-Aldrich) was used as the reactive extractant and was mixed with the solvents to a concentration of 15% (w/w). After membrane extraction the pH of the hydrolysate was about 3 and was then adjusted to pH 5.7 with ammonium hydroxide. Ammonium hydroxide volume for organic phases during membrane extraction consisting of octanol and oleyl alcohol respectively were 6 and 12 mL per 1 liter hydrolysate.

Fermentation of hydrolysates using different conditioning steps as well as control experiments were conducted as summarized in Table 1. A combination of both detoxification methods was also tested to determine if fermentation performance could be further enhanced with multiple treatments. Hydrolysate was treated by liquid-liquid extraction method and then conditioned using ammonium hydroxide. Additionally, olive oil (used oil from a restaurant at 1% w/w) was added to several of the liquid-liquid extracted hydrolysate to scavenge the small amount of solvent that was presence in this material.

4.2.4 Fermentation

Fermentations were conducted using *Z. mobilis 8b,* a glucose-xylose fermenting bacterial strain (Mohagheghi et al., 2004). *Z. mobilis 8b* was stored in cryovials at -70°C in rich media (RM, 10 g/L yeast extract, 2 g/L KH_2PO_4), 20 g/L glucose, 10 g/L xylose in 20% (v/v) glycerol.

Inoculum was produced using the following procedure. A cyrovial of *Z. mobilis 8b* culture (0.1 mL) was transferred to a 15-mL Falcon tube (Becton Dickinson, Franklin Lakes, NJ) containing 10 mL RM with 50 g/L glucose and 10 g/L xylose. The tube was incubated at 30ºC in an incubator (Precision Gravity Convection incubator, Model 2, Precision Scientific, Chennai, India.). After 8-10 h, the culture was transferred to a 250-mL baffled shake flask containing the same media found in the 15-mL tube. The flask was incubated in a shaking incubator (Innova 4000, New Brunswick, NJ) at 37°C and 150 rpm. Glucose and cell density were monitored using a glucose analyzer (YSI 2300 STAT Plus, YSI Incorporated, Yellow Springs, Ohio) and spectrophotometer (Spectronic 601, Milton Roy Ivyland, PA). After about 12 h, when glucose concentration had dropped to 30-35 g/L and optical density (OD) was 4.0 to 4.5 (absorptance units at 600 nm), a 10% (v/v) inoculum was transferred to the fermentation flask.

Fermentations were performed in 125-mL shake flask at a 100 mL working volume. The media contained hydrolysate liquor diluted to 55% (v/v) of its original strength and RM. The concentration of glucose in the fermentation flask was increased by an additional 50 g/L. The additional glucose represents glucose that would have been produced by enzymatic hydrolysis of the cellulosic solid had the solids been present. For simplicity, enzymatic hydrolysis was not performed. A positive control fermentation was performed in media containing RM with 50 g/L, 30 g/L and10 g/L of glucose, xylose and acetic acid, respectively. The pH of the control

fermentation was adjusted to 5.7 using potassium hydroxide. Fermentation flasks were loosely capped and placed in the shaking incubator (Innova 4000) at 37°C and 150 rpm for 72 h. Each condition was performed in triplicate. Samples were taken at 4 h intervals for the first 12 h and then every 24 h thereafter.

4.2.5 Analysis

Glucose, xylose, ethanol and acetic acid concentrations were determined by High Performance Liquid Chromatography (HPLC) using a Biorad Aminex HPX-87 H (Bio-Rad Laboratories, Hercules, CA, USA) column equipped with a Cation H^+ guard cartridge (BioRad). The HPLC was an Agilent 1100 (Santa Clara, CA, USA) equipped with a refractive index detector. The injection volume was 6 µL. The mobile phase was 0.01 N sulfuric acid and column flow rate and temperature were 0.6 mL/min and 55°C, respectively. All measurements were performed in triplicate and average results are reported.

4.2.6 Calculations

Ethanol yield (Y) was calculated using Equation 1.

$$Y = \frac{\Delta E}{S_i (0.51)} \qquad (1)$$

ΔE (g/L) is the final minus the initial ethanol concentration and S_i (g/L) is the sum of the initial concentration of glucose and xylose at the beginning of the fermentation.

Ethanol productivity was calculated from the difference between ethanol concentrations at adjacent data points divided by the time interval and only the maximum value is reported.

4.3 Results and Discussion

In our previous work, we investigated reactive-membrane extraction as a technique to remove organic compounds from dilute-acid-pretreated hydrolysates (Grzenia et al., 2007, 2008). We were able to remove a large fraction of the acetic acid, furfural and 5-hydroxymethylfurfual from hydrolysate liquors using a cross-flow membrane module and an organic solvent containing a tertiary aliphatic amine. The primary goal of this work was to ferment membrane-extracted hydrolysate liquors and compare ethanol yields and rates to those achieved in AHC hydrolysate liquors.

Figure 1 shows glucose, xylose and ethanol concentration data for a well-performing membrane-treated liquor (Condition 2 in Table 1) and a pH-adjusted liquor (Condition 6 in Table 1) that did not perform as well, but was also not the worst performing condition. Glucose in the extracted liquor was consumed by 8 h into the fermentation; while glucose in the pH-adjusted liquor was not fully consumed until 30 h into the fermentation. The xylose concentration data showed a similar trend for the two processes, except that not all of the xylose was consumed for either condition. Xylose concentration in the pH-adjusted liquor decreased slowly from 43 to 35 g/L over the 48 h fermentation, but nearly all of the xylose was consumed in the membrane-extracted liquor. The initial xylose concentration in the membrane treated hydrolysate was about 8% lower than the initial concentration in the pH-adjusted hydrolysate. This is most likely due to small sugar losses in the organic phase. Importantly these losses are not greater than the losses that occur in current detoxification operations (Mohagheghi et al., 2006). The difference in glucose concentrations is less noticeable because additional glucose was added to each fermentation flask. Ethanol concentrations reach a maximum value of 46 g/L for the extracted liquor and a maximum value of 35 g/L for pH-

adjusted liquor. The ethanol present at time zero was from ethanol carried over in the inoculum. After 8 h of fermentation, the extracted liquor produced more ethanol than the pH-adjusted liquor because of greater xylose consumption. Data from the other conditions showed similar behavior to these results, except for octanol-extracted hydrolysates where little glucose was consumed. This behavior is typical of detoxified hydrolysates and has also been observed in AHC and overlimed hydrolysates (Jennings and Schell, 2010, Alriksson et al., 2005, 2006).

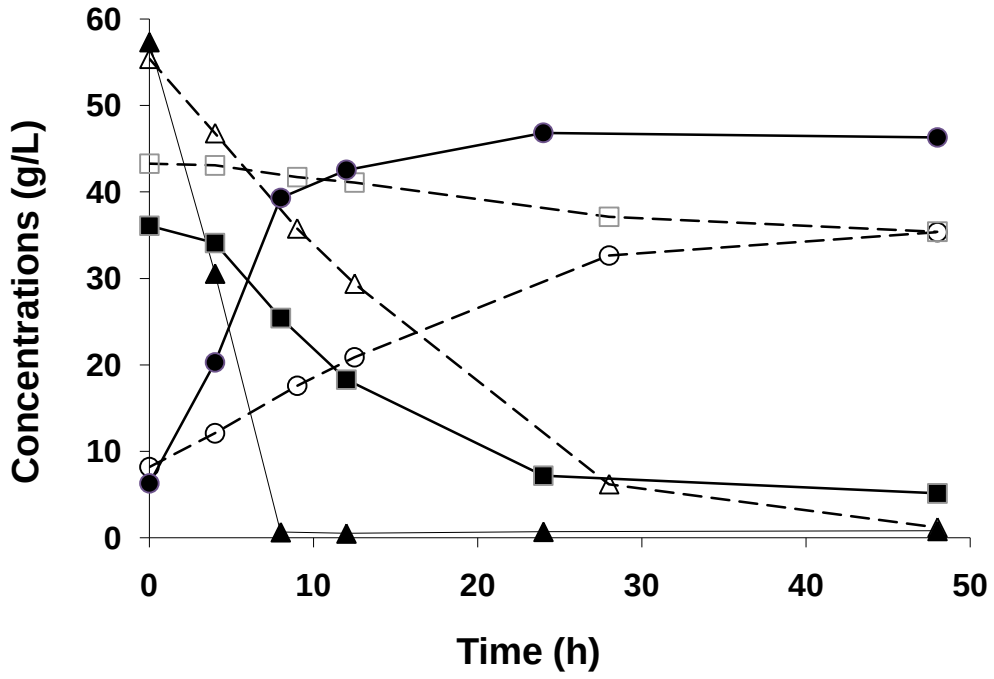

Figure 1 Glucose (triangles), xylose (squares) and ethanol (circles) concentrations during fermentation of a membrane-extracted hydrolysate using an oleyl alcohol organic phase followed by AHC (filled symbols, solid line, Condition 2 in Table 1) and pH-adjusted hydrolysate (open symbols, dashed line, Condition 6 in Table 1)

Table 1 shows ethanol yields, concentrations, and maximum productivity, as well as ammonium hydroxide use for each method if appropriate. The different treatment conditions are ranked in the Table 1 from highest to the lowest ethanol yield. As expected, the control fermentation achieved the best performance because no inhibitors are present. Hydrolysate extracted with oleyl alcohol (Condition 2-3) performed well achieving ethanol yields from 0.83-0.85 and productivities very near the value (approximately 5 g/(L•h)) achieved by the control fermentation. AHC of oleyl alcohol-extracted hydrolysates (Condition 2) did not significantly improve performance over the unconditioned oleyl alcohol-extracted material. A positive outcome since conditioning is undesirable because of the cost for additional ammonium hydroxide.

As seen in Table 1, the lowest ethanol yield and productivities were produced on hydrolysate extracted with octanol (Conditions 9 and 10). AHC of these materials did not significantly improve ethanol production. Both octanol and Alamine 336 were measured in the aqueous phase suggesting that both compounds could cross the membrane during the extraction process. This finding has been previously noted (Grzenia et al., 2008, 2010). In theory, when hydraulic pressure is higher on the aqueous side of the membrane than on the organic phase side of the membrane, there should be no leakage of organic compounds into the aqueous phase. However, the hydraulic pressure can be too high and exceed the breakthrough pressure forcing solvent molecules out of the pores, which causes leakage of water phase into the organic phase. It has been reported that tight control of the pressure difference between the aqueous and organic sides of the membrane is important to avoid leakage of compounds across the membrane, which if occurs leads to emulsion formation (Gawronski and Wrzesinska, 2000; Lee et al., 2001; Wang et al., 2002). We maintained excellent pressure control during our

experiments and used gear pumps to obtain nearly pulse-less flow. No emulsion formation was visible during our work, but octanol and Alamine 336 were still detected in the hydrolysate. Total suppression of transport organic phase into the aqueous phase is not possible due to the very low solubility of the organic phase in the aqueous phase. While transport due to convection can be suppressed slow diffusion across the membrane will occur.

Kapacu et al. (1998) reported that alcohols with 2-12 carbons were strong inhibitors of *Saccharomyces cerevisiae*. Offeman et al. (2008) screened several organic solvents (octanol, decanol, isofol, etc.) for extractive removal of ethanol during fermentation. In shake flasks experiments, he exposed *S.* cerevisiae to 20 % (v/v) solvent concentrations and reported strong inhibition. Zautsen et al. (2008) removed ethanol during fermentation of lignocellulosic sugars by liquid-liquid extraction using various solvents and concluded that solvents with the highest partition coefficient ($P_{ow,}$ partition coefficient of a solvent over water and octanol) had better biocompatibility. Octanol was also used to extract toxic compounds from hydrolysate, but was not considered for use in fermentation due its toxicity. Oleyl alcohol showed the best extractive performance among biocompatible solvents tested. Chan et al. (2010) performed fermentations on Alamine 336/octanol treated bio-oil hydrolysates. The yeast strain, *Saccharomyces cerevisiae T2*, was not able to produce ethanol until it was adapted to the treated material. Octanol is likely the toxic compound in our fermentations.

Alamine 336 is also reported to be a possible toxin to microorganisms. Yabannavar et al. (1991) noted that Alamine 336 is soluble in water at less than 5 ppm, but probably is toxic below this level. He immobilized *Lactobacillus delbrueckii* into k-carrageenan beads to protect the microorganism from solvents and investigated the addition of soybean oil to the gel-cell matrix. He hypothesized that oil acts as a scavenger for Alamine 336 effectively reducing its diffusion

rate into the gel matrix. He showed that the oil protected the cells during direct exposure to a 15% Alamine 336 (v/v)/oleyl alcohol solution.

We also explored the potential of adding 1% (v/v) olive oil to treated hydrolysate to determine if this procedure improved ethanol yields. As shown in Table 1, olive oil was added to hydrolysate extracted with octanol (Condition 8) and further conditioned with ammonium hydroxide (Condition 4). The addition of oil for both conditions clearly increased ethanol yield and rates compared to the corresponding conditions without oil. We did not measure the concentration of octanol before and after addition of olive oil, but suspect that the oil is scavenging octanol and worked well to improve ethanol yields on octanol-extracted hydrolysate. The results also suggest that Alamine 336 was not impacting fermentation performance since oleyl alcohol-extracted hydrolysate performed well. Kapucu et al. (1998) also produced results supporting the ability of oil to scavenge alcohols by showing that addition of 30% sunflower oil to a fermentation broth reduced the toxicity of decanol. However in our experiments, the microorganism was in direct contact with the solvents and addition of oil into octanol-treated hydrolysate (Condition 4) may saponificate octanol molecules protecting the microorganism from octanol's toxic effects.

Ethanol yields for hydrolysate extracted with a 1:1 (v/v) mixture of octanol and oleyl alcohol (Condition 7) were between ethanol yields produced by each solvent used alone, further suggesting that octanol is highly toxic to the microorganism. Finally, AHC (Condition 5) and pH-adjusted hydrolysate (Condition 6) produced ethanol yields and rates substantially better than octanol extracted hydrolysate, but less than oleyl alcohol extracted hydrolysate.

