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Enhancing Bioethanol Fermentation through Removal of Acetic Acid Using Liquid-Liquid Extraction

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ENHANCING BIOETHANOL FERMENTATION THROUGH REMOVAL OF ACETIC ACID USING LIQUID-LIQUID EXTRACTION

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TABLE OF CONTENTS

Page

Page

LIST OF TABLES

Appendix Table Page

LIST OF FIGURES

Figure Page

Appendix Figure

ABSTRACT

Aghazadeh Mahdieh Ph.D., Purdue University, May 2016. Enhancing Bioethanol Fermentation Through Removal of Acetic Acid Using Liquid-Liquid Extraction . Major Professor: Abigail S. Engelberth.

The concern for the ever growing human population as well as the depletion of fossil fuel resources and their impact on global warming have long been motivations for the researchers to investigate means for sustainable producing carbon-neutral energy. Second-generation biofuel refers to liquid fuels that are produced from non-food resources and reduce the total greenhouse gas emission by at least 60 %.

Acetic acid has been shown to be one of the most ubiquitous fermentation inhibitors in a bioethanol production facility which slows down the bioethanol production and reduces its yield through inhibition of the ethanol producing microorganisms.

The use of liquid-liquid extraction has shown to be a viable tool to remove the acetic acid from corn stover hydrolysate. Extraction coupled with a solvent recovery unit enhances the bioethanol production through improving the product yield as well as its production rate.

Economic assessment of the proposed system showed that incorporating the extraction unit within an industrial scale corn stover bioethanol production plant is a feasible option which can drop the MESP by up to $0.35/gal.

CHAPTER 1. INTRODUCTION

1.1 Research Motivations

1.1.1 Bioethanol

Growing population and fast pace of industrialization are strong contributors to the quickly diminishing fossil fuel resources. Limited oil and gas reserves, combined with environmental concerns, and more dramatic consequences of global warming have pushed many researchers to investigate sustainable and renewable resources for energy. Second generation biofuels, i.e. bioethanol produced from lignocellulosic biomass through biochemical or thermochemical routes, can reduce the greenhouse gas emission by at least 60 % (M. Ladisch, Ximenes, Engelberth, & Mosier, 2014). The biochemical conversion of non-food cellulosic biomass to bioethanol is a multi-step process (M. R. Ladisch, Mosier, Kim, Ximenes, & Hogsett, 2010). The biochemical option for bioethanol formation involves the following:

1.Preparation of the biomass: biomass is collected post-harvest, stored properly to be used continuously over the year. Often in this step the size of biomass is reduced through grinding and milling and then it is dried.

2.Pretreatment: the crystallinity of the feedstock is attacked to make the cellulose and hemicellulose more accessible. High pressure and temperature, along with acid or base in some methods, are the necessary elements.

3.Enzymatic hydrolysis: it is stated that this is the most cost intensive stage in the process due to high enzyme cost. Six and five carbon sugars are being formed from the hydrolysis of cellulose and hemicellulose.

4.Fermentation: microorganisms are added to the hydrolysate and consume the sugars in their metabolism; ethanol is a byproduct of this mechanism.

5.Ethanol separation and recovery: common practice is to use distillation to recover the ethanol that has been produced during the fermentation.

1.1.2 Fermentation inhibitors

In second-generation bioethanol production, pretreatment is necessary to enhance the accessibility of cellulose and hemicellulose for enzymes. Fermentation inhibitors are compounds that are inherently present in the after pretreatment lignocellulose biomass (Klinke, Thomsen, & Ahring, 2004; Maiorella, Blanch, & Wilke, 1983b; Palmqvist & Hahn-Hagerdal, 2000). Examples of the inhibitors include weak acids - like acetic, formic, and levulinic- furans and phenols (Almeida et al., 2007). Figure 1-1 summarizes the source for the major inhibitory compounds.

The inhibition effect of acetic acid on fermentation has been widely studied; biomass growth, ethanol production, and the conversion efficiency in presence of acetic acid are the most scrutinized parameters. Casey *et al*. (2010) demonstrated that the yeast growth, substrate consumption, and ethanol volumetric productivity decrease in presence of acetic acid.

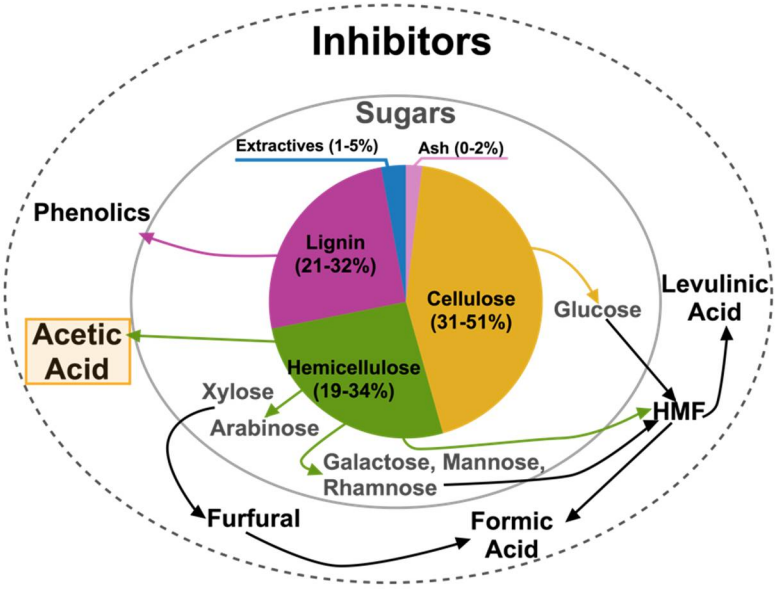

Figure 1-1: Plant component sources and their resulting fermentation inhibitors.

Acetic acid, 5-hydroxymethyl furfural (HMF), furfural, and formic acid are among the most well studied of these inhibitors. Inhibition slows down the fermentation and reduces the production of ethanol (Almeida et al., 2007; Delgenes, Moletta, & Navarro, 1996; Jönsson, 2013; Phowchinda, Deliadupuy, & Strehaiano, 1995). Fermentation in an environment free of inhibitors will progress significantly faster with higher final ethanol yield (Kim, Kreke, Hendrickson, Parenti, & Ladisch, 2013, Nilvebrant, Reimann, Larsson, & Jonsson, 2001).

Concentrations of inhibitory compounds in the corn stover hydrolysate depend on the type of the plant and the pretreatment process. Öhgren *et al* (2006) reported 1.6 g/L acetic acid, 0.06 g/L HMF, 1.1 g/L furfural, 1.4 g/L formic acid for steam pretreated corn stover; Mancilha *et al*. (2003) 1.06 g/L of acetic acid, 0.0034 g/L HMF, and 2.2 g/L furfural from dilute acid pretreated corn stover; while Zhao *et al.* (2013) stated that the

concentrations of acetic acid, HMF, and furfural are 4.7 g/l, 1.2 g/L, and 1.1 g/L respectively with dry dilute acid pretreatment. Much higher concentration of acetic acid has been reported when the solid loading in the pretreatments are higher; in Humbird *et al*. work the acetic acid concentration reached 16.1 g/L at 30 % solid loading dilute acid pretreatment (2010).

Quantitative studies about the toxicity effect of the inhibitors on different strains of yeast are extensive. The inhibition effect can be divided in four major categories:

1- Ethanol yield

2- Ethanol production rate

3- Growth rate

4- Substrate consumption.

Figure 1-2 summarizes data from a review study on furans inhibition effect on different strains of *S. cerevisiae* (2007). Table 1-1 includes more examples of the effect of some fermentation inhibitors on different strains of *S. cerevisiae*.

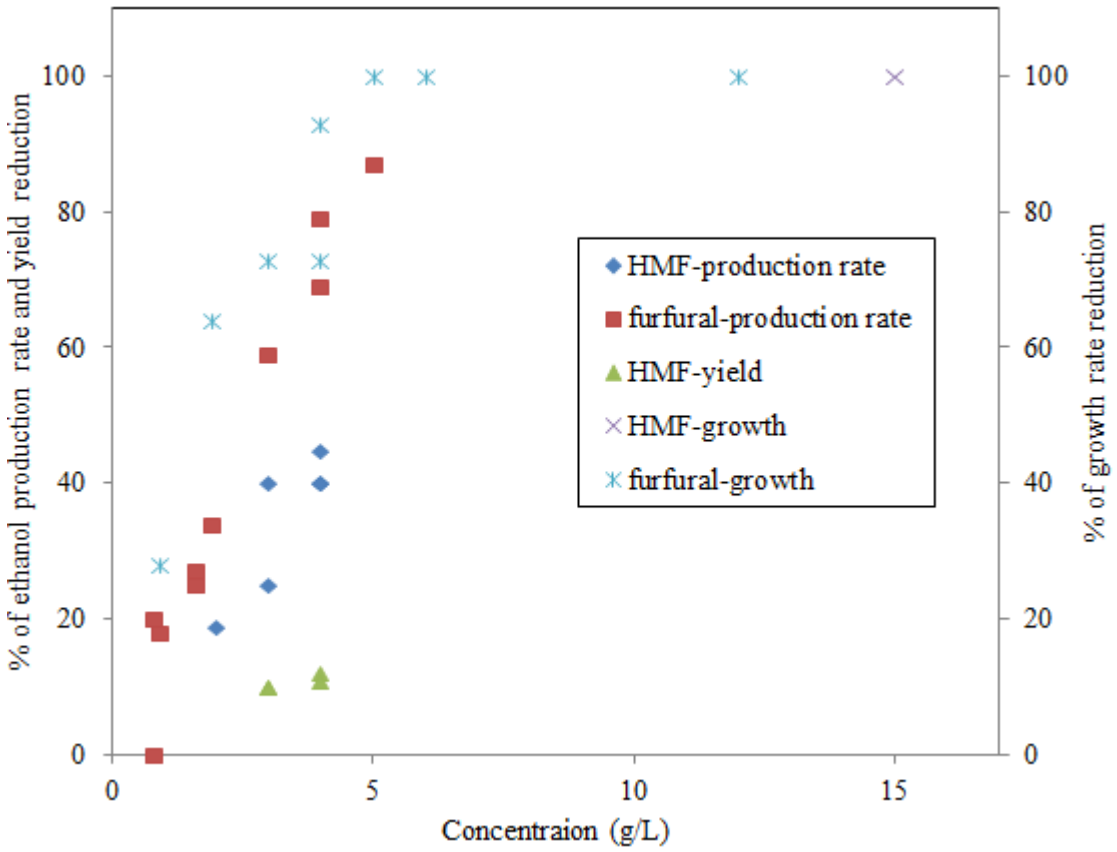

Figure 1-2: The effect of HMF and furfural concentration on the yeast growth, ethanol production rate, and yield (data obtained from Almeida *et al.* (2007))

It is evident from Figure 1-2 that the inhibition effect of furfural and HMF results in the inability of the yeast to reproduce at concentrations above 5 g/L and 15 g/L, respectively. Presence of furfural in the fermentation medium drops the ethanol production rate up to 90 %, whereas HMF drops it to almost 50%. However, HMF does not cause a substantial difference in the yield of the fermentation.