Both AHC and oleyl alcohol-extraction are effective methods of detoxifying hydrolysates, but both methods produce some loss of sugars. However, a significantly larger amount of ammonium hydroxide is required for AHC compared to the amount required from extracted hydrolysates (see Table 1). Extracted hydrolysates are very near pH 4 after extraction and only a small amount of caustic is required to further adjust pH to the value required for fermentation. It is unclear if the saving in chemical cost outweighs the additional operating cost of the membrane extraction equipment. We must also assume for the extraction process that is no or little loss of solvents. Nevertheless, an economic analysis of both methods would clearly determine if membrane extraction is economically viable and this work is in progress.

Table 1 Fermentation performance results and ammonium hydroxide use for treated hydrolysate liquors

Condition Number	Hydrolysate Treatment	Ethanol Yield	Final Ethanol Concentration (g/L)	Maximum Ethanol Productivity (g/(L•h))	NH_4OH Use (mL/L liquor)
1	No treatment, pure sugars	0.91	51.0	4.98	-
2	ME: Oleyl alcohol[a]/AHC	0.85	46.9	4.75	20
3	ME: Oleyl alcohol	0.83	46.4	4.94	-
4	ME: Octanol/AHC/olive oil[b]	0.77	45.2	1.86	15
5	AHC	0.76	45.9	2.54	48
6	Neutralization	0.54	35.3	1.09	33
7	ME: mixture[c]/AHC	0.41	21.9	2.04	15
8	ME: Octanol/olive oil[b]	0.09	10.1	0.33	-
9	ME: Octanol/AHC	0.04	10.0	0.14	15
10	ME: Octanol	0.00	0.0	0.00	-

[a]ME: solvent-membrane extracted and associated organic phase

[b]1% (v/v) olive oil added to shake flasks containing detoxified hydrolysate

[c](1:1) mixture of oleyl alcohol and octanol

4.4 Conclusions

Detoxification of dilute-acid-pretreated corn stover hydrolysate can be accomplished using reactive membrane extraction. Depending on the solvent used, the technique improves ethanol yields and rates compared to AHC. It is also possible to regenerate and recover most of the solvent. But reactive membrane extraction process is more complicated and likely has higher capital and operating cost. Additionally, the extraction process requires 6 hours of contact time whereas the residence time for AHC is one-half hour. Further optimization is necessary as well as economic analysis to assess performance and potential to replace AHC.

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Chapter 5

MEMBRANE EXTRACTION FOR BIOFUEL PRODUCTION

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Abstract

Dispersion-free membrane-based solvent extraction has been shown to overcome many of the disadvantages associated with dispersion-based contacting devices used for conventional solvent extraction. Here, we have explored the use of membrane-based solvent extraction for the production of biofuels. Given the non-volatile nature of most biomass components it is

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likely that solvent extraction will dominate in future biorefineries. Three potential applications for membrane-based solvent extraction are considered.

Production of biofuels from lignocellulosic biomass may be achieved through three main routes: gasification, pyrolysis or liquefaction, and hydrolysis. Hydrolysis of the lignocellulosic biomass leads to an aqueous sugar-rich solution which may be fermented to biofuels such as ethanol. The use of membrane based solvent extraction has been investigated for removal of acetic acid from hydrolysates derived from sugar cane bagasse, sorghum, oats, coffee husks, corn fiber and corn leaves prior to fermentation. Acetic acid is one of the products of hydrolysis that is toxic to the microorganism used to ferment the sugars to ethanol. The result obtained here indicate that membrane based solvent extraction is a flexible unit operation that may be used to remove acetic acid from a variety of hydrolysates.

Biomass hydrolysates may also be converted to fuels by aqueous phase processing. Hydroxymethylfurfural (HMF) is an important intermediate in this process. Membrane based solvent extraction has been used to recover this valuable intermediate from an aqueous phase. In addition membrane based solvent extraction has been used to remove glycerol from butanol. This later separation could be of significance in the production of biodiesel.

The results for the three extractions investigated here highlight the versatility of membrane based solvent extraction for the production of biofuels. Experimentally determined overall mass transfer coefficients agree well with those predicted from theory. These mass transfer coefficients could be used to guide the design of larger scale processes.

Key words: Acetic acid; Biodiesel; Bioethanol; Biofuel; Dispersion-free extraction; Glycerol; Hollow fiber; Hydrolysate; Hydroxymethylfurfural; Lignocellulosic biomass; Mass transfer coefficient; Membrane contactor

5.1 Introduction

Development of energy-efficient process for sustainable production of fuels and chemicals is critical, given declining world petroleum reserves, increasing energy needs by emerging economies, and political and environmental concerns associated with using fossil fuels[1]. Figure 1 is a summary of the breakdown of the US energy supply for 2003[2].

Fossil fuels (petroleum, coal, and natural gas) accounted for about 86% of the U.S. energy supply in 2003, while renewable sources accounted for 6% combined, 47% of which comes from biomass. In addition, energy demand is projected to grow by more than 50% by 2025[3]. Clearly, finite petroleum resources cannot meet the increasing energy demand.

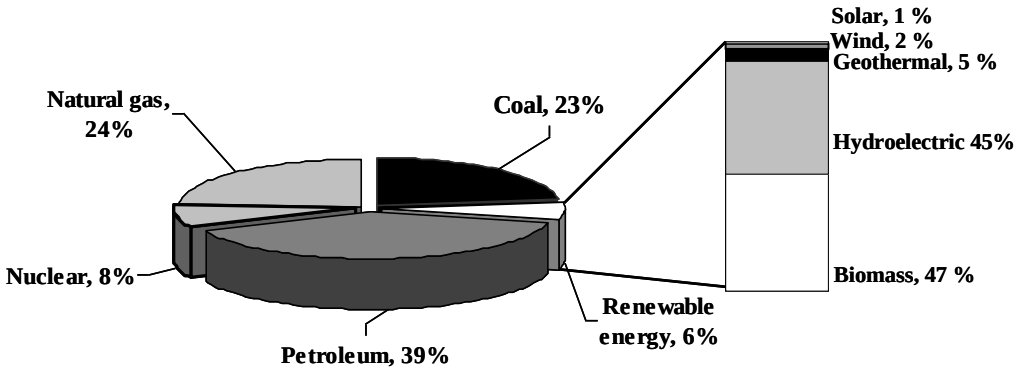

Figure 1 Summary of biomass resource consumption modified from [2]

The form of renewable energy that can contribute substantially to energy needs at costs competitive to fossil fuels is the solar energy captured by photosynthesis and stored in biomass[4]. Currently, the United States produces about 4.5 billion gallons of ethanol annually from about 90 corn grain-to-ethanol refineries (noncellulosic). It is estimated that 130 billion gallons of fuel ethanol could be produced from lignocellulosic biomass in the United States4. Improving biomass to ethanol conversion processes to minimize processing costs will have a significant impact on our economy.

A major obstacle to the large scale conversion of lignocellulosic biomass to fuels and chemicals is the lack of cost effective separations for product isolation and purification as separations can account for up to 60-80% of the processing costs. While distillation dominates in petroleum refinery separations, given the non-volatile nature of most biomass components it is likely that solvent extraction will dominate in future biorefineries[5].

Solvent (liquid-liquid) extraction involves the transfer of the solute species from the feed to the extracting solvent[6]. Two immiscible phases are brought together to promote good mass transfer. This is achieved by dispersing one phase in the other. Consequently, the dispersed phase must be coalesced and the phases separated after extraction of the solute. The amount of solute transferred to the solvent will be limited by its partition coefficient between the feed and solvent phases. Solvent extraction is generally conducted using a number of mixer settlers in series, or a continuous countercurrent extraction column. However, irrespective of the equipment used, conventional extraction operations suffer from a number of disadvantages. These include dispersion of one phase in the other, which requires subsequent coalescence and phase separation, emulsification problems, flooding and loading concerns and scale up related issues[7].

Membrane based solvent extraction, using microporous hollow fibers, overcome all of these disadvantages. The membrane physically separates the two immiscible phases. Thus, independent variation of the two phase flow rates over a large range of flow rates is possible without regard to flooding or loading limitations. The hollow fiber membrane immobilizes the liquid-liquid interface. In the experiments described here hydrophobic polypropylene hollow fibers are used. Thus the membrane pores are filled with the organic phase. Loss of organic phase is prevented by ensuring the aqueous side pressure is higher than the organic side pressure. In addition, extraction is achieved without dispersion of one phase in the other, thus no coalescence step is required.

Schlosser et al.[8] have reviewed the use of membrane-based solvent extraction for recovery and separation of organic acids. The theory of membrane-based solvent extraction is well developed[9]. Transfer of the solute species from the feed to the solvent phase through the membrane is described by an overall mass transfer coefficient. The overall mass transfer coefficient depends on three individual mass transfer coefficients which describe mass transfer across the concentration boundary layer in each of the phases and through the membrane pores. Numerous correlations are available in the literature for predicting the mass transfer coefficient inside the fibers, outside the fibers and in the membrane pores[10].

Here, we present three applications for membrane-based solvent extraction in the manufacture of biofuels. Table 1 summarizes these applications. The first application focuses on production of bioethanol by fermentation of lingocellulosic biomass. The second application explores the feasibility of extraction of 5-hydroxymethylfurfural (HMF) from an aqueous solution into methyl isobutyl ketone (MIBK). While the first two examples focus on extraction of a target compound into an organic phase, the third application involves the extraction of glycerol from 2-

butanol into an aqueous phase. Efficient removal of glycerol during the manufacture of biodiesel is essential for the development of cost-effective technologies for production of biodiesel. Below the potential role for membrane extraction in each of these examples is described in more detail.

5.1.1 Removal of acetic acid from biomass hydrolysates

Lignocellulsoic biomass consists of three main polymers: cellulose, hemicellulose and lignin. There are three main routes for conversion of lignocellulosic biomass into liquid fuels: gasification, pyrolysis or liquefaction and hydrolysis1. Here we focus on hydrolysis. Hydrolysis involves the combination of thermochemical and biochemical processing steps to convert the cellulose into glucose and hemicellulose in to 5 carbon sugars (mainly xylose)[11]. Dilute sulfuric acid is used to hydrolyse the lingocellulosic biomass, as it has been shown to effectively hydrolyze the hemicellulose and increase the enzymatic digestibility of the cellulose[12]. All of the different lingocellulosic biomass samples were provided by the School of Engineering of Lorena (EEL), University of São Paulo, Lorena, SP., Brazil. Dilute sulfuric acid was used to pretreat the lignocellulosic biomass.

Table 1 Summary of membrane based solvent extraction experiments conducted. Pretreatment was conducted at 120 °C

Feed	Target Compound	Comment		
		Pretreatment conditions Initial		pH
		Pressure (atm)	Holding time (min)	
Sugar cane bagasse	Acetic Acid	1	10	1.15
Sorghum		1	10	1.3
Oat		1	10	1.37
Coffee Husk		1	30	1.32
Corn Fiber		0.5	10	0.84
Corn Leaves		1	10	0.6
		Organic phase 85:15 octanol: Alamine 336 (weight ratio)		
HMF in water or water/DMSO mixture	HMF	Organic phase MIBK		
Glycerol in 2-butanol	Glycerol	Glycerol extracted from 2-butanol		

During pretreatment compounds are produced that are toxic to the microorganism used to ferment the sugars to the desired products (ethanol). Here, we focus on removal of acetic acid which is produced as a result of hydrolysis of acetyl groups present in the hemicellulose. In its protonated form, acetic acid can diffuse through the cytoplasmic membrane of cells affecting the cell metabolism[13,14]. It can further diffuse through the cell cytoplasm where it lowers the intracellular pH, resulting in impaired transport of various ions and increased energy requirements and reduced ethanol yields[15]. This is particularly important as the viability of a cellulosic ethanol plant will depend on maximizing ethanol yields.

In our earlier work, we have explored the feasibility of using membrane-based solvent extraction for removal of acetic acid from corn stover hydrolysates[16,17]. Here, we present results

for extraction of acetic acid from a number of different hydrolysates readily available in Brazil. As described in our earlier work, the organic phase consists of Alamine 336, a long chain water insoluble tri-octyl/decyl amine dissolved in octanol. Long chain aliphatic amines may be used for extraction of carboxylic acids for dilute aqueous solutions[18,19,20,21,22,23,24,25,26,27]. Table I summarizes the various lingocellulosic biomass hydrolysates we have investigated. In addition, pretreatment conditions are listed. These pretreatment conditions were developed by EEL and were found to produce a xylose-rich hydrolysate.

There is a considerable amount of literature available on the extraction of acetic acid in the presence of sulfate and bisulfate anions using aliphatic tertiary amines[16]. Briefly, for effective extraction of acetic acid the aliphatic amine (Alamine 336 used here) must be insoluble in the aqueous phase. Further, for the hydrolysates listed in Table 1, after pretreatment, the pH will be between 0.6 and 1.4. Thus the sulfuric acid will be present almost entirely as HSO_4^- and SO_4^{2-}. Eyal and Canri[27] describe 4 main mechanisms for acid extraction by amine-based extractants. In the case of sulfuric acid, a strong mineral acid, ion pair formation occurs where the amine binds a proton to form an ammonium cation. As shown in our earlier work, this mechanism leads to significant extraction of sulfuric acid according to the following mechanism:

$$R_3N_{(organic)} + H^+_{(aq)} \rightarrow R_3NH^+_{(organic)}$$

$$R_3NH^+_{(organic)} + HSO_4^-{}_{(aq)} \rightarrow R_3NH^+HSO_4^-{}_{(organic)}$$

Alamine 336 will extract the protonated form of acetic acid. Barrow and Yerger[28] indicate that the acetic acid molecule reacts with the amine to form an ion pair. As we have shown in our earlier work[16], sulfuric acid will be extracted preferentially. This leads to a rapid increase in pH

till the pH reaches a value of 2, the pKa value for the second dissociation constant for sulfuric acid. After the hydrolysate pH goes above 2, the rate of extraction of acetic acid increases. As the pH approaches 4.75, the pKa of acetic acid, the rate of extraction of acetic acid rapidly decreases. Here, we extend our previous results obtained for corn stover-based hydrolysates to the hydrolysates listed in Table 1. We show that membrane extraction is a highly flexible unit operation that may be used to remove acetic acid from a variety of lignocellulosic biomass hydrolysates. Further, the rate of extraction may be correlated using mass transfer coefficients.