Table 1-1: Inhibition effect on *S. cerevisiae* in the fermentation of bioethanol

Inhibitor	Amount	The strain	The effect	Reference
Acetic acid	15 g/L	424A (LNH-ST)	67% drop in ethanol volumetric production rate	(Casey et al., 2010)
Acetic acid	15 g/L	424A (LNH-ST)	~ 20 % drop in cell growth	(Casey et al., 2010)
Formic acid	2 g/L	ATCC 4226	67 % drop in cell concentration	(Maiorella, Blanch, & Wilke, 1983a)
HMF	4 g/L	Y-1528	45 % drop in ethanol production rate	(Keating, Panganiban, & Mansfield, 2006)
Furfural	42 mM	baker's yeast	69 % drop in ethanol production rate	(Palmqvist, Almeida, & Hahn-Haegerdal, 1999)
Syringaldehyde	1.5 g/L	CBS 1200	67 % drop in ethanol production rate	(Delgenes et al., 1996)

Inhibition of acetic acid largely depends on the pH of the medium. Undissociated acetic acid can enter through the cell wall and dissociates due to higher pH inside the cell. The plasma ATPase hydrolyzes the ATP to pump the proton outside the cell leaving less ATP for the cell reproduction (Casey et al., 2013). Formic acid has higher level of toxicity because of a different inhibition mechanism due to its smaller size. Narendranath *et al*. (2001) reinstated this mechanism for acetic acid and showed that acetic acid starts to change the intracellular pH at concentration 0.25 % w/v and above. Other studies are available on the pH and acetic acid concentration effect on *S. cerevisiae.* The findings of

Matsushika *et al.* (2012) demonstrated negligible inhibition of acetic acid at pH 6 but significant inhibition (especially on xylose consumption) at lower pH. Almost all of the studies in this area have mentioned synergistic effects from these inhibitors, especially between the furan compounds (Matsushika & Sawayama, 2012).

1.2 Research Objectives

1.2.1 Solvent selection for the liquid-liquid extraction separation

Solvent selection study starts with obtaining ternary phase diagrams of water, acetic acid, and the organic solvent (Sorensen, 1980). The ternary phase diagrams determine whether or not the system forms a biphasic regime and that acetic acid has high solubility in the organic solvent. The solvents that have these properties will be used to simulate the liquid-liquid extraction unit in Aspen Plus™ software. Based on the extraction yield, consumption rate of the solvent, the miscibility with aqueous solutions, and their non-attraction to the sugars, the original list of the fifty organic solvents will be narrowed down to a number of solvents that can be tested in laboratory experiments. Split fraction of acetic acid in organic phase should also be measured in both model solutions with glucose and xylose, and liquid part of pretreated corn stover to validate the simulation results. The performances of the selected solvents are to be tested to extract the other known inhibitors using Aspen Plus™ simulation.

1.2.2 Fermentation performance after applying liquid-liquid extraction

Dilute acid pretreatment at 140 °C will be used to prepare the corn stover and the liquid part will be filtered for the extraction. Liquid-liquid extraction experiments will be

conducted with selected solvents to analyze the extraction efficiency of the acetic acid removed from the biomass hydrolysate. Further fermentation experiment will be carried out with the collected lower phase and USDA NRRL Y-1546 (a strain of *S. cerevisiae*) as the yeast to quantify the impact of liquid-liquid extraction on different fermentation parameters. The interactive inhibition of the solvent and acetic acid needs to be studied in order to specify the level of the acetic acid and solvent that are tolerable to the yeast. Solvent recovery is an essential step to make LLE economically feasible and also reduce the solvent content below the inhibition threshold. In this part of the study the impact of removing the acetic acid with means of liquid-liquid extraction on the bioethanol production performance during the fermentation will be assessed.

1.2.3 Techno-economic analysis of the liquid-liquid extraction system

Incorporating extraction column and the solvent recovery step in a biorefinery will change the dynamic of the plan. The size of the extraction column can be estimated using the flowrate of the feed and solvent streams. The characteristics of the extract stream exiting the column will determine the size of the flash drum to evaporate the solvent. The size, the material of the equipment, and the type are the key parameters to estimate their purchasing and installing costs. The manufacturing cost of the system mainly includes the cost of the solvent which is a strong function of solvent recyclability. On the other hand adapting this system increases the revenue of the biorefinery by increasing the ethanol production rate and ethanol yield.

CHAPTER 2. REVIEW OF FERMENTATION INHIBITOR REMOVAL TECHNIQUES

2.1 Inhibition mechanism of acetic acid

Many approaches have been taken to manage acetic acid inhibition on *S. cerevisiae*. The effect of acetic acid on *S. cerevisiae* is a strong function of the pH of the fermentation medium (Graves, Narendranath, Dawson, & Power, 2006; Pampulha & Loureirodias, 1989; Thomas, Hynes, & Ingledew, 2002). The dependence on pH suggests that it is the undissociated form of acetic acid that diffuses through the plasma membrane of the cell and causes the chemical disturbance (Casey et al., 2010).

Acetic acid reduces cell growth rate thereby reducing the substrate – glucose and xylose –consumption rate which result in decrease of the production of ethanol (Casey et al., 2010; Graves et al., 2006; Thomas et al., 2002).

The mechanism of acetic acid inhibition has been widely published and there is a consensus that the diffusion of undissociated acetic acid into the cell lowers the intracellular pH (Ullah et al., 2012, Zhao et al., 2008, Narenranath et al., 2001, Maiorella et al. ,1983, Pampulha et al. ,1989)

Higher proton content inside the cell facilitates the hydrolysis of ATP which results in lower amount of ATP for the cell reproduction (Carmelo, Santos, & Sá-Correia, 1997).

To overcome the effects of the fermentation inhibitors there are two main strategies: directed evolution and the removal of inhibitors. Directed evolution occurs through adaptation or gene modification to increase the tolerance of the microorganisms to harsher environment (Almeida et al., 2007; Wright et al., 2011; Zheng et al., 2011).

The directed evolution of different strains of *S. cerevisiae* has been well researched. The programmed cell deaths of *S. cerevisiae* caused by acetic acid can be prevented by prior adaptation of the cells to acetic acid (Giannattasio, Guaragnella, Corte-Real, Passarella, & Marra, 2005). Short-term adaptation of *S. cerevisiae* at pH optimal for cell growth can improve the fermentation at lower pH and presence of 6 g/L acetic acid (Sànchez i Nogué, 2013). This adaptation was done by pre-treating the cells at low pH acidic medium for 200 minutes increased the cell viability up to 180 % and improved the ethanol production. In an attempt to specify the genes that cause the weak acid inhibition, Fernandes *et al*. (2005) identified the transcription factor in *S. cerevisiae* that contributes to the yeast adaptability to short chain carboxylic acids. Overexpression of HAA1 gene in *S. cerevisiae* enhanced the cell growth under acetic acid environment specially at medium level of the acid (Tanaka, 2012).

Most of the published research has been strain specific. To perform gene modification, the inhibition mechanism and the "-omics" analysis have to be thoroughly understood. Conversely, removing inhibitors can have the added benefit of producing a value-added bio-product along with the production of bioethanol from the biorefinery.

2.2 Separation methods to remove the inhibitors

2.2.1 Membrane Separation

Membrane separations use a selective permeable medium to recover a product of interest from a mixture. In general, membrane separations are controlled by some type of size exclusion as depicted in Figure 2-1. Ion exchange, vacuum, and evaporation are also implemented in some systems to enhance the driving force (Mulder, 1996).

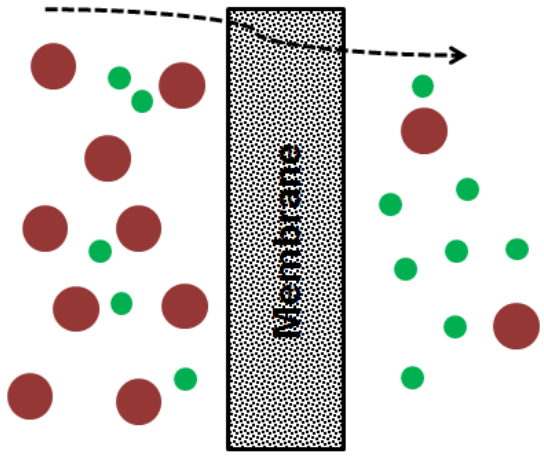

Figure 2-1: Schematic membrane separation system. The porous and selective membrane allows certain compounds to diffuse to the permeate side.

The selection of the commercial or synthetic membrane is a vital design factor to determine the feasibility of this technique to remove the acetic acid from biomass hydrolysate.

Hollow fiber membrane combined with extractive effect of a mixture of tertiary amines and octanol, as diluent, was used to remove the acetic acid from the liquid hydrolysate collected from dilute acid pretreatment of corn stover. Although the membrane extraction removed the sulfuric acid and some other phenolic inhibitors, the extracted acetic acid could only reach 60 % (Grzenia, Schell, & Wickramasinghe, 2008).

Successful research has been performed with nano-filtration membrane to remove acetic acid from rice straw hydrolysate prior to fermentation. This method used a membrane with 150-300 Da cut-off to allow acetic acid to permeate through while prohibiting the passage of sugars (Weng et al., 2009). This separation resulted in up to 95% of acetic acid content in the retentate side from xylose solution, at pH 9 and pressure of 24.5 bars, but a build-up of sugars decrease the amount of acetic acid that passes through and eventually lead to membrane fouling. Later a nano-filtration membrane was also used on wood-based hydrolysate to remove acetic acid, methanol, furfural, and HMF from sugars (Weng et al., 2010). The results indicated that repeated dilution and concentration were necessary to wash the inhibitors from the sugars.

Other membrane separation methods have been tested. Pervaporation, which is a technique that combines membrane permeation with an evaporator apparatus, with

grafted PVA membrane has a clear trade-off between separation factor and the permeation rate; the former increases with the thickness of the membrane while the latter decreases at higher membrane thickness (Al-ghezawi, Sanli, & Isiklan, 2006). The separation factor ranged between 3.64 to 14.6 for 10-90 wt. % of acetic acid content in the feed stream. This range is much higher than the common acetic acid content in the liquid biomass hydrolysate – 1 to 2 wt. % – (Almeida et al., 2007).