5.2.1 Extraction of 5-hydroxymethylfurfural

The previous example focused on fermentation of lignocellulosic biomass hydrolysates into bioethanol. Detoxification (conditioning) of the hydrolysate was required in order to remove compounds that are toxic to the microorganism. Alternatively aqueous phase processing could be used to chemically convert the aqueous sugar rich hydrolysate into potential transportation fuels such as dimethylfuran (DMF). Aqueous phase processing, pioneered by Dumesic and co-workers, involves the catalytic conversion of sugars, sugar alcohols and polyols into hydrogen or alkanes[29,30,31,32,33,34,35,36,37].

Huber et al. 1 describe a self-sustaining biomass biorefinery for conversion of biomass into liquid alkanes using aqueous phase processing. Acid dehydration of 6 carbon sugars can lead to the production of HMF. HMF is a critical and versatile intermediate in the conversion of biomass to liquid biofuels such as DMF, liquid alkanes and many other value-added products. Removal of HMF from the aqueous reaction media will greatly enhance HMF yield by driving the forward reaction and preventing HMF degradation. We have explored the feasibility of HMF removal by membrane based solvent extraction.

Extraction of HMF is complicated by the fact that HMF is highly hydrophilic. An ideal extractant should have low water solubility. In addition, the extractant should be polar in order to efficiently extract the highly polar HMF. We have used MIBK (methyl isobutyl ketone) as the organic extractant. Previous investigators have used MIBK as the organic phase in a biphasic reactor designed to produce HMF from fructose[38,39,40]. Aqueous phases consisting of mixtures of water and DMSO (dimethyl sulfoxide) have been used. Use of a phase modifier such as DMSO has been shown to improve reaction yields[38]. The results again highlight the versatility of membrane based solvent extraction. Our results may be correlated using mass transfer coefficients.

5.3.1 Glycerol extraction

Biodiesel is a "green fuel" that has several advantages over conventional petroleum based diesel. Engines using biodiesel will have a significant decrease in carbon dioxide and many other green house gas emissions. Current commercial biodiesel production uses base catalyzed transesterification to convert triglycerides to biodiesel. Each triglyceride molecule reacts with three molecules of alcohol (commonly methanol) to produce one ester and glycerol molecule. NaOH and KOH catalysts are commonly used. Removal of glycerol from the reaction mixture is essential. Base catalyzed transesterification reactions have little tolerance for free fatty acids (FFA) and water. FFAs will cause soap formation and generate water. In turn, water will cause ester hydrolysis to produce more FFAs. Consequently, product recovery can be difficult and costly.

While acid catalysis is slower than base catalysis, the fact that acid catalysts can catalyze esterification of FFAs and do not lead to saponification reactions is a major advantage[41]. Further

use of higher alcohols such as butanol rather than methanol is an advantage as butanol may be produced via biomass fermentation leading to a greener process. Additionally, it is hydrophobic and has a higher boiling point allowing the transesterification reaction to be conduced at higher temperature.

Development of alternative transesterification schemes will require separation of glycerol from a reaction medium containing different components. Here, we have investigated glycerol extraction from an organic phase consisting of butanol as would be the case if butanol were the alcohol used for the transesterification reaction. Unlike the previous examples, membrane-based solvent extraction is used to extract glycerol from an organic phase into an aqueous phase. However, the same mass transfer correlations apply.

5.2 Material and Methods

The membrane extraction set up used for all the experiments is given in Figure 2. Two Liqui-Cel membrane contactors MiniModule® 1 x 5.5 and MiniModule® 1.7 x 5.5 (Membrana, Charlotte, USA) were used for extraction of acetic acid and glycerol respectively. The 1 x 5.5 and 1.7 x 5.5 modules contain 0.18 and 0.58 m^2 of active membrane surface area. HMF extraction was conducted using a Liqui-Cel® Extra-Flow 2.5 x 8 module. This module contains 1.4 m^2 of membrane surface as well as a central baffle to promote mixing of the shell side fluid. All three modules contained polyporpylene fibers; 300 μm OD, 220 μm ID, porosity 40%, pore size 0.04 μm. The larger Extra-Flow 2.5 x 8 module was compatible with MIBK which was used as the organic phase for extraction of HMF.

Flexible chemical resistant Masterflex, precision silicone 6410-18 and tygon 2075 tubing (Cole-Parmer, Vernon, IL) was used to connect a Watson Marlow 505 U and 505 S peristaltic

pump (Cole-Parmer, Vernon, IL) to the MiniModule 1 x 1.5. For the Minimodule 1.7 x 5.5 and the Extra-flow 2.5 x 8 module a Masterflex 77601-10 pump head equipped with a 20-650 RPM drive (Nr 7591-50, Cole-Parmer) and a Watson Marlow 503 U peristaltic pump were used with Masterflex 96410-73 precision silicone tubing (Cole-Parmer). Pressure gauges, 0 -100 kPa (McMaster-Carr, Atlanta, GA) were used to monitor the aqueous and organic phase pressures.

All experiments were conducted at 25 °C. The system was started by first turning on the aqueous and then the organic phase pumps. The aqueous phase was pumped through the fiber lumen while the organic phase, was pumped on the shell side. Table 2 gives the various aqueous and organic phase flow rates investigated. In all experiments the aqueous side pressure was above the organic phase pressure at any given point in the module to prevent passage of the organic phase into the aqueous phase. For extraction of acetic acid, pH was monitored using a Thermo Orion 520 pH meter (Thermo Fisher Scientific, Waltham MA) equipped with a Metler Toledo pH probe (Cole-Parmer). Samples were taken from the aqueous phase at frequent intervals for high pressure liquid chromatography (HPLC) analysis (extraction of acetic acid and HMF) or refractive index measurement (extraction of glycerol) as described below.

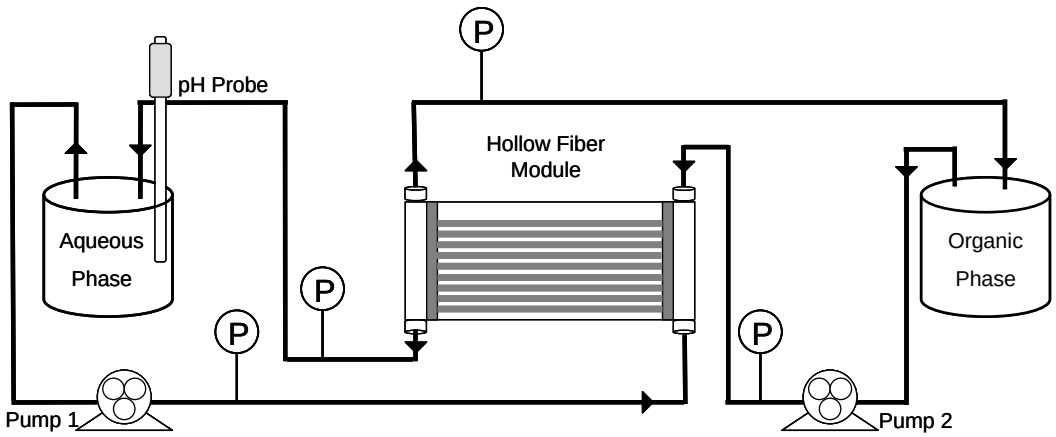

Figure 2 Schematic representation of experimental setup

5.2.1 Removal of acetic acid from biomass hydrolysates

The various biomass samples listed in Table 1 were air dried and milled to a mesh size of 10. Water was added such that the solid : liquid ratio was 1:10. Hydrolysis of the biomass was conducted using dilute sulfuric acid at a ratio of 100 mg of acid to 1 g of biomass in a 200-mL stainless steel container. Holding times are given in Table 1. After reaction, the remaining solids were removed by filtration using 0.45 μm pore size filter paper (Millipore, Bedford, MA).

Extraction of acetic acid from different biomass hydrolysates was conducted using 333 g of organic phase consisting of 85% octanol (Sigma-Aldrich Corporation, St. Louis, MO) and 15 % Alamine 336 (w/w) (Cognis, Cincinnati, OH). The aqueous phase consisted of 500 g of hydrolysate. The aqueous and organic phase flow rates were 48 and 23 L hr^{-1} respectively. The aqueous side inlet and outlet pressures were maintained at 21 and 3.5 kPa while the organic side inlet and outlet pressures were maintained at 14 and 2 kPa.

During extraction, 2 mL samples of the aqueous phase were removed at frequent intervals for the HPLC analysis (2424 HPLC system equipped with a refractive index detector, Waters Corporation, Milford, MA). An Aminex HPX-87 H column (Biorad Hercules, CA), was used, with a mobile phase consisting of 0.01N sulfuric acid at a flow rate of 0.6 mL min^{-1}. The column temperature was set to 45 °C with an injection volume of 20 µL.

5.2.2 HMF extraction

HMF was obtained from SAFC© (St. Louis, MO). The initial feed concentration was ~5 g/L. The aqueous phase (500 mL) comprised either DI water or 15:1 DI water:DMSO (molar ratio)mixture. Dimethyl sulfoxide (DMSO) was purchased from Thermo Fisher Scientific. The organic phase consisted of 500 ml of MIBK (Sigma-Aldrich). Aqueous and organic phase flow rates from 16-64 L hr^{-1} were investigated (see Table 2). The HMF concentration in the aqueous phase was measured using HP 1050 HPLC equipped with a refractive index detector HP 1047A (Agilent Technologies, Santa Clara, CA). The column temperature was set to 55 °C with an injection volume of 6 µL.

5.2.3 Glycerol extraction

Glycerol (Mallinckrodt Baker, NJ) was extracted from 2-butanol (Sigma-Aldrich). The organic phase consisted of 86.2 g glycerol dissolved in 340.8 g 2-butanol. Organic phase flow rates between 27.7 and 10.8L hr^{-1} were investigated. The inlet and outlet organic phase pressures were controlled at 14 and 3.5 kPa. The aqueous phase consisted of 275.0 g of 2-butanol saturated DI water. Aqueous flow rates between 10 and 28 L hr^{-1} were investigated. The aqueous phase inlet and outlet pressures were set at 21 and 9.5 kPa, respectively. Samples (1 mL) were taken at frequent intervals from the aqueous phase for glycerol analysis using a refractive index meter (Bausch & Lomb, Rochester, NY) at 20 °C.

Table 2 Summary of experimental conditions used for extraction of acetic acid, HMF and glycerol

Feed	Aqueous flow rate ($L\ hr^{-1}$)	Organic flow rate ($L\ hr^{-1}$)	Initial target compound concentration ($g\ L^{-1}$)	Comments
Sugar Bagasse	48	23	2.93	Hydrolysate filtered using 0.45 μm filter
Sorghum			3.61	
Oat			5.94	
Coffee Husk			0.45	
Corn Fiber			2.74	
Corn Leaves			1.21	
HMF in water and water/DMSO mixture				Water:DMSO (Mole ratio)
	32	32	4.6	1:0
	64	16	4.4	1:0
	48	24	4.5	1:0
	32	16	4.2	15:1
Glycerol in 2-butanol	10.8	24.5	2	2-butanol was saturated with water
	27.7	24.5		
	12.7	49		
	27.7	49		

5.3 Results

Acetic acid extraction from various biomass hydrolysates is shown in Figures 3-4. Filled symbols linked by a line represent the change in acetic acid concentration in the hydrolsyate and are read using the left hand side y-axis. To compare results for the three different extractions considered here (ethanol, HMF, and glycerol), the results are normalized by dividing the measured acetic acid concentration in the aqueous phase ($g\ L^{-1}$) by the molecular weight of acetic acid ($60.05\ g\ mol^{-1}$) and the membrane surface area ($0.18\ m^2$) and multiplying by the

organic phase flow rate (23 L h^{-1}). Thus the change in acetic acid concentration is given in terms of an acetic acid flux. Open unconnected symbols give the variation of hydroylsate pH with time and are read using the right hand side y-axis. Figures 3 and 4 indicate that the initial acetic acid concentration varies considerably between the different hydrolysates. The initial acetic acid concentration depends on the lingocellulosic biomass as well as the severity (temperature, sulfuric acid concentration, and time) of the pretreatment[11,42]. Consequently, the development of a hydrolsyate detoxification process depends on the lignocellulosic biomass and the hydrolysis conditions. All six hydrolysates indicate an increase in pH during extraction. As described in our earlier work[16,17] for corn stover hydrolysates, sulfuric acid will be preferentially extracted over acetic acid which results in an increase in hydrolysate pH.

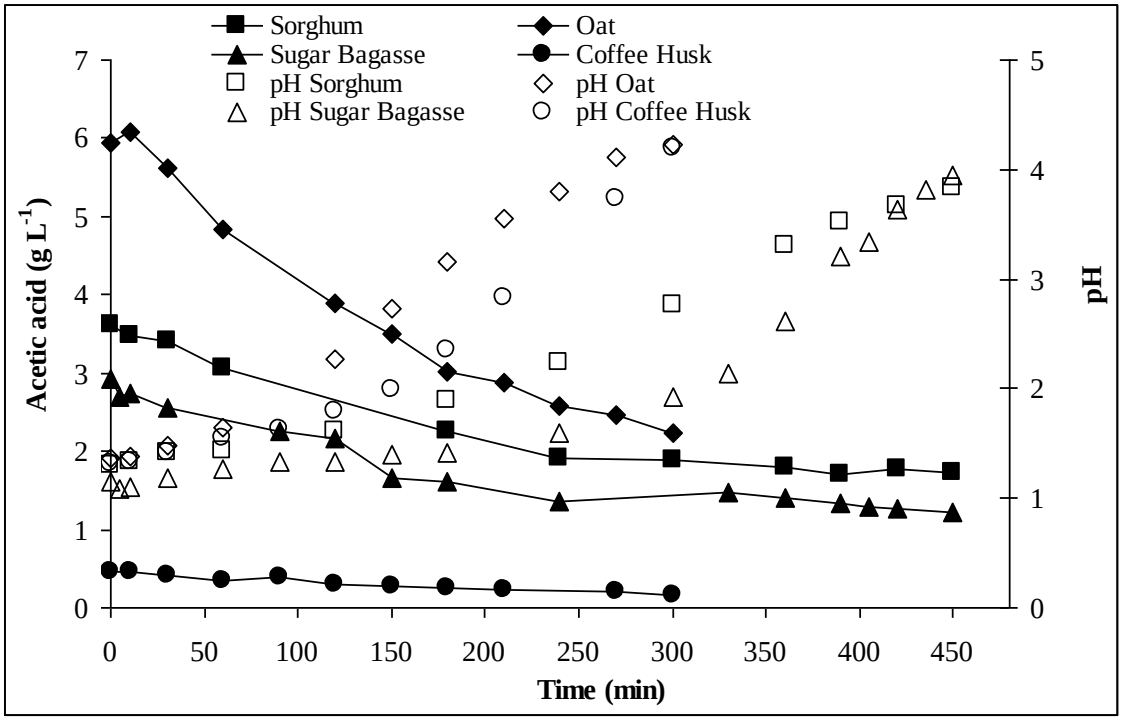

Figure 3 Acetic acid extraction from sorghum, oat, coffee husk and sugar bagasse hydrolysates using 15% Alamine 336 in octanol as the organic phase

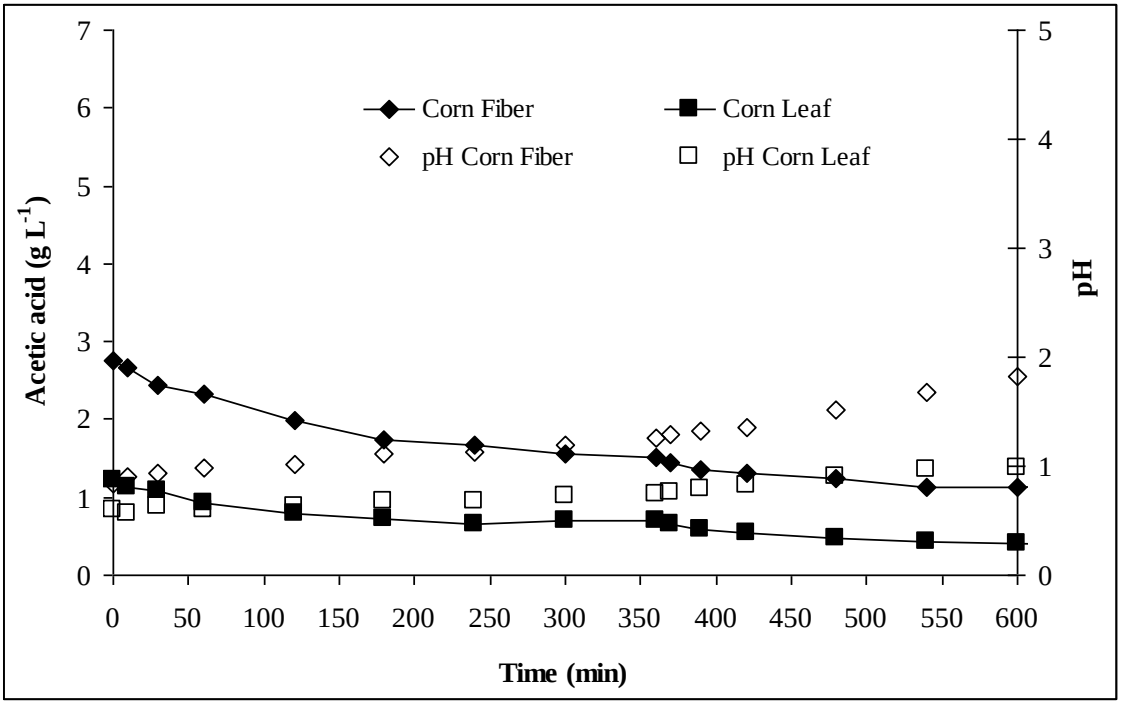

Figure 4 Acetic acid extraction from corn fiber and corn leaf hydrolysates 15% Alamine 336 in octanol as the organic phase

A mass balance around the aqueous feed reservoir for acetic acid gives:

$$V\frac{dC}{dt} = -KA(C - C^*) \qquad (1)$$

where V is the volume of hydrolysate (500 mL), K is the overall mss transfer coefficient based on the aqueous phase, A is the membrane surface area (0.18 m^2) C is the acetic acid concentration in the hydrolysate and C^* is the acetic acid concentration in the hydrolysate that would be in equilibrium with the concentration in the organic phase. Since fresh organic phase was used for each experiment, initially $C^*$is zero. We also assume the aqueous and organic phase reservoirs are fully mixed, and the rate of change of acetic acid concentration in the hydrolysate per pass through the module is small. Integration of Equation (1) leads to:

$$Ln\left(\frac{C}{C_0}\right)=\frac{KAt}{V} \qquad (2)$$

Plotting the left hand side of Equation (2) against time should lead to a straight line the slope of which is proportional to the overall mass transfer coefficient as given in Figure 5. For short extraction times the results for all six hydrolysates fall on the same straight line. However, for longer run times significant deviations from this straight line are observed. For hydrolysates containing higher initial concentrations of acetic acid such as oat and sorghum, assuming the acetic acid concentration in the organic is zero will not be valid at longer run times.

The decrease in the overall mass transfer coefficient at longer run times may also be due to fouling of the membrane by particulate matter in the hydrolysate. In this work the hydrolysate was prefiltered using a 0.45 μm filter prior to membrane extraction. Our results indicate that inclusion of a membrane detoxification step will lead to modifications in the unit operations before and after the membrane step and the effect of these changes must be accounted for when determining the economic viability of membrane detoxification.

The overall mass transfer coefficient based on the aqueous phase is made up of three individual mass transfer coefficients:

$$\frac{1}{K}=\frac{1}{k_a}+\frac{m}{k_m}+\frac{m}{k_o} \qquad (3)$$

where k_a, k_m and k_o are the aqueous, membrane, and organic phase mass transfer coefficients, respectively, and m is the distribution coefficient of acetic acid between the phases defined as the acetic acid concentration in the aqueous phase divided by the concentration in the organic phase. The aqueous and organic phase mass transfer coefficients depend on the aqueous and

organic phase flow rates[43] ,while the membrane mass transfer coefficient does not depend on the phase flow rates. The membrane mass transfer coefficient is given by:

$$k_m = \frac{D\varepsilon}{\tau l} \qquad (4)$$

where D is the diffusion coefficient of acetic acid in the membrane pores, ε is the membrane porosity (40%), τ is the membrane tortuosity, and *l* is the wall thickness of the hollow fibers (40 μm). Membrane tortuosity factors ranging from 3-12 have been reported[44]. Here we use a value of 3 as is commonly used for polypropylene membranes[45].

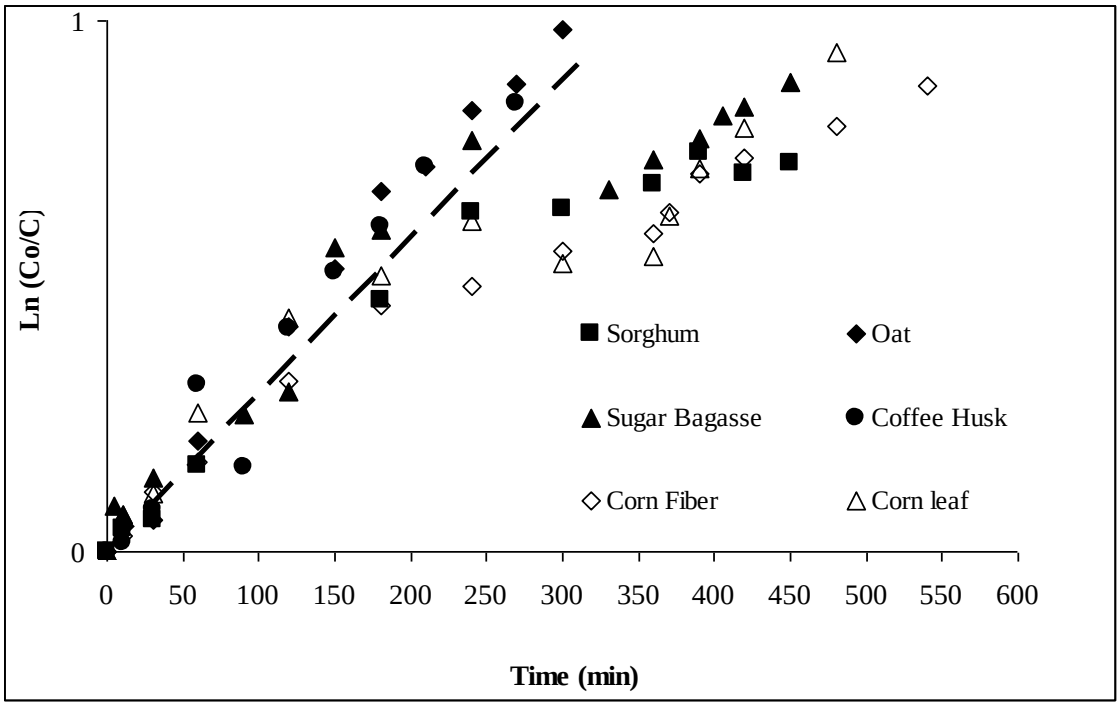

Figure 5 Determination of overall mass transfer coefficient for removal of acetic acid from biomass hydrolysates

In our earlier work[17] ,we have shown that, for extraction of acetic acid, the membrane mass transfer coefficient controls the rate of acetic acid extraction. Thus the overall mass transfer coefficient is independent of the aqueous and organic phase flow rates over a large range flow rates. Consequently, the overall mass transfer coefficient may be approximated by k_m/m (Equation (3)). The experimentally determined value of the overall mass transfer coefficient from Figure 5 is given in Table 3.

The membrane mass transfer coefficient may be calculated using Equation (4). The diffusion coefficient of acetic acid is estimated using the Wilke-Chang equation[46,47].

$$D = \frac{7.4x10^{-8}\left(\phi M_2\right)^{0.5} T}{\mu V^{0.6}} \qquad (5)$$

where ϕ is the association parameter assumed to be 1.5 for alcohols, M_2 is the molecular weight of octanol (130 g mol^{-1}), T is the temperature (295 K), μ is the viscosity of the solvent and V is the molar volume of the solute. The presence of Alamine 336 is ignored and the viscosity of octanol at 25 °C is used (6.09 x 10^{-3} Pa s). The molar volume of acetic acid is calculated from its density and molecular weight (see Table 4)[48]. The calculated value of the overall mass transfer coefficient is also given in Table 3.

Table 3 Calculated and experimentally determined mass transfer coefficients

Compound	KA / V (s^{-1})	Experimental mass transfer coefficient (ms^{-1})	Calculated mass transfer coefficient (ms^{-1})
Acetic acid	0.003	1.39E-07	1.49E-06
Glycerol	0.0526	4.16E-07	5.88E-07
HMF	0.1415	8.42E-07	7.08E-06

The experimentally determined mass transfer coefficients lower than the calculated mass transfer coefficient. There are, however, a number of simplifications that have been made in estimating the mass transfer coefficient. Errors in diffusion coefficients predicted by the Wilke-Chang Equation are much higher for non-aqueous solutions[47]. Further, the viscosity of octanol was assumed though the organic phase contains 15% Alamine 336 which has a much higher viscosity.

The experimentally determined mass transfer coefficient is based on the aqueous phase concentrations. However, the membrane pores are filled with the organic phase. Thus, a distribution coefficient for acetic acid between the two phases should be included in the calculated overall mass transfer coefficient[49]. Estimation of the distribution coefficient for acetic acid is complicated as the concentration of the dissociated species in the aqueous phase as well as acid bound to amine in the organic phase must be included, resulting in the distribution coefficient being a function of pH. Consequently, the effect of the distribution coefficient on the membrane mass transfer coefficient has been ignored, and the membrane mass transfer coefficient is used to approximate the overall mass transfer coefficient. In addition, fouling of the membrane by particulate matter that passed through the 0.45-mm filter used to prefilter

the hydrolysate could explain the lower experimentally determined mass transfer coefficient compared to the calculated mass transfer coefficient. In earlier work[17] for corn stover-based hydrolysates, the experimental and calculated mass transfer coefficients were within 60% of each other. In this work, the hydrolysate was prefiltered using a 0.22-mm filter. It is also worth noting that mass transfer coefficients are typically accurate to within 40%[47] .Thus, Table 3 indicates that the mass transfer coefficient for acetic acid may be estimated using equation 3 and 4.

Table 4 Values of parameters used to calculate diffusion coefficients. Physical data from references [48,50,51]

Compound	Molecular weight of Solute (g mol^{-1})	Molecular weight of Solvent	Density of Solute (g cm^{-3})	Molar volume of Solute (cm^3 mol^{-1})	Association parameter	Viscosity of Solvent (mPas)	$D(cm^2s^{-1})$
Acetic acid	60.1	130.2	1.0	57.2	1.5	6.1	4.50E-06
Glycerol	92.1	100.2	1.3	73.0	1.5	3.0	5.90E-06
HMF	126.1	74.1	1.2	104.6	1.0	0.5	2.50E-05

Figure 6 gives results for extraction of HMF from water into MIBK. Analogous to Figures 3 and 4, to compare results for HMF extraction with extraction of acetic acid and glycerol, the results are normalized by dividing the measured HMF concentration in the aqueous phase (g L^{-1}) by the molecular weight of HMF (126.11 g mol^{-1}) and the membrane area (1.4 m2) and multiplying by the organic phase flow rate. If the rate of transfer of HMF across the membrane is independent of the aqueous and organic phase flow rates, since the measured HMF concentration in the aqueous phase is being multiplied by the organic phase flow rate to normalize the results, the HMF flux should be directly proportional to the organic phase flow rate for the same extraction time. Figure 6 indicates that this is in fact the case. In addition, the rate of HMF extraction from either water or a 15:1 mol ratio water:DMSO solution is the same. Thus the presence of DMSO in the feed has no effect on the rate of HMF extraction. Consequently, as was the case for acetic acid extraction from biomass hydrolysates, the membrane mass transfer coefficient dominates and we approximate the overall mass transfer coefficient by km/m. Thus Equations (1-4) apply for the extraction of HMF from water and water DMSO solutions.