Grafted co-polymer membrane composed of polyvinyl alcohol and polyacrylamide in a pervaporation system were examined to find an optimal acetic acid separation with respect to temperature and separation factor of 23was achievable (T. A. Aminabhavi & Naik, 2002).

Vacuum membrane distillation with hollow fiber membrane was verified for the separation of acetic acid and furfural from water solutions at elevated temperatures (Chen et al., 2013). Acetic acid removal percentage reached about 30 % at 70 °C, and was much lower than furfural.

A pervaporation system was modified to avoid the contact of the feed stream to the membrane by placing the evaporation step prior to the permeation (evapomeation) to gain better results of up to 52 separation factor (Isiklan & Sanli, 2005). Although the acetic acid concentration range was above the average acetic acid content in biomass hydrolysate, this synthetic membrane separation work demonstrated higher separation factor at higher acetic acid concentration.

Membrane separation has the advantage of minimal contamination to the feed stream. Even though there have been promising results for fermentation inhibitors removal using various membrane separation methods, high purchasing cost of

commercial membranes and frequent fouling during these processes are the major drawbacks of adopting this method at the industrial scale. As many studies have shown, the flux and selectivity have opposite trends with respect to the membrane thickness; therefore to get the desired selectivity considerable pressure drop is inevitable in these types of processes.

2.2.2 Adsorption studies

Adsorption is one the most highly selective separation processes (Seader, Henley, & Roper, 1998). Adsorption operates by flowing the feed, mixed in a mobile phase, over an adsorbent (Crittenden, 1998). The adsorbent has various affinities to the different compounds present in the feed stream which result in varying retention times. As a result, at the outflow stream these compounds can be detected and collected at different times corresponding to their retention times on the adsorbent. In Figure 2-2 the green compound has the higher affinity and therefore higher retention time while the yellow compound has the lower affinity and lower retention time on the surface of the adsorbent.

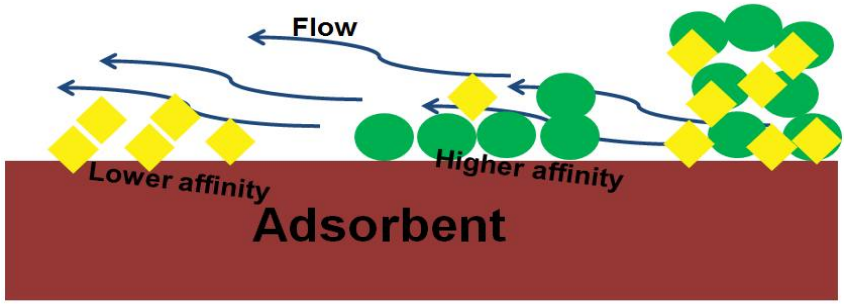

Figure 2-2: Schematics adsorption separation. Different compounds can be separated from the feed stream based on their retention time on the adsorbent.

The use of adsorption to remove fermentation inhibitors from the broth have been studied by numerous research groups (Carvalho, 2006; Mancilha & Karim, 2003; Nilvebrant, Reimann, Larsson, & Jonsson, 2001; Sainio, Turku, & Heinonen, 2011).

The isotherms of acetic acid adsorption from a water solution on five different types of synthetic activated carbon were plotted and characterized and it was determined that activated carbon is an adequate adsorbent material for acetic acid removal from aqueous solutions at lower concentrations (Dina, Ntieche, Ndi, & Mbadcam, 2012).

100 % removal of acetic acid was achievable by combining the evaporation with activated charcoal adsorption followed by resin adsorption in a study by Carvalho *et al.* (2006) which compared fermentation inhibitors removal from eucalyptus hydrolysate using vacuum evaporation versus adsorption with activated charcoal, diatomaceous earth, ion exchange resin, and adsorbent resins. The downside of their technique was high sugar loss (of about 30 %) during the adsorption process.

Isotherm characterization and the regeneration performance were used to compare the cation exchange adsorbents with neutral polymeric adsorbent and activated carbon in removing acetic acid from water solutions that modeled the biomass hydrolysate. Acetic acid had the highest adsorption efficiency compared to furfural and HMF but selecting the most efficient adsorbent depends on the feed composition and initial concentration of the inhibitors (Sainio et al., 2011).

Seven other resins to remove acetic acid, HMF, and furfural from aqueous solutions were tested for the purpose of maximizing the xylose recovery for xylitol fermentation in Mancilha *et al.*'s study. According to their study, weak-base anion exchange commercial resin was the best performing adsorbent to remove acetic acid from corn stover hydrolysate (2003).

Adsorption of sugars instead of the inhibitors has also been primary focus of some publications. The adsorption of the five and six carbon sugars on two different polymeric adsorbents, Dowex 99 and poly 4-vinylpyridine (PVP), from corn stover hydrolysate yielded in a higher final ethanol yield compared to the overliming technique (Xie, Phelps, et al., 2005). In a later study a five zone simulated moving bed (SMB) system, loaded with aforementioned adsorbent, showed 85-92% fermentability of the sugars that were recovered at different zones of the SMB (Xie, Chin, et al., 2005). The acetic acid in these studies was co-eluted with the sugars but its low concentration (3.37 g/L) had no negative impact on the fermentation. Cost analysis, ignoring the utility cost, was also showed minimal impact on the manufacturing cost of the fermentable sugars.

Different types of ion exchange columns can be used in the removal of the majority of the inhibitors from a dilute acid hydrolysate of spruce to increase the ethanol production in the downstream process; despite high pressure drop and frequent need to recharge of the column (Nilvebrant et al., 2001).

Adsorption technologies exhibit high efficiency in respect of either fermentation inhibitors or fermentable sugars recovery. Using stationary phase systems also have

minimal toxicity effect on any of the downstream processes mainly fermentation. On the downside, column regenerations, which are inevitable parts of any adsorption systems, are extremely energy intensive. Furthermore the use of synthetic adsorbents makes the biorefinery less sustainable and adding a chromatography column in the process contributes to additional pressure drop in the system.

2.2.3 Liquid-liquid extraction studies

Liquid-liquid extraction is a well-known process that is frequently used in chemical and petrochemical plants. This technology involves adding an immiscible solvent to the liquid system. The desired component has more affinity to the solvent and when the equilibrium is reached the solvent (extract phase) is rich with the desired compound (Figure 2-3). Solvent selection is a prominent part of this technology, since purification of the desired compounds and recycling the solvent back to the system are essential steps for this process.

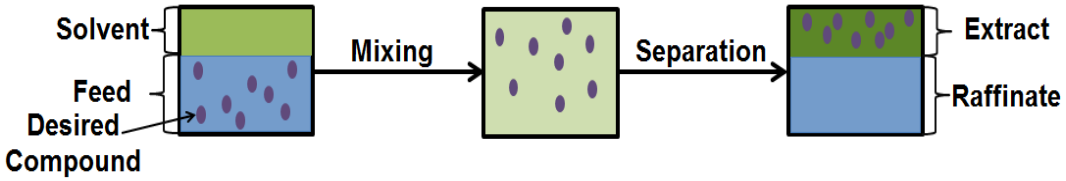

Figure 2-3: Schematic liquid-liquid extraction separation which is a separation method in which an immiscible solvent with high affinity to the desired compound is added to the mixture

Laboratory experiments were conducted to extract acetic acid via liquid-liquid extraction with trioctylphosphine oxide (TOPO) followed by distillation to remove

the acetic acid from the solution and recycle the solvent. TOPO binds with the acetic acid and removes it from the water solution; however it shows poor capability for repeated recycle, which greatly increases the cost of using this solvent for an large scale extraction (Um, Friedman, & van Walsum, 2011).

The partition coefficient of acetic acid, furfural, HMF, vanillin, syringeldehyde, and coniferyl aldehyde in eleven different organic solvents, ranging from C6 alkene to C18 alcohol, from synthetic solution were measured by Zautsen *et al.* (2009). Considering the biocompatibility of the solvents in fermentation experiments, it was indicated that the extractability of the solvents have negative correlation with the biocompatibility of them.

High liquid-liquid extraction efficiencies to recover acetic acid from pre-hydrolysis liquor from a Kraft pulp process were recorded using tri-n-octylamine with octanol as the diluent and sodium hydroxide in water solution for back extraction and recovery (Ahsan, Jahan, & Ni, 2013). Increasing the salt loading in the back extraction step kept the first and back extractions efficiency as high as almost 100 %.

Development of a mathematical model for pilot-scale liquid-liquid extraction of acetic acid followed by a stripping step to recover the solvent, demonstrated that higher flowrate of the solvent increases the efficiency of the process. Ethyl acetate was the best performing solvent compared to diethyl ether, and a mixture of the two (Jipa, Dobre, Stroescu, & Stoica, 2009).

Liquid-liquid extraction with varying solvent loading, feed concentration, and pH with Alamine-336 as the solvent and 2-ethyl-1-hexanol as diluent was optimized at

85 % efficiency at pH of 3.5 with initial concentration as high as 45 g/L acetic acid (Katikaneni & Cheryan, 2002).

Table 2-1 summarizes the literature regarding the usage of membranes, adsorbents, and liquid-liquid extraction to remove acetic acid from either model aqueous solutions or biomass hydrolysate.