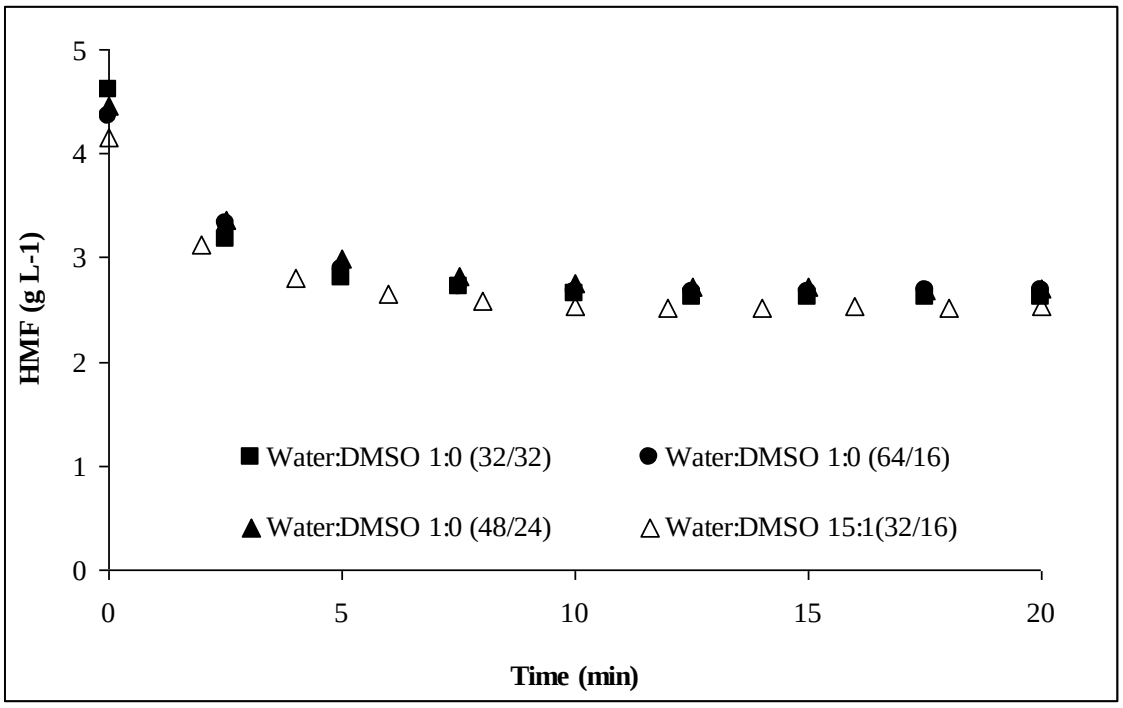

Figure 6 HMF extraction results from water and water/DMSO mixture (mol fraction) at various (aqueous/organic) phase flow rates in L hr^{-1}

Figure 7 is a plot of the left hand side of Equation (2) against time. The results fall on approximately the same curve. As was the case for extraction of acetic acid, the overall mass transfer coefficient may be determined by fitting a straight line to the initial data. Table 3 gives the experimentally determined overall mass transfer coefficient for extraction of HMF.

Equation (4) may be used to calculate the overall mass transfer coefficient. Table 4 gives values for the molecular weight and viscosity of MIBK and the density, molecular weight, and molar volume of HMF[48,50]. The association parameter □ was taken to be 1.0 as is usually assumed for organic compounds[46]. The distribution coefficient, m, for HMF was experimentally determined to be 1.17 (concentration of HMF in the water and water/DMSO mixtures divided by concentration in MIBK). Reasonable agreement is obtained between the predicted and

experimentally determined mass transfer coefficients given the numerous assumptions that have been made, as discussed above.

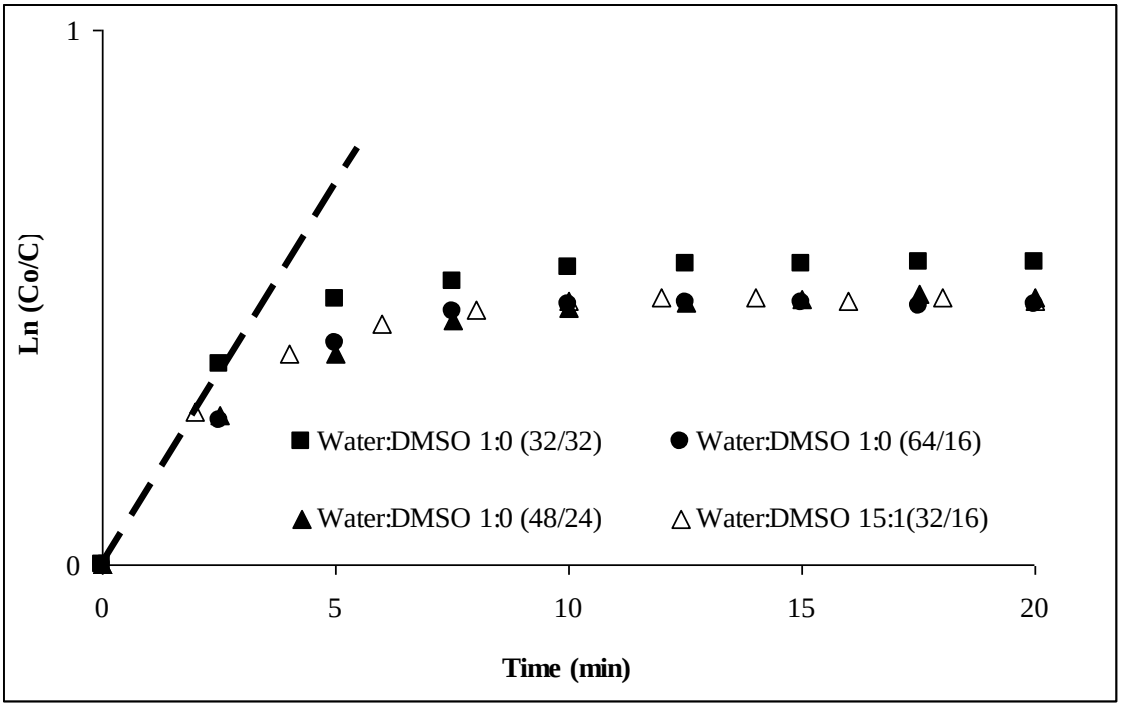

Figure 7 Determination of overall mass transfer coefficient for removal of HMF from water and water/DMSO mixtures (mol fraction) at various (aqueous/organic) flow rates in L hr^{-1}

Results for glycerol extraction from butanol into water are given in Figure 8. The measured glycerol concentration in water is divided by the molecular weight of glycerol (92.1 g mol^{-1}) and the membrane surface area (0.58 m^2). However, unlike extraction of acetic acid and HMF, the aqueous extractant phase flow rate is used to normalize the measured glycerol concentration.

Analogous to extraction of HMF, if the transfer of glycerol across the membrane is independent of the aqueous and organic phase flow rates, since the measured glycerol concentration in the aqueous phase is being multiplied by the aqueous phase flow rate to normalize the results, the glycerol flux should be directly proportional to the aqueous phase flow rate for the same extraction time. Figure 8 indicates that this is in fact the case. As can be

seen for a range of aqueous (10–28 L h^{-1}) and organic (24–49 L h^{-1}) phase flow rates tested, no effect is observed on the rate of mass transfer. Thus, again the overall mass transfer coefficient may be approximated by km/m (Equation (3)). Since glycerol is being extracted from the organic phase into the aqueous phase, a mass balance around the aqueous feed reservoir for glycerol leads to a slightly modified form of Equation (1)

$$V\frac{dC}{dt} = KA(C^* - C) \qquad (6)$$

where V is the volume of water, (275 mL), K is the overall mss transfer coefficient based on the aqueous phase, A is the membrane surface area (0.58 m^2) C is the glycerol concentration in aqueous phase and C^* is the glycerol concentration in the aqueous phase that would be in equilibrium with the concentration in the organic phase. Integration of Equation (6) leads to

$$Ln\left(1-\frac{C^*}{C}\right) = -\frac{KAt}{V} \qquad (7)$$

Plotting $Ln\left(\frac{C-C^*}{C^*}\right)$ against time should lead to a straight line the slope of which is proportional to the overall mass transfer coefficient as given in Figure 9. The experimentally determined overall mass transfer coefficient is given in Table 3. Equation (4) is again used to calculate the membrane mass transfer coefficient. Table 4 gives values for the molecular weight and viscosity of butanol and the density, molecular weight, and molar volume of glycerol[48,51]. The association parameter ϕ was taken to be 1.5 as is usually assumed for alcohols[46]. The distribution coefficient, m, for glycerol was experimentally determined to be 3.34 (concentration of glycerol in the water divided by concentration in 2-butonol). As can be seen,

good agreement is obtained between the predicted and experimentally mass transfer coefficients.

Taken together, our results highlight the versatility of membrane extraction as a unit operation in future biorefineries. The overall mass transfer coefficient may be predicted using Equations 3 and 4. These mass transfer coefficients will be important when estimating the rate of removal of the target compound, in scaling up the process and when correlating the experimental data. This in turn will enable easy design and scale up of larger extraction systems.

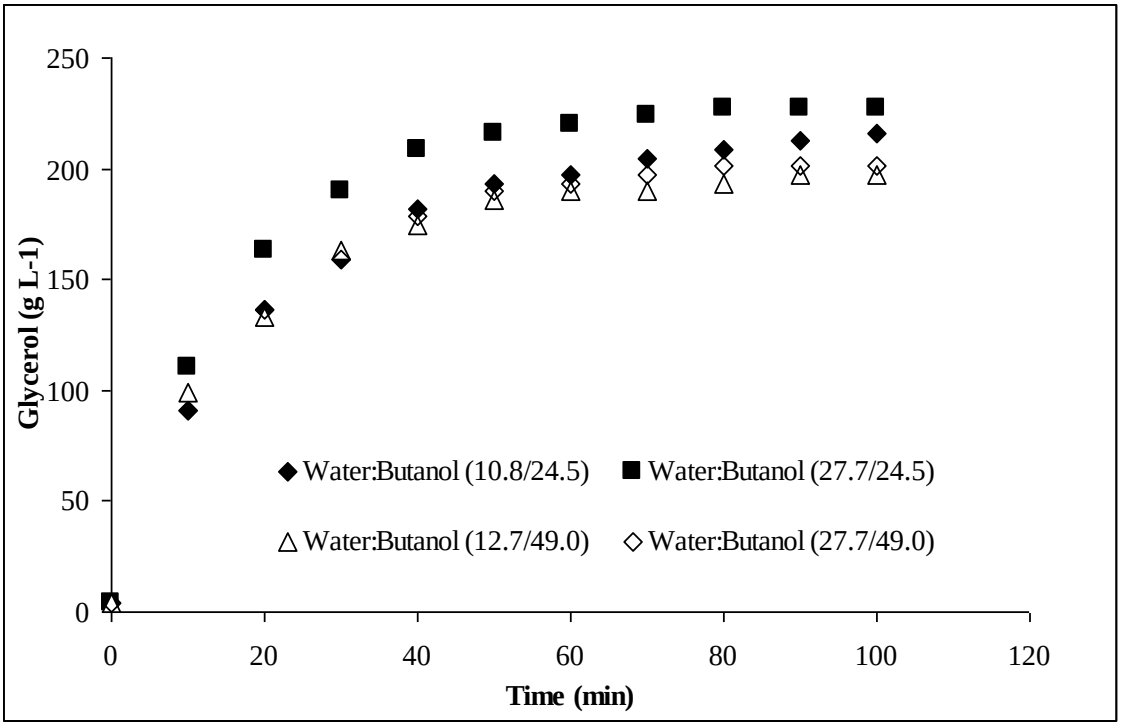

Figure 8 Glycerol extraction results from 2-butanol into water at various (aqueous/organic) flow rates in L hr^{-1}

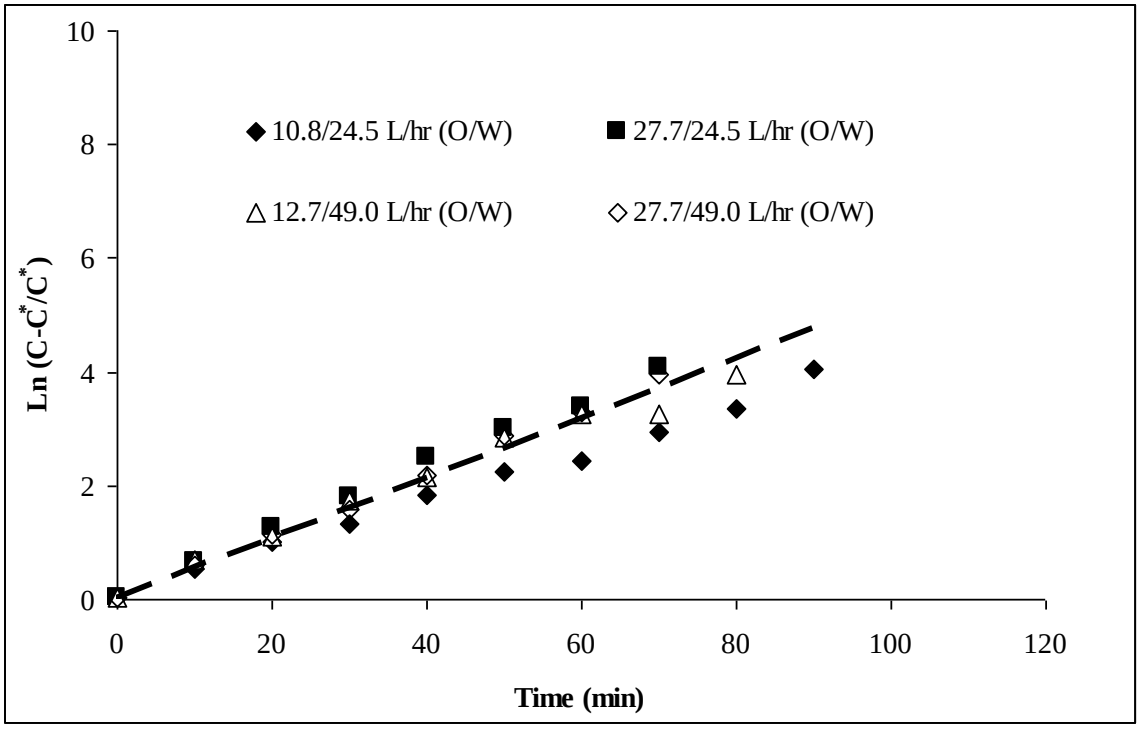

Figure 9 Determination of overall mass transfer coefficient for removal of glycerol from 2-butanol at various (aqueous/organic) flow rates in L hr^{-1}

When using membrane-based solvent extraction to detoxify biomass hydrolysates, it is essential to minimize losses of the organic phase in the hydrolysate, as the organic phase is toxic to the microorganism used for subsequent fermentation. The fact that membrane-based solvent extraction is dispersion-free is therefore a significant benefit[17]. The actual economic viability of an extraction process, however, depends on the actual extraction being conducted and integration of membrane-based solvent extraction into the overall manufacturing process.

5.4 Conclusions

Extraction of acetic acid from aqueous biomass hydrolysates into an organic phase consisting of a mixture of octanol and Alamine 336 has been investigated. Removal of acetic acid from biomass hydrolysates is important to maximize ethanol yields, as it is toxic to the microorganisms used to convert the sugars present in the hydrolysate to ethanol. Extraction of

HMF from an aqueous phase into MIBK has been investigated. HMF is a very valuable intermediate during the thermochemical conversion of sugars present in biomass hydrolysates into transportation fuels. Extraction of glycerol from an organic phase consisting of 2-butanol into water has also been investigated. This extraction could be of significance in the production of biodiesel. For each of the extractions studied in this work, the overall and calculated mass transfer coefficients are in good agreement. These mass transfer coefficients are important when estimating the rate of extraction which will be essential in designing a larger-scale process. They will also be important when correlating the experimental data. Our results highlight the versatility and tremendous possibilities for dispersion-free membrane-based solvent extraction as a unit operation in future biorefineries.

5.5 Acknowledgements

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Chapter 6

COMPUTATIONAL EXPLANATION OF EXTRACTION MECHANISM

Introduction

Tertiary long chain aliphatic amines can extract carboxylic acids at relatively low concentrations present in wastewater streams, fermentation broth or in any other aqueous environments present as products or byproducts[1,2]. A mixture of extractant and organic solvent is used to extract acids. The properties of pure amine extractants would be too viscous to be suitable for extraction. Therefore mixtures of amines with polar or non-polar solvents are used to enhance extraction by reducing viscosity.

Several factors have been recognized to influence the mechanisms of extraction and extraction efficiency for acid extraction using amine –solvent mixtures. These factors include the properties of the acid, the concentrations of the extractant and acid, and type of solvent used. Two of the most important properties of the acid are its acidity, represented through pK_a value, and the hydrophobicity. Solvent is considered the other controlling factor that can enhance the extraction power of amines. The solvent can form hydrogen bonds with the carboxylic acids, leading to a stabilization of the formed acid-amine complexes in the organic phase. Polar solvents tend to enhance the extraction effectiveness of amines as compared to

non-polar solvents. Additional controlling factors such as co-extraction of water into the organic phase, or a third phase formation[3, 4] can occur using amines.

Eyal et a.l[5] proposed four mechanisms of extraction for the acid by amine including ion-pair formation, H-bond formation, solvation and anion exchange, all depending on the acidity and basicity of the solute and amine-based extractant respectively. Ion-pair formation based on acid-base reaction between the carboxylic acid and amine provides a high distribution of acids into the organic phase. At low pH conditions, where the amine is more basic than the conjugate base of the extracted acid or at higher pH where the pK_b of the amine is higher than the pK_a of the acid, the dominant extraction mechanism is via ion-pair formation[5]. Pure distinction between these mechanisms, especially for carboxylic acids is not clear and one or two mechanisms could contribute to the extraction[5]. However, in the literature common explanation of extraction weak carboxylic acids using amine compounds is through amine–acid ion pair mechanism.

Here we present three mechanisms on extraction of acids using aliphatic tertiary amine mixed in octanol organic phase. The mechanisms are of acid-base reaction and hydrogen bonding type and are a combination of each other in extracting multi-mixtures of acids. The selected acids represent three classes of acids; strong acid, weak acids and acids in between the two cases. Support for these mechanisms is obtained through computer simulations and shaking experiments.

6.1 Methods

6.1.1 Quantum Mechanical Calculations

6.1.1.1 Gas Phase Free Energy Calculations

The initial structures of carboxylic acid, its dimers and amine complexes as well as HMF, furfural and their amine complexes were constructed using VMD, a molecular dynamics visualization software. The optimized structures of these compounds and their free energies and associated solvation free energies in both the aqueous and organic phases were determined using Gaussian 03. The gas phase free energies were determined using density functional theory (DFT) based hybrid B3LYP method with 6-31+G(d) basis set.

6.1.1.2 Complex Formation and Solvation Free Energy Calculations

Free energy of complex formation in solution was calculated using a thermodynamic cycle, as shown in Figure 1, using acetic acid-alamine complex as an example. As shown in Figure 1, free energy of complex formation in solution ($\Delta G_{solvent}$) is directly related to the gas phase free energy change (ΔG_{gas}) and the solvation free-energies of reactants and products (ΔG_{sol}).

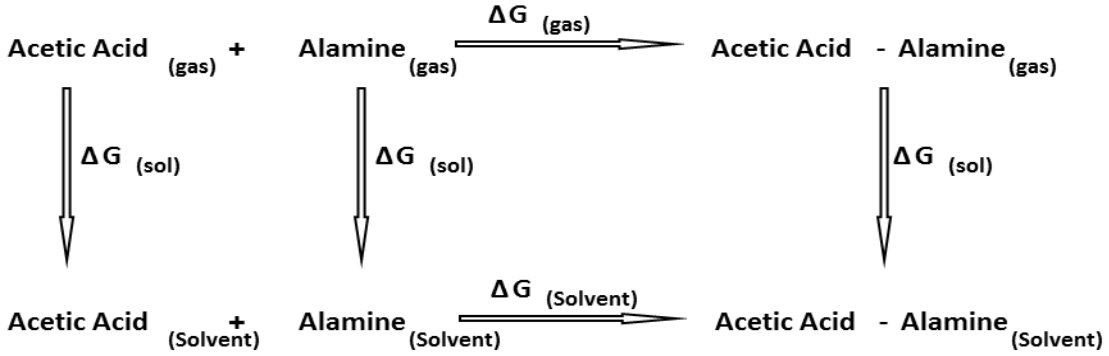

Figure 1 Thermodynamic cycle for the free energy of complex formation in solution between acetic acid and alamine

The free energy of complex formation in solution can be calculated using the following equation:

$$\Delta G_{(solvent)} = \Delta G_{(gas)} + \Delta G_{(sol)\ Products} - \Delta G_{(sol)\ Reactants} \quad (1)$$

Solvation free energies were calculated using the PCM model with UAHF atomic radii based on HF/6-31+G(d)-optimized gas-phase structures. Water and octanol were the solvents for the aqueous and organic phases. Standard solvent parameters for these two solvents were used in Gaussian03. The calculations were carried out at room temperature in agreement with the experimental extraction conditions.

6.2 Extraction Experiments

Liquid-liquid extraction experiments were conducted by contacting known concentrations and volumes of organic and aqueous phase solutions. In 15-mL centrifuge tubes (Thermo Fisher Scientific Inc. PA, U.S., Cat. Nr.: 03395-119), a 5 mL aqueous phase containing a mixture of acetic acid (Mallinckrodt Baker, Inc. NJ, U.S., CAS No.:64-19-7) , levulinic acid (98+% pure, Acros Organics, NJ, U.S., CAS No.: 123-76-2) and formic acid (98+ % pure, Acros Organics, NJ, U.S., CAS No.: 64-18-6.) was mixed with 5 mL of Alamine 336 (Cognis, Cincinnati, OH) solved in octanol (Sigma-Aldrich Corp. St. Louis, MO, cat. Nr.: 279307). The acetic acid concentration was 0.2 M or 0.5 M, whereas the other two acid concentrations varied in the same solution from 0.005 M, 0.05 M to 0.02 M. The Alamine 336 concentration was 0.0104×10^{-3}M, 0.52×10^{-3}M, or 1.04×10^{-3}M. The solutions were shaken manually for a few seconds and then left still overnight for equilibration. Pipetting removed the lower aqueous phase and the remaining acid

concentrations were measured using a 1100 HP HPLC (Agilent, Santa Clara, CA) equipped with a Biorad Aminex HPX-87 H column (Bio-Rad Hercules, CA) and a HP refractive index detector.

Distribution coefficient of the solute (acetic acid) between the two phases is described by the distribution ratio of the overall extracted solute to the remaining concentration of solute in the aqueous phase.

$$D=\frac{\overline{C}_A}{C_A} \qquad (2)$$

,where $\overline{C}_A$ describes the acetic concentration in the organic phase.

6.3 Results and Discussion

The liquid portion of biomass hydrolysate possesses carboxylic acids, furan derivatives and phenolics. Depending on the pretreatment method alkaline or inorganic acids are also present. The results of extraction of acetic acid from hydrolysate using Alamine 336 (tri-n-octyl amine (TAO)) in octanol phase were divided in two experimental series, where the first series investigated the effects of sulfuric acid on acetic acid alone and second series focused on other carboxylic acid effects on acetic acid extraction.

Figure 2 shows the distribution coefficient of acetic acid versus Alamine concentration in the presence of various sulfuric acid concentrations. As noticed, the sulfuric acid concentration has a significant impact on the distribution coefficient, where K_d decreases when sulfuric acid concentration increases. The distribution coefficient is also strongly dependent on the acetic acid concentration. It appears that the lower the acetic acid concentration, the better the extraction. High distribution coefficient of 21 was observed when sulfuric acid concentration is

0.005 M at low acetic acid concentration of 0.05 M. Distribution coefficient values for acetic acid in the range of 3 to 4 in octanol phase using Alamine 336 were shown by several earlier studies[6,7]. High distribution coefficient values in the range of 20 were observed typically for di-carboxylic acids, and not for short mono-carboxylic acids. Di-carboxylic acids have two possible binding sites for amines, forming a 1:2 acid/amine complex. The polar diluent has a stabilizing effect on the complex as also reported by Tamada et al.[8] for extraction of succunic acid using Alamine 336 and octanol. However, there could be another extraction mechanism acting during the acetic acid extraction in the presence of sulfuric acid, as described later.

The preferable extraction of sulfuric acid to acetic acid was already shown in previous publications. The high acidity of the sulfuric acid with first and second pK_a values at -3 and 1.99 was mostly responsible for preferable extractions as compared to acetic acid with a pK_a value of 4.75[9] .

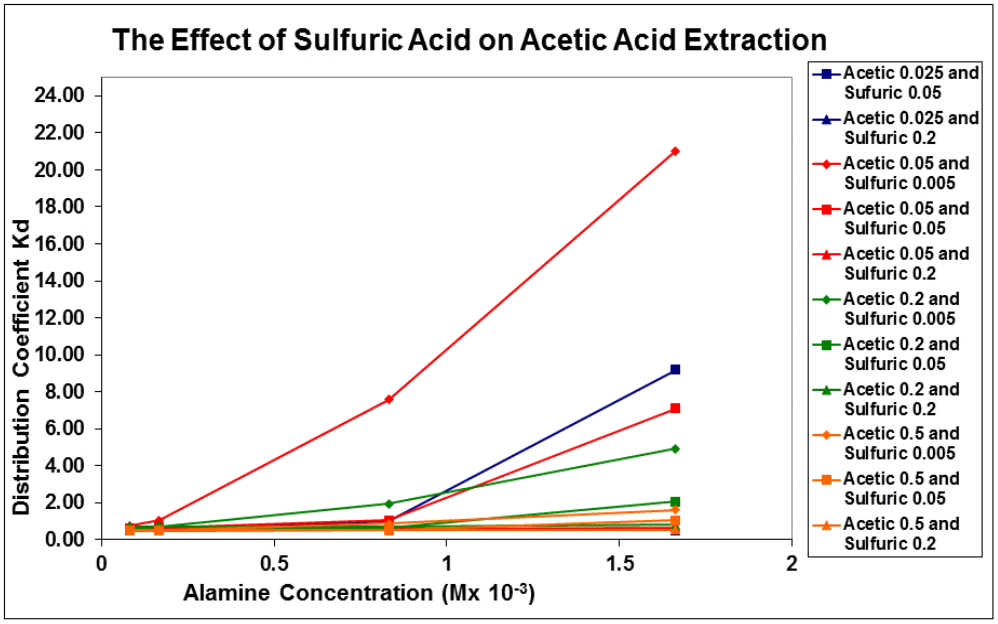

Figure 2 Distribution coefficient versus Alamine 336 concentration of acetic acid in the presence of sulfuric acid with various concentrations

The extraction coefficients of acetic acid are shown in Figures 3 and 4 as a function of Alamine 336 concentration in the presence of two other carboxylic acids of formic and levulinic acids. Figure 3 has a lower initial loading of acetic acid of 0.2 M as compared to Figure 4 with 0.5 M. Both figures show at very low concentrations of TAO, an acetic acid distribution number, which is over three and increases with increasing initial acetic acid loadings. In the past, distribution factors of three and more were known for increasing TAO concentration (0.2-1.2 M) and for polar solvents[10,11]. The extraction increased to a concentration of 40 % TAO and decreased again due to the low solubility characteristics of TAO for the polar complexes[12]. The extraction coefficient of acetic acid appears also to depend slightly on the concentrations of the formic and levulinic acid as well as the concentration of Alamine 336. The K_d values are all below 1 except for a few points varying the Alamine concentration as well as the formic or levulinic acid concentrations. It appears that at lower Alamine concentration as well as at lower formic/levulinic acid concentration, the extraction coefficient is substantially higher reaching about 3. However, increasing the Alamine concentration as well as formic or levulinic acid will rapidly decrease the acetic extraction coefficient. It is more apparent for higher acetic acid loadings.

The physical chemistry for the solvation of formic and levulinic acid is very different. Formic acid has no large R-chain, is a very polar molecule (1.41 D) with stronger acidity than the levulinic acid, its extraction is mostly based on ion-pair formation via acid–base reaction. Levulinic acid has a longer R-chain and is more hydrophobic and weaker in acidity. The extraction of levulinic acid is most likely via the formation of a hydrogen bonded complex. Therefore, the extraction of acetic acid from a solution containing both acids is chemically very interesting and important in mimicking real extraction conditions.