Table 2-1: Summary of separation methods used for acetic acid removal from aqueous systems

Membrane Studies		
The method	The finding	Reference
Hollow fiber membrane with mixture of tertiary amine and octanol	60 % acetic acid removal	(Grzenia et al., 2008)
Nano filtration	95 % acetic acid removal	(Weng et al., 2009)
Grafted PVA membrane for pervaporation	Up to 14.6 separation factor for acetic acid	(Al-ghezawi et al., 2006)
Grafted co-polymer for pervaporation	Up to 23 separation factor for acetic acid	(T. M. Aminabhavi & Toti, 2003)
Vacuum membrane distillation with hollow fiber membrane	30 % acetic acid removal	(Chen et al., 2013)
Evapomeation	Up to 52 separation factor for acetic acid	(Isiklan & Sanli, 2005)
Adsorption Studies		
The method	The finding	Reference
Activated carbon	Development of isotherms	(Dina et al., 2012)
Vacuum evaporation + activated charcoal + adsorbent resin	100 % acetic acid removal with the combination of methods	(Carvalho, 2006)
Cation exchange resin	Higher acetic acid adsorption compared to HMF and furfural	(Sainio et al., 2011)
Weak-base anion exchange resin	100 % acetic acid removal	(Mancilha & Karim, 2003)
Dowex 99 and PVP as adsorbent in SMB	Recovered sugars have enhanced fermentation performance	(Xie, Chin, et al., 2005)
Ion-exchange column	Complete removal of inhibitors	(Nilvebrant et al., 2001)

Table 2-1 continued

Liquid-liquid Extraction Studies		
The method	The finding	Reference
TOPO as the organic solvent	Low recyclability of the solvent	(Um et al., 2011)
Eleven different organic solvents	Low biocompatibility	(Zautsen et al., 2009)
Tri-n-octylamine as organic solvent and octanol as the diluent	100 % removal of acetic acid	(Ahsan et al., 2013)
Ethyl acetate, diethyl ether, and their mixtures as organic solvents	Ethyl acetate shows the highest performance	(Jipa et al., 2009)
Alamine-336 as organic solvent with 2-ethyl-1-hexanol as the diluent	85 % efficiency at lower pH	(Katikaneni & Cheryan, 2002)

2.3 Challenges regarding liquid-liquid extraction

There are many challenges facing the use of liquid-liquid extraction systems for acetic acid removal from biomass hydrolysate to enhance the industrial scale bioethanol production. Even though many well-studied separation techniques have been successful in removing the fermentation inhibitors from the biomass hydrolysate, the shortcoming in this area is an integrated and structured study that quantifies and reports a systematic liquid-liquid extraction experiments along with the solvent recovery and its economic impact. Therefore this work addresses these three major issues which are associated with an extraction system.

1- Solvent selection: is an area where most of the previous studies have paid little attention to; and therefore there is a need for a strategic plan to select the most efficient and sustainable organic solvent for acetic acid removal.

2- Biocompatibility and solvent recovery: another gap in the current state-of-the-art in the extraction is whether the better performing solvents have any impact on the bioethanol-producing microorganisms and also if they are recyclable.

Biocompatibility of alkanes and alcohols, that were used for fermentation inhibitors removal, is measureable by the carbon dioxide emitted during the fermentation (Zautsen et al., 2009). Limited up-to-date publications are available regarding the toxicity mechanism of the organic solvent except for toxicity effects study of organic solvents on the *S. cerevisiae* cell membrane as chromosome loss inducers (Mayer, Goin, Arras, & Taylor-Mayer, 1992; Zimmermann, Scheel, & Resnick, 1989). Most of the published studies on organic solvent toxicity or biocompatibility on fermentative yeasts are focused on the solvents which are suitable for simultaneous product extraction (Roy, 2013). The mixture of Alamine336 and oleyl alcohol showed toxicity to the lactic acid fermentation due to the small amount of solubility in water and therefore it can be lowered by decreasing the miscibility (Yabannavar & Wang, 1991). Tertiary amines with different carriers and diluent demonstrated medium toxicity in lactic acid fermentation process and the use of any of the modifiers intensified this effect (Martak et al., 1997). An innovative computer based simulation to select the most biocompatible solvent for fermentation processes ranked esters are as the most toxic solvents compared to alkanes, ketones, and alcohols (Cheng & Wang, 2010).

The biocompatibility study of the selected solvents, in the previous section, to the ethanol fermenting yeast is essential for bioethanol production enhancement.

When the organic solvents are used for extraction, a proper recovery stage has to be added to the process in order to reutilize the solvent. Therefore this work aims to characterize a solvent recycling unit that would follow the extraction. After an ideal recycling step:

- The solvent is prepared to re-enter the extraction column and perform with the same extractability characteristics as before. At this point any solvent loss can be compensated from the solvent storage unit to maintain the flowrate.
- The level of the organic solvent in the aqueous phase would drop below the inhibition threshold of that solvent.
- The energy and cost balance of the overall system are not affected significantly.

3- Economic feasibility: the last area that needs further study to evaluate the LLE viability is to estimate the economic impact of liquid-liquid extraction. It includes techno-economic evaluation of the fermentation inhibitors removal to estimate the capital and manufacturing cost associated with constructing a separation unit in an existing second generation bioethanol plant.

2.4 Conclusion

Studying the mechanism of bioethanol fermentation inhibition has persuaded many research groups to investigate the adaptation and gene modifications of the yeast. Various separation methods have been used to remove the inhibitors and many of them have proven to be efficient for removing many of the known inhibitors.

Literature review reveals that liquid-liquid extraction is an alternative separation method that needs to be further tested for biocompatibility with yeast and also for its economic feasibility. LLE can be improved for the purpose of fermentation inhibitor removal by incorporating a systematic solvent selection to select the most efficient solvent to decrease acetic acid content present in the biomass hydrolysate below its inhibition threshold. Testing the solvent for its compatibility with the fermentation yeast is the second part of this work. The practicality of LLE is dependent upon a recovery stage to recycle the solvent. After proving the concepts with model simulation and lab scale experiment, the potential economic impact of this process on industrial scale biorefinery needs to be addressed.

CHAPTER 3. EXPLORATION INTO LIQUID-LIQUID EXTRACTION SOLVENTS TO REMOVE *SACCHAROMYCES CEREVISIAE* INHIBITORS[1]

3.1 Abstract

The process of converting lignocellulosic biomass bioethanol involves pretreatment of the woody structure of the biomass. Pretreatment allows for better accessibility of the polymeric sugars for enzyme digestion, but also results in the release of detrimental compounds that can inhibit fermentation.

Laboratory results indicate that liquid-liquid extraction (LLE) is able to remove the common fermentation inhibitors and reduce the concentration in a pre-fermentation broth below an inhibitory threshold. Bench top studies were used in conjunction with process simulations to select an organic solvent for use in LLE. The goal was to identify an organic extraction solvent with the lowest miscibility with the biomass liquid hydrolysate while allowing the sugars and water to remain in the stream destined for fermentation.

Through careful study, an initial list of fifty solvents was narrowed to nine using an Aspen Plus™ simulation. Laboratory experiments were then conducted to demonstrate that the affinity of each of the nine solvents to sugars is negligible.

[1] Chapter 3 is adapted from the conference proceeding "Aghazadeh M, Engelberth AS. Exploration into Liquid-Liquid Extraction Solvents to Remove *Saccharomyces cerevisiae* Inhibitors. In: 2015 AIChE Annual Meeting. Salt Lake City, UT, USA: American Institute of Chemical Engineering; 2015".

One solvent proved that it was also able to achieve complete extraction of acetic acid – the most ubiquitous inhibitor – from biomass liquid hydrolysate. Further simulation clarified the impact of LLE on the remaining known inhibitors.

3.2 Introduction

The biochemical option for bioethanol formation involves: preparation of the biomass, pretreatment, enzymatic hydrolysis, fermentation, and ethanol separation and recovery (Ladisch, Ximenes, Engelberth, & Mosier, 2014). Pretreatment is necessary to make the cellulose and hemicellulose accessible for enzymatic hydrolysis. Fermentation inhibitors are compounds that are inherently present in the solution after pretreatment of lignocellulose biomass. Examples of inhibitors include weak acids (e.g. acetic, formic, and levulinic), furans and phenols. All biomass is comprised of some combination of cellulose, hemicellulose, and lignin. When the weak ester bonds in the hemicellulose breaks, the acetyl groups are easily liberated during the biomass pretreatment, therefore acetic acid is present in all pretreated biomass (Grzenia, Schell, & Wickramasinghe, 2008).

Inhibition slows down the fermentation and reduces the yield of ethanol produced (Kim, Kreke, Hendrickson, Parenti, & Ladisch, 2013; Nilvebrant, Reimann, Larsson, & Jonsson, 2001). Casey *et al*. (2010) demonstrated that the yeast growth, substrate consumption, and ethanol volumetric productivity decrease in presence of acetic acid.

Studies have been performed to determine the extent of removal of fermentation inhibitors prior to fermentation using ion exchange column, nano-filtration, and hollow fiber membranes (Grzenia et al., 2008; Nilvebrant et al., 2001; Weng et al.,

2009; Weng et al., 2010). Liquid-liquid extraction (LLE) is a well-known process that has not been applied to this particular extraction issue. LLE uses the addition of an immiscible solvent to the liquid system that has higher affinity to the desired compounds. Solvent selection is a prominent part of this technology since purification of the desired compounds and recycle of the solvent are essential steps in this process.

Laboratory experiments have been performed to extract acetic acid via liquid-liquid extraction with trioctylphosphine oxide (TOPO) (Um, Friedman, & van Walsum, 2011). Zautsen *et al*. (2009) measured the partition coefficients of inhibitors in many organic solvents and found that the better performing solvents may not be biocompatible with the fermentation microorganisms. Ahsan *et al*. (2013) found high liquid-liquid extraction efficiencies in their work to recover acetic acid from pre-hydrolysis liquor from a Kraft pulp process. Katikaneni and Cheryan (2002) conducted a comparative study to select the most effective method between LLE and esterification to recover acetic acid from an acetic acid fermentation process. They found that at higher acetic acid content and lower pH the extraction efficiency increases.

The research reported in this manuscript was conducted in an effort to identify the most efficient solvent to extract fermentation inhibitors using LLE and determine the subsequent economic impact on an existing biorefinery. This study examined multiple organic solvents using Aspen Plus™ simulations, quantified the extraction

performance on both a test solution and on biomass hydrolysate; and finally calculated the economic impact of this process on a biorefinery.

3.3 Materials and Methods

3.3.1 Simulation

Aspen Plus™ version 7.3 (Aspen Technology Inc., Burlington, MA) was used to simulate the extraction of acetic acid, glucose, and xylose from a water solution using a 10 stage LLE column. The column was sized for economic purposes using simple flooding calculations.

3.3.2 Laboratory Experiment and Analysis

The LLE method was modified from the experimental method of Katikaneni and Cheyran (2002). LLE was performed in 50 mL vials with 10 mL of an aqueous feed and 10 ml of the solvent. Two types of solutions were created: a test solution containing only the compounds used in the simulation, and a corn stover hydrolysate solution with concentrations adjusted to match the simulation. The test solutions were made with 30 g/L glucose, 25 g/L xylose, and 10 g/L acetic acid. These concentrations were picked based on literature review on common acetic acid, glucose, and xylose concentrations in hydrolysate from lignocellulosic biomass (Casey et al., 2010; Garlock et al., 2011; Grzenia et al., 2008; Mao, Genco, van Heiningen, & Pendse, 2010). Biomass hydrolysate was the result of pretreating corn stover (40 mesh sieve) with 1% wt. sulfuric acid at 140 °C for 40 minutes. Pretreatment was conducted in 1” OD × 0.083” wall thickness, 316 stainless steel

tubing capped with 1” Swagelock tubes and fittings (Swagelock, Indianpolis, IN). After adding the 10 mL of the organic solvent (solvent to feed volume ratio of 1:1), the vials were shaken for 5 minutes, left to equilibrate at room temperature for 12 hours, and then centrifuged for 20 minutes at 5000 rpm. All chemicals were purchased from Sigma (Sigma Aldrich, St. Louis, MO).