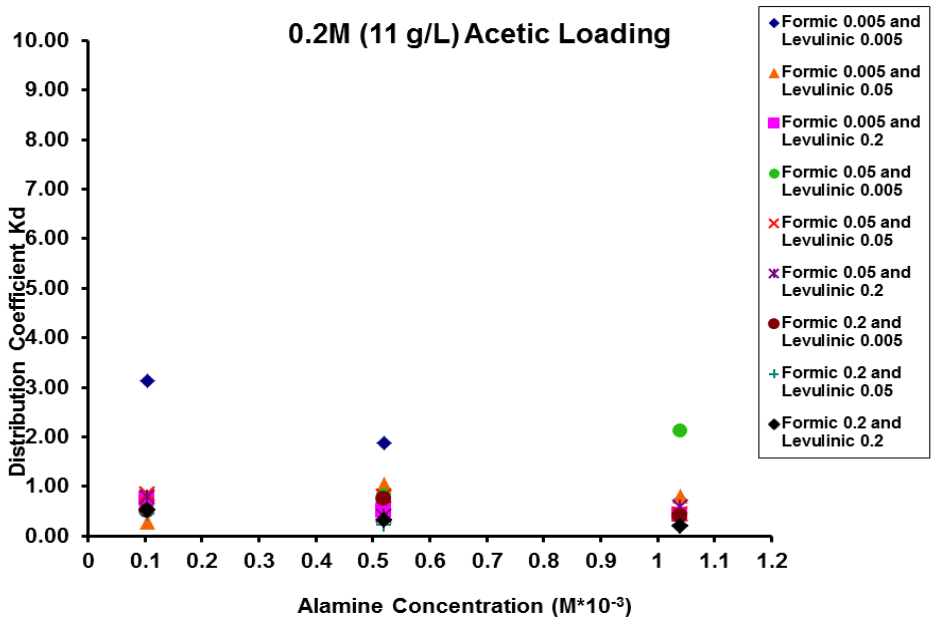

Figure 3 Distribution coefficient versus Alamine 336 concentration of acetic acid (0.2M) in the presence of various formic and levulinic acid concentrations

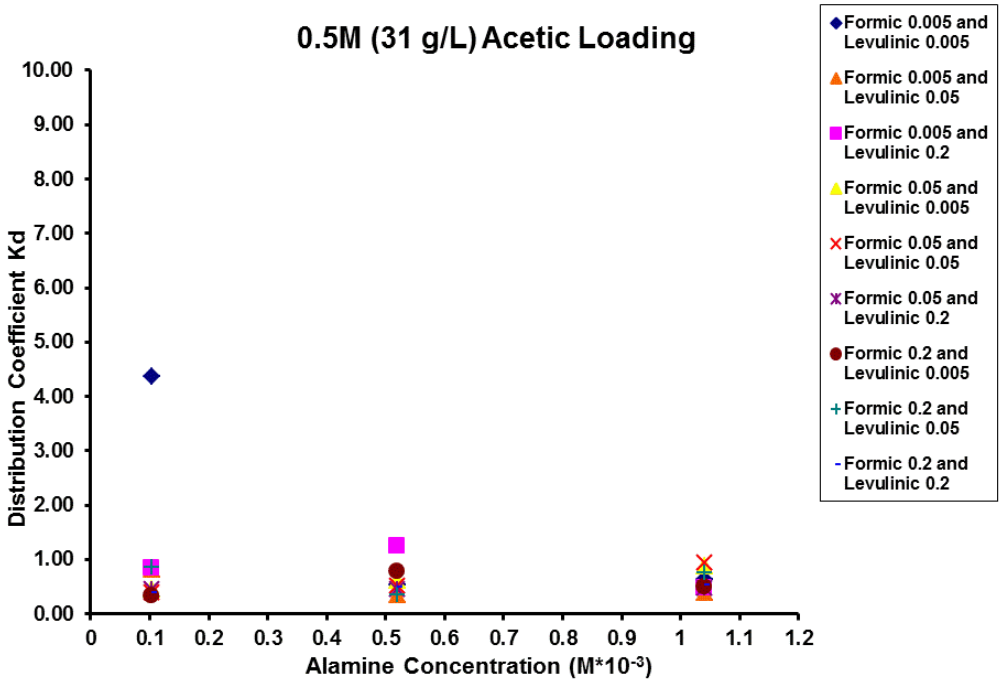

Figure 4 Distribution coefficient versus Alamine 336 concentration of acetic acid (0.5 M) in the presence of various formic and levulinic acid concentrations

Based on experimental observations, we propose three possible mechanisms for acetic acid extraction in the presence of other strong or weak acids. In the presence of a strong acid such as sulfuric acid, the strong acid is extracted first based on the acid-base reaction extraction. Once the strong acid is extracted to the organic phase, the base amine could be partly regenerated due to the recombination of the proton with the conjugated base of the acid. This is due to the fact that the acidity of acids is solvent dependent. In organic solvent with a low dielectric constant, the acidity is reduced compared to the aqueous phase[13]. As a result, recombination of the dissociated proton and the conjugated base will occur. After the strong acids are extracted, the freed amine will be able to form a complex with the acetic acid which will be discussed in more detail in the modeling section. This acetic-amine complex will then be extracted to the organic phase. This is more apparent when both the acetic acid and the strong acid concentrations are relatively low as can be seen in Figure 2. At high sulfuric acid concentration, the amines are mostly associated with sulfuric acid. As a result acetic acid extraction is not efficient. At higher acetic acid concentration, the extraction is also low since there is not enough amine to complex with the acetic acid to be able to extract to the organic phase.

The second mechanism involves the weak acid extraction with amine. In the case of weak acid, the extraction is due to the formation of the acid-amine complex. Once this complex is formed, it has a more favorable solvation free energy in the organic phase and will be distributed more favorably in the organic phase. In the case of acetic acid, it is a weak acid with a pK_a value of 4.76. The COOH acid group will form a hydrogen bond with the N atom of the tertiary amine in the case of Alamine 336. The interaction of the polar functional groups between the acid and amine will make the complex more favorable in the organic phase. Once

the complex is transported to the organic phase, it will be relatively stable. Thus acetic acid extraction by Alamine will be concentration dependent. Higher concentration of acetic acid will reduce the extraction coefficient as shown in Figure 5. The acetic acid extraction could be better described by using the acetic acid/amine concentration ratio $C_r=C_{aa}/C_{am}$, where C_{aa} and C_{am} represent the acetic acid and amine concentration respectively. When C_r is lower but still much larger than 1 at lower acetic acid or higher amine concentrations, the complex formed is relatively stable and extraction coefficient increases with the increasing of the amine concentration as shown in Table 1. However, at even higher acetic acid concentration, another mechanism will start to affect the extraction. That is the formation of acetic acid dimer in the organic phase. The dimer formation will improve the acetic acid extraction. This will be discussed in more detail later.

Extraction Coefficients of Acetic Acid

Alamine (M) →	0.00005	0.0001	0.0005	0.001
Acetic Acid (M) ↑				
0.025	1	1.5	8.4	9
0.05	0.9	1.0	3.3	4.8
0.2	0.7	0.6	1.7	3.7
0.5	0.6	0.7		

Figure 5 Distribution coefficient of various acetic acid and Alamine 336 concentrations

The third extraction mechanism involves an acid with acidity lying between the two previous cases such as formic acid with a pK_a of 3.75. In this case, both the acid-base reaction extraction process and complex formation extraction process will occur. For acetic acid

extraction in the presence of formic acid, formic acid will be extracted first due to more favorable reaction free energy of acid-base reaction/extraction process. However, the amine can be easily freed in the organic phase due to the very low acidity of formic acid in the organic phase. Since both acid-base reaction extraction as well as complex formation extraction exist for the formic acid, the dependence of the acetic acid extraction in the presence of formic acid is more complex. In the case of levulinic acid with a pK_a value similar to acetic acid, levulinic acid extraction will compete with the acetic acid extraction.

The influence of solvents is present in all three mechanisms. Polar solvents provide a good solvation medium for the ion-pair formation of amine and weak acids, wherein octanol builds a hydrogen bond to the carbonyl group of the acid. As shown in Figure 3, the TAO to acid ratio is 1:5000 for the highest acetic acid loading (0.5M) at the lowest TAO concentration yielding a distribution of four. This phenomenon cannot be explained through characteristics of solvent, or extractant interaction. Further, a dimer formation of acetic acid in the organic phase could lead to this high distribution coefficient at the mentioned conditions in Figure 3 and 4. Open or cyclic dimer formations were detected through spectroscopic studies in nonpolar solvents by King et al.[8] or Ziegenfuss et al.[14] and in high acetic acid concentrations ranging from 3.9 M to 17.9 M, as shown by Irish et al. [15,16].

Recently, new mathematical models of carboxylic acid extraction, using amine in solvents, incorporated 1:1, 2:1 and 3:1 acid –amine complexes and showed great extraction prediction[17,18]. Quantum chemical calculations in the gas phase and in the nonpolar solvent showed that a dimer formation is possible[19]. According to new quantum calculations strong hydrogen bonds are formed in a cyclic acetic acid dimer, where polar solvents stabilizes the hydrogen bonds better than nonpolar solvents[20]. As seen in Table 1 the extraction of acids

from water to octanol phase shows a positive $\Delta\Delta G_{sol}$ free energy of solvation. This is consistent with the low distribution coefficients observed for acetic, formic and levulinic acid being 0.48, 0.28 and 0.28, respectively[12]. A dimer formation and a possible extraction from the water to the octanol phase is indicated though the high negative free energy of dimerization values ($\Delta\Delta G_{solvent}$) for acetic, formic, and levulinic acid. Additionally, the influence of TAO shown in form of trimethylamine on dimers is shown in Table 1, and indicates no better stabilization of the dimer complexes. This result is in agreement with data shown in Figure 3 and 4 where at low amine concentrations a higher distributions coefficient was shown for acetic acid. A possible dimer formation at low amine concentrations can explain this behavior.

Table 1 Free energies of solvation ($\Delta\ \Delta G_{sol}$) for single molecules and free energy of dimer complex formation ($\Delta\Delta G_{solvent}$) for acetic, formic and levulinic cyclic dimers. Free energy of complex formation of dimers with trimethylamine molecule

Single Molecule	**$\Delta\ \Delta G_{sol}$ (Water to Octanol) (kJ/mol)**
Acetic Acid	32
Formic Acid	30.1
Levulenic Acid	52.6
Dimer	**$\Delta\ \Delta G_{solvent}$ (Waterto Octanol) (kJ/mol)**
Acetic Acid	-29.8
Formic Acid	-30.4
Levulinic Acid	-29.8
Dimer-Ammonia	
Acetic Acid-trimethylamine	-9.2
Formic Acid-trimethylamine	-8.9
Levulinic Acid-trimethylamine	-11.5

During experiments presented in Figure 3 and 4, a noticeable reduction of the water phase and an increase of organic phase was observed. In the past, water extraction was reported for TOA –butyric, TOA- acetic or TOA-succinic acid extractions in n-alkanes[14,21,22] . The TOA-butyric system seems to influence the co-extraction of water, where one mole of water was co-extracted with one mole of complex. In the TOA-acetic acid or TOA-succinic acid complexes, TOA seemed not to have any impact. Rather it was decided that water co-extraction was influenced by the acid concentration and the solvent.

Computational simulations using quantum mechanical simulations suggested a water co-extraction with acetic acid dimers with one or two water molecules per dimer[19]. Simulations presented in Table 2 agree with the co-extraction of one water molecule per acetic acid dimer, however, it unlikely predicts a two water molecule extraction per dimer complex.

Table 2 Free energy of complex formation for Alamine-water complexes ($\Delta\Delta G_{solvent}$)

Dimer	**$\Delta\ \Delta G_{solvent}$ (Water to Octanol) (kJ/mol)**
Acetic Acid- 1 H2O	-9.6
Acetic Acid- 2 H2O	16.3

The influence of Alamine on HMF and furfural extraction is explored in Table 3. Both molecules show similar chemical appearance, where the additional hydroxymethyl group increases the hydrophilic character of HMF as compared to furfural. However, on their own, both molecules are not preferably extracted into the organic phase. Alamine displayed in form of an ammonia molecule with three methyl groups enhances the extraction of both molecules. The simulated hydrophobic amine molecule interacts better with the more hydrophobic furfural than the more hydrophilic HMF. Experimental data verified this behavior for extraction of

furfural and HMF from biomass hydrolysate. In the absence of Alamine 336, the distribution coefficient in octanol for HMF and furfural was 0.68 and 1.94 and increased in the presence of 5 % (v/v) Alamine 336 to 0.82 and 3.25 respectively. Further increase of Alamine 336 concentration to 15 % (v/v) decreased the furfural distribution coefficient to 2.87 and increased slightly the HMF value to 0.89. The decrease of distribution coefficient for furfural could be due to the changes of octanol phase containing acids, making the octanol phase more hydrophilic.

Table 3 Free energies of solvation ($\Delta \Delta G_{sol}$) for HMF and furfural and free energy of complex formation for Alamine-HMF and Alamine-furfural ($\Delta\Delta G_{solvent}$)

Molecule	**$\Delta\Delta G_{(Sol)}$ Water to Octanol (kJ/mol)**
HMF	52.6
Furfural	33.7
Molecule-complex	**$\Delta\Delta G_{(Sol)}$ Water to Octanol (kJ/mol)**
HMF - trimethylamine	-14.2
Furfural - trimethylamine	-27.2

6.4 Conclusions

Extraction of acids form hydrolysate using amines has to be separately examined based on the present amine-acid complexes. Three mechanisms of acid extraction in the presence of amine were suggested, verified though shaking experiments, and supported using quantum mechanical simulations. Strong acids are extracted though acid base mechanism. Co-extraction of weak acids in the presence of strong acid is possible due to influence of solvent on strong acids.

Extraction of acetic acid in the presence of weak acids such as levulinic or formic acid is also hindered at concentrations of 0.05 M indicating an occurrence of complex formation and acid-base type reactions. Formic and levulinic acid are preferably extracted in the presence of acetic acid. However a high extraction of acetic acid at very low concentration of formic, levulinic and extractant, was observed. It increased with increase content of acetic acid. Computational simulations propose a cyclic dimer formation of acetic acid and explain possible water co-extraction experienced during experiments. To verify the observations, spectroscopic analysis of solutions having distribution coefficients of three to four have to be conducted in the future.

Enhanced HMF and furfural extraction in the presence of Alamine 336 was verified through computational simulations.

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Chapter 7

COST ESTIMATIONS

7.1 General setup implementation considerations

Aspen Plus (Aspen Plus Technologies Inc., USA) software was used to simulate the implementation of membrane supported liquid extraction method into NREL's detoxification unit. As shown in Figure 1, the currently considered ammonia detoxification process is replacing the overliming method, which increases the benefits of the overall process in not considering a solid/liquid separation unit. A membrane process requires a solid/liquid separation unit for the production of a particle free stream. Particles would immediately plug the membrane and decrease the extraction of chemical compounds to undesirable rates. Thus, the membrane process has to compete economically with a simple ammonia conditioning process.