HPLC (Waters 2695 Separation Module, Waters Corporation, Milford, MA) was used to measure the glucose, xylose, and acetic acid contents of aqueous phase (Aminex HPX-87H Column, Bio-Rad, Hercules, CA – 300 x 7.8 mm pre-packed HPLC carbohydrate analysis column). The mobile phase was 5 mM sulfuric acid with a flow rate 0.6 mL/min, an internal temperature 35 °C and external temperature 65 °C.

3.4 Results and Discussion

Removal of fermentation inhibitors, especially acetic acid, using liquid-liquid extraction (LLE) is the focus of this work. To establish the best performing solvent to extract acetic acid and other fermentation inhibitors in an LLE apparatus, a series of AspenPlus™ simulations and experiments were conducted. An initial list of fifty solvents were selected for screening based on the ternary phase diagrams of acetic acid, water and the solvent (Sorensen, 1980). To narrow down the fifty solvents to a more reasonable list, four benchmarks were developed. These benchmarks took into account both process and environmental constraints. The benchmarks are: 1) the consumption rate of the solvent should be as low as possible while achieving a high acetic acid yield, 2) water content in the extract stream should be as low as possible, 3)

the sugar initially entering with the feed stream should leave the column in the raffinate stream and 4) the solvent must not be carcinogenic or toxic. Based on the fourth benchmark, eighteen solvents were immediately discarded due to their inherent toxic properties.

3.4.1 Simulation: Narrowing Down the Solvents

For the remaining thirty-two solvents, an AspenPlus™ simulation was developed to test the split fraction, SF= $\left(\frac{\text{acetic acid in the extract}}{\text{acetic acid in the feed}}\right)$, of the acetic acid between the water and the third solvent. Each of the solvents were run through the simulation varying the solvent volumetric flow rate until the split fraction of acetic acid was equal to a desired set point of 0.99. This meant that some solvents required a high solvent consumption rate to achieve the chosen SF and were thus eliminated, as they did not fit the first benchmark. The simulation allowed for the elimination of nine additional solvents; twenty-three of the solvents were able to achieve the desired SF within a ten-stage extraction column. Figure 3-1 displays the solvent flow rate and the water content in the extract stream for the twenty-three viable solvents. It is apparent in Figure 3-1 that there is a trade-off when selecting the ideal extraction solvent, as the solvent consumption rate decreases, the water content in the extract stream increases. The optimum range, based on Figure 3-1, includes solvents between ethyl acetate to methyl butyrate; these nine solvents consumed less than 150 kmol/hr for 500 kmol/hr of aqueous feed, while allowing the water content in the extract to remain low (less than 25 kmol/hr). The gray box in Figure 3-1 clearly displays the nine solvents that fit the first two benchmarks, which include: ethyl acetate, *n*-butyl acetate, cyclohexyl

acetate, ethyl propionate, isobutyl acetate, *n*-pentyl acetate, isopentyl acetate, ethyl butyrate, and methyl butyrate.

3.4.2 Experimental: Acetic Acid Extraction

The nine solvents that were selected via simulation were then subjected to a single-stage LLE experiment to determine their ability to remove acetic acid from the aqueous solution. The LLE was necessary to test the solvents with the aqueous solution because the destination stream of the sugars could be easily manipulated in the simulation based on the chosen property method. An initial LLE experiment with a test solution was conducted to evaluate the simulation results. Figure 3-2 shows the experimental results of the test solution for SF of acetic acid for each of the nine solvents tested along with the percent of xylose and glucose that remain in the aqueous phase (labeled as % Recovery). For all nine solvents, sugars remained predominately in the aqueous phase; 92%-95% for glucose, and 93%-99% for xylose.

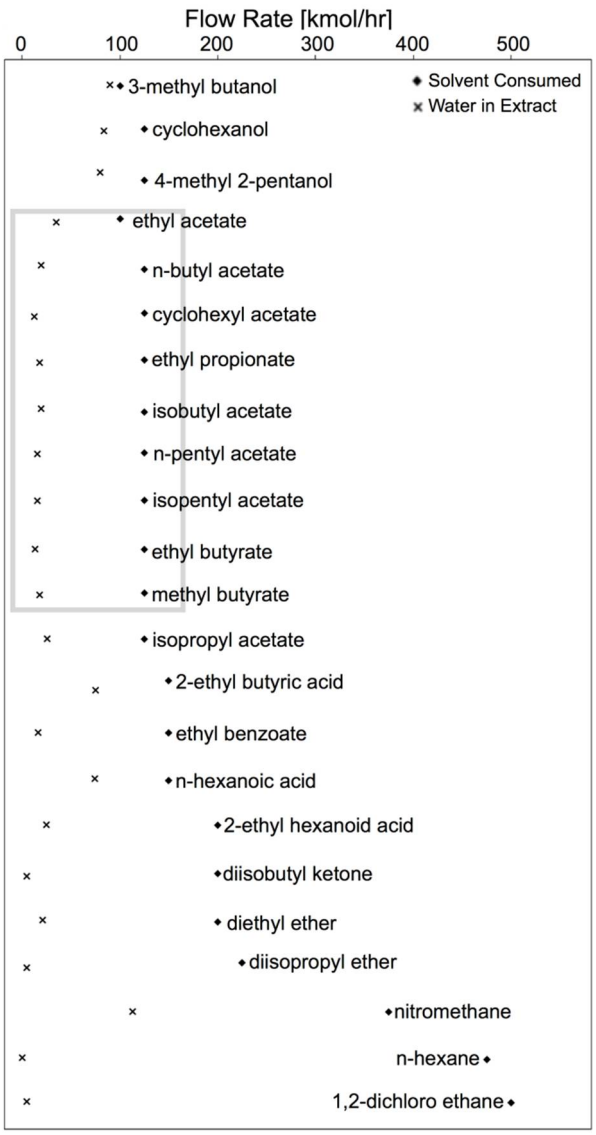

Figure 3-1: Solvent consumption rate and water content in the extract stream from the simulation results of the solvents extracting 99% acetic acid from a solution of water, acetic acid, glucose, and xylose. The compounds within the box were chosen as the best performing solvents based on their low solvent consumption while keeping the water in the extract as low as possible.

To gain a more complete understanding of the performance of each of the nine solvents the LLE experiment was performed on a solution of corn stover hydrolysate.

The procedure to make the hydrolysate was described in the material and methods section. A summary of the LLE experimental results comparing the test solution with actual corn stover liquid hydrolysate is shown in Figure 3-2. Note that the performance of the nine solvents is the same for the test solution as for the liquid hydrolysate. Using the data gathered from the LLE experiment, the nine solvents were ranked based on their ability to meet the benchmarks previously stated. The ranking of nine solvents, from best performing to unsatisfactory are: ethyl acetate, butyl acetate, isobutyl acetate, ethyl propionate, methyl butyrate, ethyl butyrate, cyclohexyl acetate, pentyl acetate, isopentyl acetate.

It was evident that ethyl acetate, butyl acetate, isobutyl acetate, and ethyl propionate were able to extract more acetic acid from the corn stover hydrolysate than from the test solution likely due to the presence of other molecules present in the hydrolysate such as sulfuric acid. The acid content, and thus lower pH can enhance the LLE yield for acetic acid (i.e. produce a salting-in effect) (Cohn, 1943; Katikaneni & Cheryan, 2002; Wheelwright, 1991). The noteworthy result from Figure 3-2 is that ethyl acetate is able to extract 85% of the acetic acid from a real solution while allowing the sugars to remain with the raffinate.

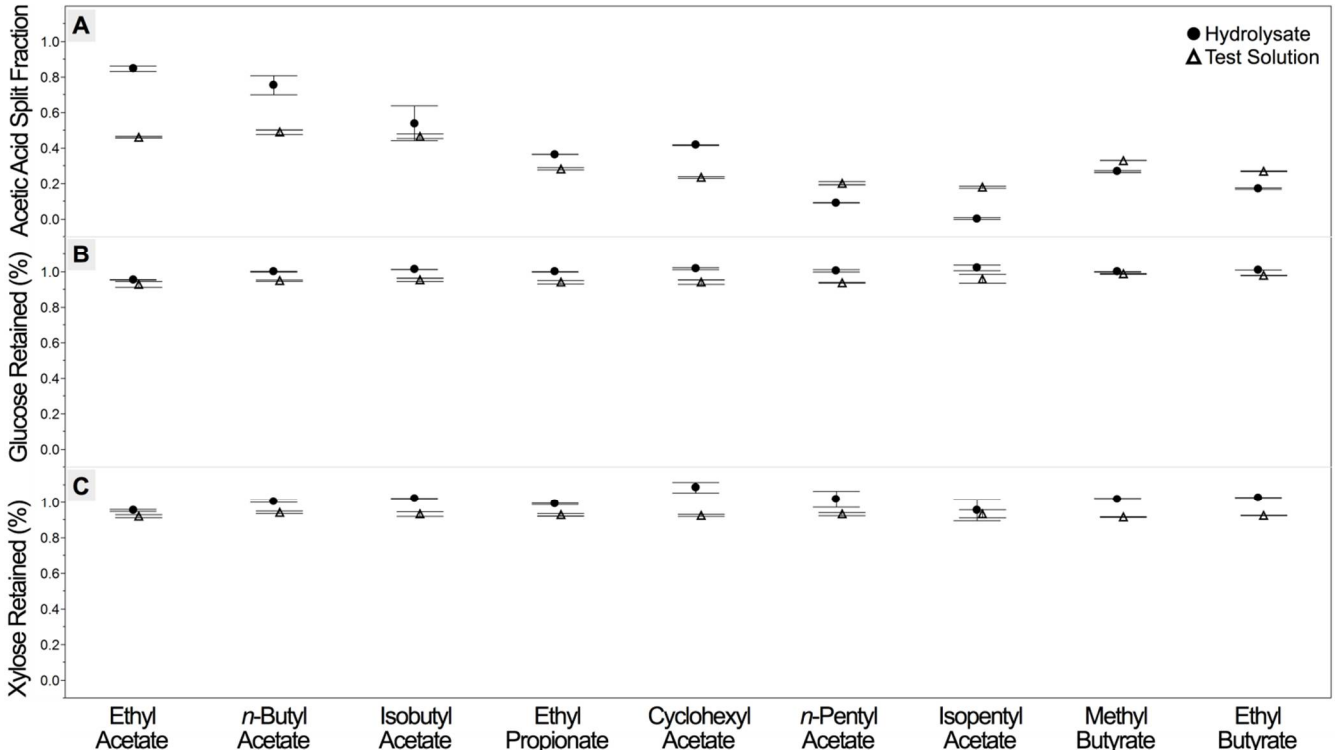

Figure 3-2: Comparison of the test solution and a real biomass hydrolysate to determine the performance of each of the nine organic extraction solvents. A) fraction of acetic acid extracted by each solvent – shown as split fraction, SF. A split fraction closer to one is more ideal as it indicates that the solvent removes more acid. B) Percent of glucose retained in aqueous phase, and C) percentage of xylose retained in aqueous phase. The ideal case is for the % retained to be as close to 100% since the goal is for the sugars to not be extracted by the solvent.

3.4.3 Simulation: Extraction of Other Common Inhibitors

Based on the results gathered for the performance of the nine solvents to remove acetic acid from a solution of corn stover hydrolysate, the next question was to see how well the solvents were able to extract other common fermentation inhibitors from biomass hydrolysate. The common fermentation inhibitors were determined from existing literature and the higher end of the reported concentration was used in our simulation. Each of the inhibitors was added to the feed stream in the Aspen Plus™ simulation. The inhibitors, along with the common concentration found in a post-pretreatment solution, are shown in Table 3-1.

Table 3-1: Concentration of additional fermentation inhibitors tested with each of the nine extraction solvents in the Aspen Plus™ LLE simulation.

Inhibitor	Concentration (g/L)	Common Biomass Source	Reference
Acetic Acid	16.1	Hemicellulose acetyl bonds	(D. Humbird, Mohagheghi, Dowe, & Schell, 2010)
Furfural	2.9	Lignin degradation	(D. Humbird et al., 2010)
Formic Acid	3.5	Carbohydrate degradation	(Martin & Jonsson, 2003)
Levulinic Acid	2.6	Carbohydrate degradation	(Almeida et al., 2007)
Vanillin	0.43	Lignin degradation	(Almeida et al., 2007)
Cinnamic Acid	0.15	Lignin degradation	(Martin & Jonsson, 2003)
4-Hydroxy benzoic Acid	0.005	Lignin degradation	(Nichols et al., 2008)
Hydroxyacetaphenone	0.004	Lignin degradation	(Almeida et al., 2007)
Acetovanillone	0.008	Lignin degradation	(Almeida et al., 2007)
3,4 dihydroxybenzoic acid	0.000005	Lignin degradation	(Nichols et al., 2008)
Syringic Acid	0.44	Lignin degradation	(Almeida et al., 2007)
Hydroquinone	0.017	Lignin degradation	(Almeida et al., 2007)
Phenol	0.035	Lignin degradation	(Almeida et al., 2007)

The results of the simulation are shown in Figure 3-3. The solvents in Figure 3-3 are arranged along the x-axis in the order of how well they extract each of the inhibitors. Ethyl Acetate is shown to be able to fully extract eleven of the thirteen inhibitors. The order of the inhibitors in the legend has been arranged to demonstrate

how likely each is to be extracted by the solvents tested. Furfural, cinnamic acid, hydroxyacetophenone, phenol, 4-hydroxybenzoic acid, and acetovanillone are all fully extracted by each solvent. The remaining seven inhibitors would require a higher solvent flow rate for better extraction. Based on the results shown in Figure 3-3, the ideal solvent is ethyl acetate due to its capacity to completely extract all inhibitors at a low solvent consumption (Flow rate = 100 kmol/hr) except acetic acid (SF =0.73) and formic acid (SF = 0.19). These findings are in agreement with the experiment results in Figure 3-2 and greatly simplify the solvent selection process as it predicts that selected solvent from the experimental results will perform well with the other inhibitors as well.

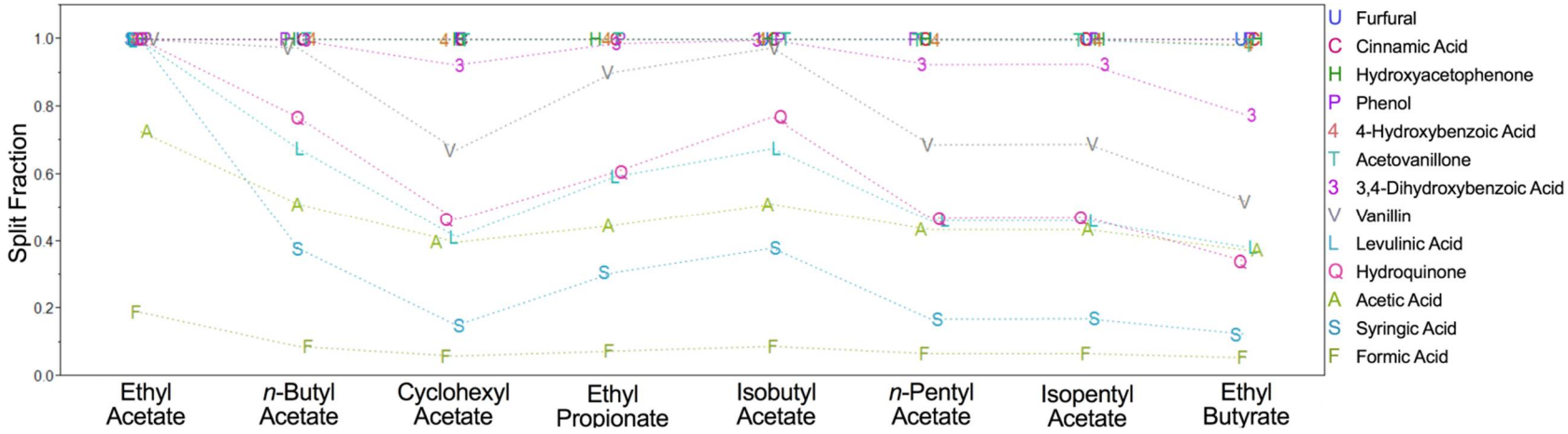

Figure 3-3: Split fraction of common fermentation inhibitors compounds using the selected nine organic solvents. Fraction of 1 indicates 100% extraction of the inhibitor. The legend explains the symbols on each line. The lines were included to more easily compare the performance of the inhibitors in each solvent.

3.5 Conclusion

A group of twenty-three solvents were ranked on their ability to extract acetic acid from biomass hydrolysate using Aspen Plus™ simulation. Laboratory results confirmed that ethyl acetate, butyl acetate, and isobutyl acetate have the capacity to reduce the acetic acid concentration below its inhibition threshold (less than 0.5 % wt.). Further simulation predicted that solvents that perform well to extract acetic acid also perform well to extract other common fermentation inhibitors. These non-toxic solvents have the capability to remove the fermentation inhibitors at low flow-rates, have no or negligible solubility with the pre-fermentation broth, and have minimum affinity to the sugars.

CHAPTER 4. ACETIC ACID REMOVAL FROM CORN STOVER HYDROLYSATE USING ETHYL ACETATE AND THE IMPACT ON *SACCHAROMYCES CEREVISIAE* BIOETHANOL FERMENTATION[2]

4.1 Abstract

Acetic acid is introduced into cellulose conversion processes as a consequence of composition of lignocellulose feedstocks, causing significant inhibition of adapted, genetically modified and wild-type *S. cerevisiae* in bioethanol fermentation. While adaptation or modification of yeast may reduce inhibition, the most effective approach is to remove the acetic acid prior to fermentation. This work addresses liquid-liquid extraction of acetic acid from biomass hydrolysate through a pathway that mitigates acetic acid inhibition while avoiding the negative effects of the extractant, which itself may exhibit inhibition. Candidate solvents were selected using simulation results from Aspen Plus™, based on their ability to extract acetic acid which was confirmed by experimentation. All solvents showed varying degrees of toxicity towards yeast, but the relative volatility of ethyl acetate enabled its use as simple vacuum evaporation could reduce small concentrations of aqueous ethyl acetate to minimally inhibitory levels. The toxicity threshold of ethyl acetate, in the presence of acetic acid, was found to be 10 g/L. The fermentation was enhanced by extracting 90% of the acetic acid using ethyl acetate

[2] Chapter 4 is in press for publication in Biotechnology Progress with the title "Acetic Acid Removal from Corn Stover Hydrolysate Using Ethyl Acetate and the Impact on *Saccharomyces cerevisiae* Bioethanol Fermentation" by Mahdieh Aghazadeh, Michael Ladisch, and Abigail S. Engelberth.

followed by vacuum evaporation to remove 88 % removal of residual ethyl acetate along with 10% of the broth. NRRL Y-1546 yeast was used to demonstrate a 13% increase in concentration, 14% in ethanol specific production rate, and 11% ethanol yield. This study demonstrated that extraction of acetic acid with ethyl acetate followed by evaporative removal of ethyl acetate from the raffinate phase has potential to significantly enhance ethanol fermentation in a corn stover bioethanol facility.

4.2 Introduction

Process optimizations and improvements are needed to strengthen the commercial viability of the second-generation bioethanol industry in the United States (Ladisch, Ximenes, Engelberth, & Mosier, 2014). The yield and the robustness of the current biochemical conversion of lignocellulosic biomass to bioethanol must improve to make this process more sustainable. Economic analysis has demonstrated that by increasing the solid loading and eliminating the effect of enzyme and fermentation inhibitors, the manufacturing cost can be reduced (Balan, 2014; Humbird, Mohagheghi, Dowe, & Schell, 2010). Removal of acetic acid will result in a higher ethanol production rate which can, in turn, lower the residence time in the fermentation bioreactor ultimately increasing the overall revenue of the biorefinery.

Biomass is comprised of cellulose, hemicellulose, lignin, extractives, and ash where the acetic acid is released from the acetyl bonds of hemicellulose during pretreatment of lignocellulosic biomass (Grzenia, Schell, & Wickramasinghe, 2008). During biomass pretreatment, acetic acid along with other inhibitors are released into

solution (Table 4-1). Acetic acid has a pronounced inhibitory effect on *Saccharomyces cerevisiae* cell growth rate and ethanol production rate (Almeida et al., 2007; Klinke, Thomsen, & Ahring, 2004; Palmqvist & Hahn-Hagerdal, 2000; Phowchinda, Deliadupuy, & Strehaiano, 1995). This inhibition impacts substrate consumption rate and ethanol production rate was quantified in earlier works (Almeida et al., 2007; Klinke et al., 2004; Palmqvist & Hahn-Hagerdal, 2000).