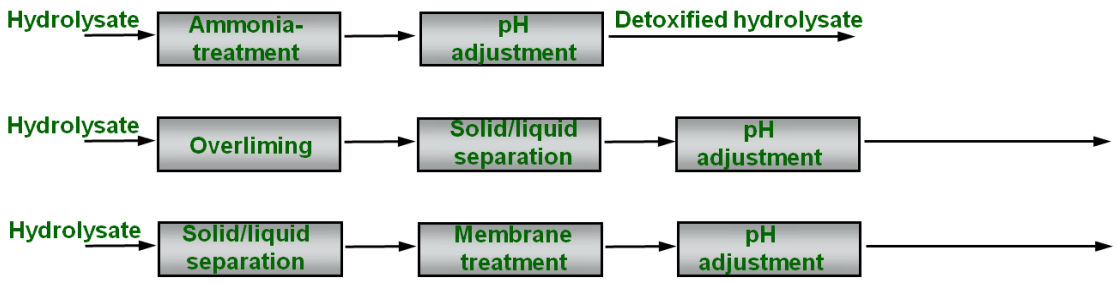

Figure 1 NREL's possible detoxification pathways

7.2 Aspen simulation procedure considerations

Due to its biocompatibility, an economic membrane process has to utilize oleyl alcohol as a solvent, for the subsequent fermentation step. Aspen simulations were conducted with following restrictions: Alamine 336 was substituted with ammonia and octanol was used as the organic phase, even though we simulate the extraction with oleyl alcohol as organic solvent. Oleyl alcohol cannot be found in the Aspen data base, therefore octanol was substituted. Chemical properties (e.g, molecular weight, density) of oleyl alcohol were added to Aspen database. Octanol and oleyl alcohol have no chemical effect during the aspen simulations on the hydrolysate or the extraction process. The higher costs of oleyl alcohol as compared to octanol are considered in the Aspen simulation.

Alamine 336 had to be substituted with ammonia because parameters in the Aspen data banks were not provided for Alamine 336. Ammonia, as a chemical, is more predefined in Aspen and its chemical structure (NH3) is similar to Alamine 336, with its nitrogen and 3 side chains. Simulations for the membrane treatment were carried out using NRTL method for calculations of activities of the liquid phase.

Table 1 shows the extraction of acetic acid, HMF and furfural from the hydrolysate using Alamine 336 (15% w/w) solved in oleyl alcohol or octanol phase.

Values of the oleyl alcohol phase are implemented into Aspen setup as shown in Figure 2. The setup in Figure 2 represents the membrane process (black square) and the regeneration process (grey square) where the solvent is regenerated using NaOH. In detail: stream HYD contains acetic and sulfuric acid and is mixed with stream SOLV, which contains octanol and 15 % ammonia. Streams are mixed in reactor REA-1 where 30 % of acetic acid and 100 % sulfuric acid are converted with ammonia to ammonium-acetate and ammonium sulfate. Ammonium-

acetate is represented in Aspen through certain key parameters, such as molecular weight or enthalpy. Resulted product stream, HYD2, is forwarded to Sep-1, where Octanol ammonium-acetate, ammonium sulfate flows to block MIX and hydrolysate including 70 % acetic acid flows out as an end product stream called PROD. The amount of octanol phase lost during membrane treatment is considered through stream SOLVLOST and is between 2-4 %. Octanol phase is contacted with sodium hydroxide in block MIX and forwarded to block REUS-REA. In this reactor ammonium-acetate plus sodium hydroxide lead to sodium-sulfate and ammonia. Product stream is sent to block SEP-3 where two streams are generated as product streams: REU4 and TRASH. REU4 stream contains octanol and ammonia and flows as regenerated stream to REA-1block, which is mixed with fresh octanol. Trash stream contains sodium-sulfate, sodium acetate and non reacted sodium hydroxide.

A simplified version of this setup is shown in Figure 3.

Table 1 Acetic acid, Furfural and HMF extraction using oleyl alcohol solvent with 15 % Alamine 336 and octanol solvent with 15 % Alamine 336

Organic phase	Acetic acid	Furfural	HMF
	%		
Oleyl alcohol	30.24	59.19	22.78
Octanol	55.90	71.61	43.87

Table 2 Stream ID and their flow rate in Aspen setup

Stream ID	Item	Flow rate kg/hr
HYD	Hydrolysate	19702
SOLV	Organic Solvent	8585
NAOH	Reg. Compound	8585
SOLVLOS	Lost Solvent	119

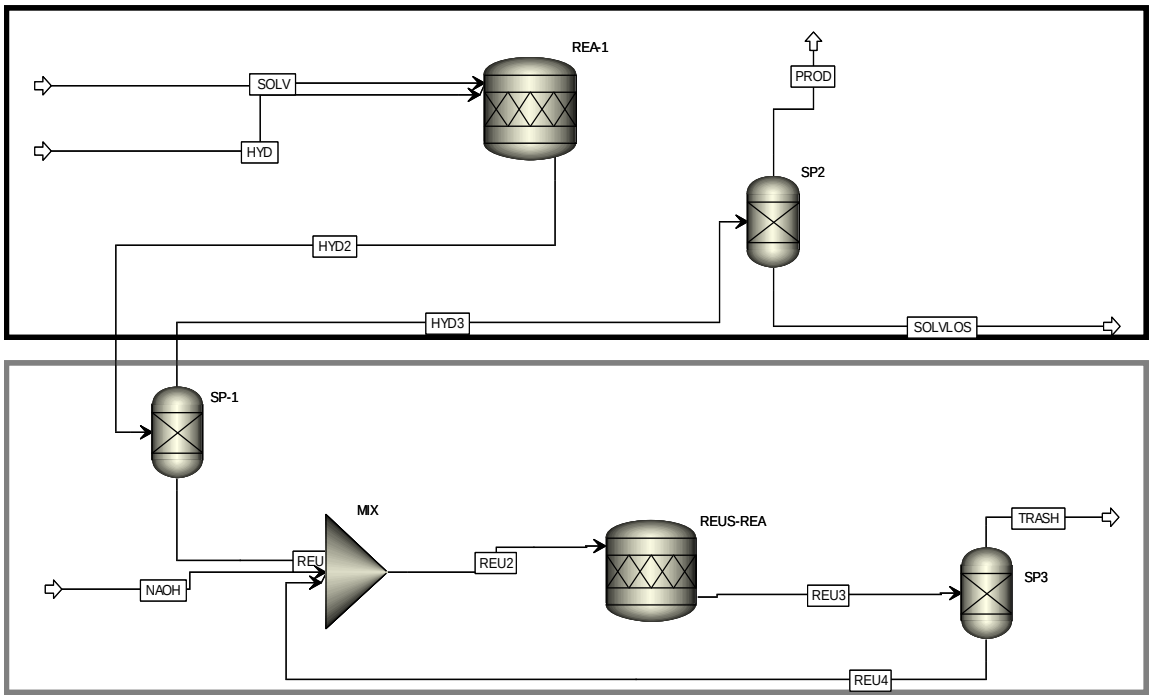

Figure 2 Aspen simulation of acetic acid extraction using octanol solvent with 15 % ammonia

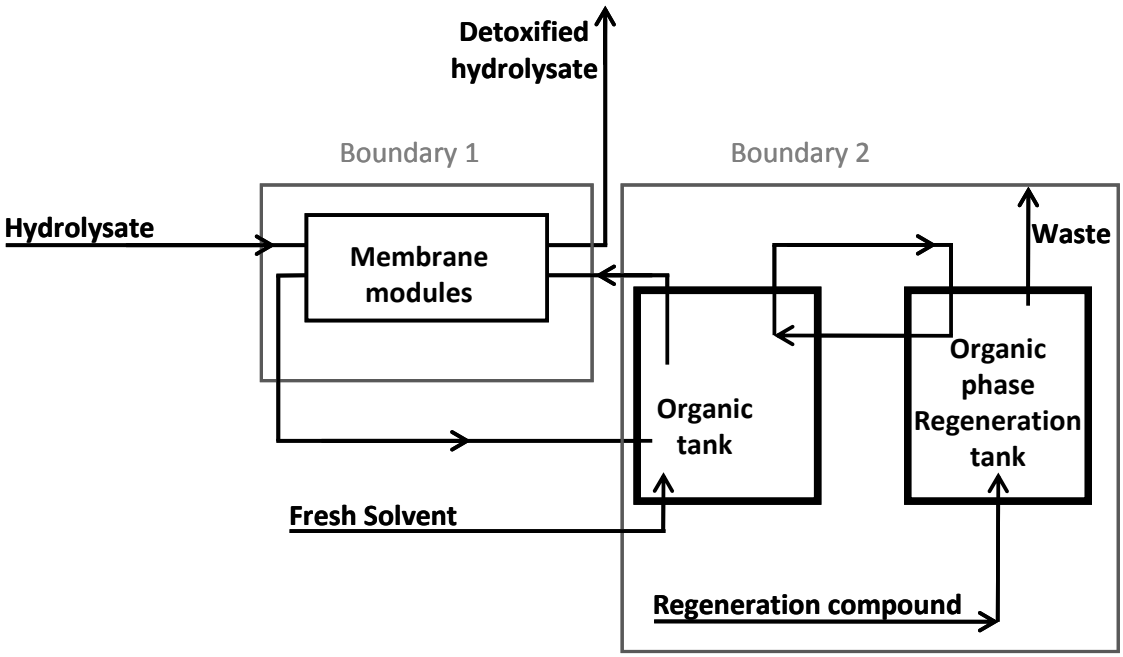

Figure 3 Overall membrane process divided in two scale up areas. Scale up of membrane modules based on hydrolysate flow rate (Boundary 1 area) and scale up of organic side based on regeneration time and flow rate of organic phase (Boundary 2 area)

7.3 Tank size and number of modules estimation

Table 3 shows the membrane characteristics of smaller and commercial scale membrane modules at certain working conditions. As noticed in Table 3, both modules use identical fibers, which exhibit at higher temperatures a lower tolerance to pressure. The model setup, using 2.5 x 8 module run at 40° C with a lumen flow rate of 0.6 gpm and 10 psig and a further increase to 2.3 gpm, resulted in a pressure of 25 psig with no noticeable rise in extraction. Flow rates of organic phase and aqueous phase will be kept similar in the industrial scale (14 x 28) membrane setup, since it uses the same polypropylene hollow fibers as the model setup.

The detoxification capacity of the model setup (2.5 x 8) on hydrolysate, is 2 L in 2 hr to concentration levels of toxic compounds that do not harm the microorganism. For reasons of

simplicity and easier scale up considerations, we can assume to be able to process 2 L in 1 hr. A scale up in membrane area from 1.4 to 200 m^2 results in a scale up factor of 157.14, which would decrease the treatment time to 0.38 min for 2 L. This results in a treatment volume of 314 L/hr.

NREL's Aspen simulation of hydrolysate flow rate, leaving the solid liquid separator, is 412 L/min or 19702 kg/hr as shown in Table 2. Therefore 63 membrane modules are needed for a continuous run.

Table 3 Characteristics of membrane module used in the model setup and available commercial scale membrane module from Membrana

Module	2.5 x 8 Extra-Flow	14 x 28 Extra-Flow
Configuration	Extra-flow, centre baffle	Extra-flow, centre baffle
Membrane	Polypropylene, X40 fibers	Polypropylene, X40 fibers
Fibre OD/ID (µm)	300/220	300/220
Porosity	40%	40%
Maximum Shellside pressure at 5-40 °C (psig)	120	120
Maximum Lumenside pressure at 15-40 °C (psig)	35	35
Active surface area (m^2)	1.4	220
Priming volume lumen/shell (mL)	150/400	21700/33500
Housing material	Polypropylene	Stainless Steal

The size of the organic and re-usage tank depends on the flow rate of the organic phase through the modules and the regeneration time in the regeneration tank. It is best to assume, that fresh organic phase is always contacted with the hydrolysate phase at the same flow rate as shown in the model setup, being 0.6 gpm. The regeneration of organic phase was conducted as

a mixer and settler setup with a mixing time of 30 min and a total residence time of 3 hr. The volumes of the two organic tanks are equal and a volume including a residence time of 4 hr in the regeneration tank is assumed. This results in tank volumes of 10000 gal each. In conclusion, we can perform a scale up in drawing boundaries around the membrane modules and around the organic tanks as seen in Figure 3.

7.4 Cost

7.4.1 Cost of Tanks

The types of tanks significantly determine the cost of the Aspen simulation. Tanks considered for the organic phase can be made out of plastic. Oleyl alcohol or Alamine do not have any effect on plastic materials. Cost were estimated with purchase cost equations obtained from in Product & Process Design Principles by D. R. Lewin et al.[1] and verified through quotes from producers.

The purchase cost equation 1 is corrected by 10-12 % as compared to the published equation by D. R. Lewin et al. in 2006 ($Cp=18*V^{0.72}$). This correction was verified through quotes from Plastic-Mart (Stand: January, 2011). Costs of organic side tanks were estimated with following specifications; open storage tank of fiberglass type.

$$C_p=19.9*V^{0.72} \qquad (1)$$

7.4.2 Cost of Materials

The cost and vendor information of oleyl alcohol, Alamine 336, and sodium hydroxide are given in Table 4. The quantity of Alamine 336 is fixed due to the possible recycle option

shown in Figure 3. Oleyl alcohol is replaced based on the quantity lost during extraction process, varying 2-4%. Sodium hydroxide is used as a regeneration agent, where the acetic acid –alamine complex is disrupted leading to sodium acetate formation. Alamine is regenerated and can be used in further detoxification steps. As shown in Table 4, costs are significant.

Table 4 Cost and vendors information (Stand: February 2011) of chemicals used in Aspen simulation

Chemical compound	Cost ($/tone)	Vendor
Alamine 336	90	Cognis
Sodium hydroxide	320	HDI Database
Oleyl alcohol	8000	Jarchem

7.5 Selling Price of Ethanol

The membrane process causes an ethanol selling price of 2.43 $/gal as compared to NREL's current estimation using ammonium hydroxide of 1.99 $/gal. Following reasons increased the ethanol price:

- The number of modules needed and its selling price of $ 16,100 (stand: February, 2011)
- The high price of sodium hydroxide used in the regeneration loop contributing alone 28.04 cents /Gallon ethanol.

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