Table 4-1: The concentration of known bioethanol fermentation inhibitors in corn stover hydrolysate categorized by their origin in the lignocellulosic biomass structure (Almeida et al., 2007; Humbird et al., 2010; Serate, 2015). It is evident that acetic acid is one of the most abundant fermentation inhibitors present in lignocellulosic biomass and hence forms the focus of this study.

Source of Inhibitor	Inhibitor	Amount (g/L)
Hemicellulose Acetyl Bonds	Acetic Acid	16.1
Carbohydrate Degradation	Formic Acid	2.7
	Levulinic Acid	1.3-2
	5-hydroxymethyl furfural	3.9
Lignin Degradation	Furfural	2.9
	Vanillin	0.5-0.9
	4-Hydroxybenzoic Acid	0.005
	4-Hydroxyacetaphenone	0.007-0.015
	Acetovanillone	0.24
	3,4 dihydroxybenzoic acid	0.000005
	Syringic Acid	0.035-0.05

Either directed evolution through adaptation and gene modification of the yeast strain or removal of acetic acid prior to fermentation may be used to decrease

inhibition. The aim of directed evolution is to render the microorganisms more amenable to the harsher environment, and has yielded positive results in: 1) improving the survival rate of *S. cerevisiae* (Giannattasio, Guaragnella, Corte-Real, Passarella, & Marra, 2005), 2) increasing the specific ethanol production rate (Sànchez i Nogué, 2013), 3) enhancing the sugar consumption rate, and 4) improving ethanol productivity (Keating, Panganiban, & Mansfield, 2006). However, the effect is strain specific and industrial strains modified in this manner are not yet in use (Balan, 2014). Furthermore residual inhibitory effects due to acetic acid are still prevalent. Reduction in inhibition by physical separation to remove the inhibitor from the broth prior to fermentation is therefore relevant because it would enhance performance of adapted yeast or reduce inhibition of yeast lacking acetate tolerant characteristics. Both solid adsorbents and liquid extractants have been used, but may introduce their own inhibitory effects.

Various approaches to remove acetic acid include anion exchange resins, membranes (Han et al., 2006), membrane distillation, and liquid-liquid extraction. The combination of a hollow fiber membrane and an organic phase mixture of octanol and Alamine 336 demonstrated 60 % removal of the acetic acid in dilute acid pretreated corn stover (Grzenia et al., 2008). Vacuum membrane distillation has also been reported to decrease the concentration of acetic acid and furfural in the corn stover hydrolysate (Chen et al., 2013). Although liquid-liquid extraction is the most efficient approach and has a prior history of use including acetic acid from aqueous solutions; toxicity of the solvent itself can result in potential limitations (Al-Mudhaf, Hegazi, & Abu-Shady, 2002; Cai, 2001; Manzak & Sonmezoglu, 2010; Matsumoto,

Otono, & Kondo, 2001). Amine and phosphine based solvents with different diluents (Ahsan, Jahan, & Ni, 2013; Lee, 2015; Ren, Wang, Li, & Wang, 2012; Um, Friedman, & van Walsum, 2011; Zautsen et al., 2009) and trioctylphosphine oxide (TOPO) are effective in extracting acetic acid from hydrolysates (Um, Friedman, & van Walsum, 2011). The better performing solvents, based on the measured partition coefficients of inhibitors in various organic solvents, were not biocompatible with the fermentation microorganisms (Zautsen et al., 2009).

Recovery of acetic acid from a prehydrolysis liquor in the Kraft pulp process showed that high liquid-liquid extraction efficiencies were achievable (Ahsan et al., 2013), however fermentation was not the goal. A comparative study to select the most effective method between LLE – using Alamine-336 and 2-ethyl-1-hexanol as the organic solvent – and esterification to recover acetic acid from an acetic acid fermentation process found that the extraction efficiency of 85% was achievable at a pH below 3.5 (Katikaneni & Cheryan, 2002). Recyclability and biocompatibility of the organic solvent with the fermentation microorganisms are key considerations for implementing solvent extraction into the bioethanol production process, and motivated the current study that combined acetic acid extraction and recovery followed by fermentation.

The goal for employing LLE is to remove acetic acid from a pre-fermentation broth while allowing the sugars to remain in the aqueous phase. Based on thermodynamic analysis of solvent/acetic acid interactions, ethyl acetate and butyl acetate were selected from twenty-three candidates for further testing. These short-chained esters have a strong attraction to protonated acetic acid and have negligible

solubility sugars with the sugars. Ethyl acetate was the least toxic, when extraction was followed by a short vacuum partitioning step, and resulted in a fermentation media that exhibited higher performance when compared to corn stover hydrolysate. This study examines the synergistic inhibition of small amounts of ethyl acetate and acetic acid that remain in the extracted broth, and fermentation conditions that are able to convert corn stover hydrolysate into ethanol at significantly higher rate and yield.

4.3 Materials and Methods

4.3.1 Materials

Corn stover was collected after the harvest in Lafayette, Indiana in September 2012. The moisture content was measured to be 9% wt. and the samples were stored in freezer at 4°C until use. Glucose, xylose, and butyl acetate were purchased from Sigma Aldrich (Sigma Aldrich, St. Louis, MO), sulfuric acid and acetic acid from Mallinckrodt (Mallinckrodt Chemicals, Phillipsburg, NJ), peptone and yeast extract from BD (Becton, Dickinson and Company, Franklin Lakes, NJ), and ethyl acetate from J. T. Baker (Avantor Performance Materials, Center Valley, PA).

4.3.2 Biomass Preparation and Pretreatment

The corn stover was hammer-milled (Model 10 HMBD, Glen Mills Inc., Clifton, NJ) with ¼″ screen and further milled by Thomas Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ) to make finer particles (~2 mm).

The dilute acid pretreatment was performed in a 2-L floor stand Parr reactor (Model 4530, Parr Instrument Company, Moline, IL). The biomass was impregnated in water solution with 0.8% wt. sulfuric acid for two hours before starting the pretreatment. The mixture was then heated from ambient to 140°C in about 30 minutes and kept at 140°C for 40 minutes. The solid loading was set at 4% to fully hydrolyze the hemicellulose and obtain maximum conversion of acetyl bonds to acetic acid. The solid and liquid were separated using cheesecloth followed by filtration with Whatman paper filter No.1. The pH of the collected liquid was 2.2 prior to neutralization of acetic acid. The glucose, xylose, and acetic acid concentrations after the pretreatment were 0, 13, and 2 g/L respectively.

After HPLC analysis of the liquid portion of the pretreated biomass, the concentration of the glucose, xylose, and acetic acid are adjusted to 100 g/L, 60 g/L, and 10 g/L (Takahashi, Takahashi, Carvalhal, & Alterthum, 1999) respectively; as indicated by Hodge *et al* (2008) an equivalent synthetic solution is a good approximation for inhibition studies and hence was used here to enable specific concentrations of fermentable sugars and acetic acid to cover a range of conditions. The condition used in this study is representative of the pretreated corn stover at its highest flowable solid loading (Casey, Sedlak, Ho, & Mosier, 2010; Kim, Kreke, Hendrickson, Parenti, & Ladisch, 2013; Schell, 2003) while remaining economically feasible, as stated by Humbird *et al*. (2010) to be 19% solid loading at enzymatic hydrolysis to minimize the ethanol selling price,.

4.3.3 Selection of Solvent Candidates Using Aspen Plus™

The solvent selection process utilized Aspen Plus™ version 7.3 (Aspen Technology Inc., Burlington, MA) to simulate the extraction of acetic acid, glucose, and xylose from a water solution using a 10-stage LLE column. Candidate extraction solvents were first selected using Aspen Plus™ software to carry out a thermodynamic calculation to predict the acetic acid extractability. A list of 23 candidates, after eliminating the more toxic solvents, were generated and evaluated based on inherent distribution coefficients and toxicity to yeast. These solvents ranged from C1 to C9 with most of them being esters, alcohols, acids, and ethers.

The nine solvents that were selected via simulation were then subjected to single-stage LLE experiments to determine their ability to remove acetic acid from the corn stover liquid hydrolysate. LLE was performed in 50 mL vials with 10 mL of sample and 10 mL of the solvent resulting in a 1:1 volume ratio. The vials were shaken for 5 minutes at room temperature, left to equilibrate at room temperature – maintained at ~ 25 °C – for 12 hours, and then centrifuged for 20 minutes at 5000 rpm. The aqueous layer was then analyzed using HPLC, and distribution of acetic acid between the two phases was calculated. Ethyl and butyl acetates were selected for further investigation.

4.3.4 Liquid-liquid extraction

The LLE experiment was adopted and adjusted from Katikaneni and Cheryan (2002). One hundred mL of liquid corn stover hydrolysate, with adjusted glucose, xylose, and acetic acid concentration, was mixed with the organic solvent at a 1:1

volume ratio. The mixture was shaken vigorously for 5 minutes and then poured in 250 mL separatory funnel and allowed to equilibrate for at least 12 hours at room temperature. For further equilibrium stages, the aqueous phase was collected from the previous stage and mixed with fresh organic solvent of same volume and same procedure was performed on the new mixture. Each sample run was tested in triplicates. The pH of the liquid hydrolysate was not adjusted until immediately before the fermentation process was initiated since previous studies that have shown that acetic acid extraction performance is enhanced at lower pH (Katikaneni & Cheryan, 2002).

4.3.5 Synergistic Inhibition Experimental Design

A full factorial experimental design was used to test the hypothesis whether or not synergistic inhibition of ethyl acetate and acetic acid exist on the final ethanol concentration and its production rate. The fermentation was performed with NRRL Y-1546 in the YEPD media (1 g/L of dry cell mass as the starting concentration) and glucose concentration of 100 g/L. Initial concentration of acetic acid in the experiments was set to 10 g/L and after a two-stage LLE the amount of acetic acid reduces to 1 g/L. Therefore, four levels of acetic acid concentrations (0, 2, 5, 10 g/L) to represent the levels of acetic acid concentration that is obtainable during different stages of LLE (data shown in *Results and Discussion*). In a separate study (data not shown) the inhibition threshold of ethyl acetate alone on NRRL Y-1546 was found to be 20+ g/L; therefore, three levels of ethyl acetate between 0 and 20 g/L were selected. The levels (0, 10, 20 g/L of ethyl acetate and 0, 2, 5, 10g/L of acetic acid)

made up the twelve combinations that are sufficient points to test for the inhibition effect on the fermentation at the mentioned conditions (Table 4-2). Each condition was tested in duplicate.

Table 4-2: The twelve combinations of different ethyl acetate and acetic acid concentrations that were used in the factorial design experiment for synergistic inhibition effect. The numbers in parenthesis are ethyl acetate and acetic acid concentrations respectively.

Combination (Ethyl acetate (g/L), acetic acid (g/L))		
1 (20,0)	2 (10,0)	3 (0,0)
4 (20,2)	5 (10,2)	6 (0,2)
7 (20, 5)	8 (10,5)	9 (0,5)
10 (20,10)	11 (10,10)	12 (0,10)

4.3.6 Evaporation

A rotary evaporator (Model R-200, Rotavapor, Büchi Labortechnik AG, Flawil, Switzerland) was used for solvent recycle. The temperature was set at 40°C with vacuum at 93 KPa (8 KPa absolute pressure) and the rotation rate set to 120 rpm. The liquid was stored in the collection flask during the evaporation process. The lost volume, of about 10%, was replaced with the adjusted liquid corn stover hydrolysate to attain the initial volume of 100 mL.

4.3.7 Fermentation

The yeast that was used for the fermentation experiments was NRRL Y-1546. The inoculum was prepared by propagating the yeast in YEPD media (1% yeast extract, 2% peptone, 2% glucose) at 28°C and 200 rpm (Casey et al., 2010).

The reference runs for yeast and the YEPD with glucose concentration was adjusted to 100 g/L. The pH, of both the liquid biomass hydrolysate and the samples collected after evaporation followed by LLE, were neutralized using potassium hydroxide. The concentration of the yeast added to each of the samples was 1 g/L of dry cell biomass. The experiment was performed at 28°C and 200 rpm for a period of 48 hours and samples were taken at equal intervals. All conditions were tested in triplicate. Detailed fermentation methods concerning the cell mass measurement and rate calculations can be found in earlier studies (Casey et al., 2013). The ethanol yield in this manuscript is defined as the ethanol concentration at each time interval divided by the maximum theoretical ethanol concentration (0.51 × initial glucose concentration).

4.3.8 HPLC Analysis

Sugars, acetic acid, ethanol, butyl acetate, and ethyl acetate in the fermentation and extraction samples were analyzed by Bio-Rad Aminex HPX- 87H ion exchange column. The method, including the analysis procedure, column characteristics, and the data storage and process tools, was adopted from Kim *et al*. (2013).

4.3.9 Statistical Analysis

Fermentation and extraction results were statistically analyzed using JMP (SAS institute, Cary, NC). The software also was used to fit the statistical model to the obtained experimental data with ANOVA. Student's t-test pairwise comparison of the different levels of the synergistic inhibition experiment was performed on the estimated data from the fitted model with JMP software.

4.4 Results and Discussion

4.4.1 Solvent Selection Using Aspen Plus™

The nine solvents were tested in the simulation and the performance of each was compared based on the partition coefficient (Equation 1). The partition coefficient was calculated based on the activity coefficient calculated from Aspen Plus™.

$$\text{Partition Coefficient } (K) = \frac{\text{Concentration in the organic phase}}{\text{Concentration in the aqueous phase}} \quad (1)$$

Equations 2 through 7 summarize the thermodynamic equations used to estimate the partition coefficients. The chemical potential of a particular component, μ_i, is the derivative of the Gibbs free energy with respect to the molar content of *i* at constant pressure, temperature, and mixture composition (Equation 2). Chemical potential in non-ideal systems is a function of fugacity (f_i)(Equation 3) which is estimated using the activity coefficient (γ_i), molar fraction (x_i), and fugacity of the compound in its pure form (f_i^*) (Equation 4).

$$\mu_i = \left(\frac{\partial G}{\partial N_i}\right)_{T,P,N_{j\neq i}} \quad (2)$$

$$\mu_i = RT\ln(f_i) \quad (3)$$

$$f_i = x_i \gamma_i f_i^* \quad (4)$$

At bi-phasic equilibrium, the chemical potential of acetic acid in the two phases is equivalent (Equations 5 and 6). Using Equation 7 to estimate the partition coefficient, K, and assuming constant temperature, it can be concluded that K of acetic acid (AA) has an inverse relationship with the ratio of the activity coefficient in the two phases (Equation 8).

$$(\mu_{AA})_{Aqueous} = (\mu_{AA})_{Organic} \quad (5)$$

$$(x_{AA}\gamma_{AA}f^{*}_{AA})_{Aqueous} = (x_{AA}\gamma_{AA}f^{*}_{AA})_{Organic} \quad (6)$$

$$K = \frac{(x_{AA})_{Organic}}{(x_{AA})_{Aqueous}} = \frac{(\gamma_{AA}f^{*}_{AA})_{Aqueous}}{(\gamma_{AA}f^{*}_{AA})_{Organic}} \quad (7)$$

$$K \propto {}^{1}/_{\gamma \text{ ratio}} \quad (8)$$

The selected property method in Aspen Plus™ is used to predict the activity coefficient of acetic acid in the two phases and therefore dictates the thermodynamic equilibrium. The partition coefficients measured by laboratory experiment are compared to the activity coefficient ratio predicted by the software and exhibit consistent ranking for the solvents studied (Table 4-3). The acetic acid present in the mixture is protonated due to the low pH (pH of the hydrolysate is 2.2 where the pKa of acetic acid is 4.75). The chemical structures of the nine solvents included ester functional group with a carbonyl oxygen that has partial negative charge. The ranking of the partition coefficients of these solvents can be explained by the density of the electron cloud on the carbonyl oxygen that is influenced by the length and symmetry of the molecule chain. The more negative carbonyl oxygen in ethyl acetate can attract the protonated acetic acid more strongly than the other the larger ester molecules that have less negative charge on their carbonyl oxygen.

Table 4-3: The ranking of the solvents based on the partition coefficient measured by laboratory experiments and the activity coefficient estimated by Aspen Plus ™ property method, the two have inverse relationship as predicted by Equation 8. Ethyl acetate and butyl acetate were selected for further studies regarding their impact on bioethanol fermentation.

Solvent	Measured Partition Coefficient (K_{AA})	Activity Coefficient Ratio $\gamma_{AA}^{Organic} / \gamma_{AA}^{Aqueous}$
Ethyl Acetate	5.67	2.74
Butyl Acetate	3.17	3.12
Iso-Butyl Acetate	3.17	3.15
Cyclohexyl Acetate	0.72	3.1
Ethyl Propionate	0.39	3.09
Methyl Butyrate	0.37	3.08
Ethyl Butyrate	0.20	3.21
Pentyl Acetate	0.10	3.27
Iso-Pentyl Acetate	0.00	3.29

4.4.2 Liquid-liquid Extraction

To assess the original goal of LLE – high extraction of acetic acid and low extraction of the sugars – the partition coefficients of these compounds were calculated. The partition coefficient, Equation 1, quantifies performance of the solvent in the extraction process. In this experiment, the impact of sequential equilibrium stages of LLE on extraction of acetic acid, sugar loss, and solvent concentration was measured. Different stages of LLE were completed using the procedure outlined in the materials and methods section; the aqueous feeds for stages II and III were obtained from the preceding stages. As the acetic acid concentration

in the aqueous phase decreases at each stage, the new composition of the system defines a new equilibrium at the organic and aqueous phase at the following stage. The partition coefficient of acetic acid increases significantly from stage I to II – from 0.52 to 9.05 – which translates to 6.6 g/L of acetic acid in the aqueous phase after the first stage decreasing to 1 g/L after the second stage, but does not increase dramatically from stages II to III (1 g/L to 0 g/L). For this reason, later extraction experiments were discontinued after two stages. The partition coefficient of sugars remained below 0.04 through all stages of LLE; which signifies that during LLE sugars were minimally transferred to the organic phase. The amount of sugar that diffused to the organic phase was 3, 2, and 0 g/L for glucose and 1, 2, and 0 g/L of xylose for the three different stages respectively; which is likely attributed to the cyclic structure of the sugars and the weak Van der Waals bonds between the sugars and ethyl acetate (an ester). The amount of ethyl acetate in the aqueous phase changes with different stages of LLE without an apparent pattern. The measured amount of ethyl acetate in this experiment (40 g/L) is lower than the reported solubility of ethyl acetate in water (8 g/100 mL at 20°C) and could be attributed to the low pH, ~ 2, of the mixture. Table 4-4 summarizes the partition coefficients of acetic acid, glucose, and xylose, along with the ethyl acetate concentration; all after three equilibrium stages of LLE. This shows that acetic acid extraction plateaus after two stages and that ethyl acetate concentration is not stage dependent.

Table 4-4: The partition coefficient of acetic acid, glucose, and xylose as well as the concentration ethyl acetate at three different equilibrium stages of liquid-liquid extraction using ethyl acetate as the organic solvent. Glucose and xylose have negligible affinity to ethyl acetate, acetic acid extraction reaches a plateau after two equilibrium stages of LLE. Ethyl acetate concentration in aqueous phase, while smaller than its recorded solubility, does not show a pattern with equilibrium stages. The errors indicate the standard deviation.

Variable	Stage 1	Stage 2	Stage 3
	Partition Coefficient		
Glucose	0.03 ± 0.01	0.01 ± 0.01	0.00 ± 0.03
Xylose	0.02 ± 0.01	0.04 ± 0.01	0.00 ± 0.02
Acetic Acid	0.52 ± 0.01	9.05 ± 0.98	∞^{*}
	Concentration (g/L)		
Ethyl Acetate	59.7 ± 0.1	35.8 ± 1.8	42.9 ± 0.5

*There is 0 g/L acetic acid present in the aqueous phase

4.4.3 Synergistic Inhibition of Ethyl Acetate and Acetic Acid

Biocompatibility of organic solvent with a strain of yeast needs to be carefully considered when designing an LLE process (Zautsen et al., 2009). Ethyl acetate and butyl acetate were able to extract acetic acid from the corn stover hydrolysate more efficiently than the other solvents tested (Table 4-3). Ethyl acetate and butyl acetate were then selected to test their biocompatibility with the Y-1546 strain of *S. cerevisiae*. Additionally, Aspen Plus™ modeling results indicated that ethyl acetate and butyl acetate are also the most effective solvents for removal of the most common *S. cerevisiae* inhibitors (Chapter 3). The LLE procedure – outlined in the materials and methods section – was carried out to extract acetic acid from biomass hydrolysate. Figure 4-1 displays the fermentation results of the aqueous phase collected from the

extraction experiment and it shows that both ethanol production and glucose consumption stop when ethyl acetate or butyl acetate is present in the broth at 40 and 4 g/L, respectively.

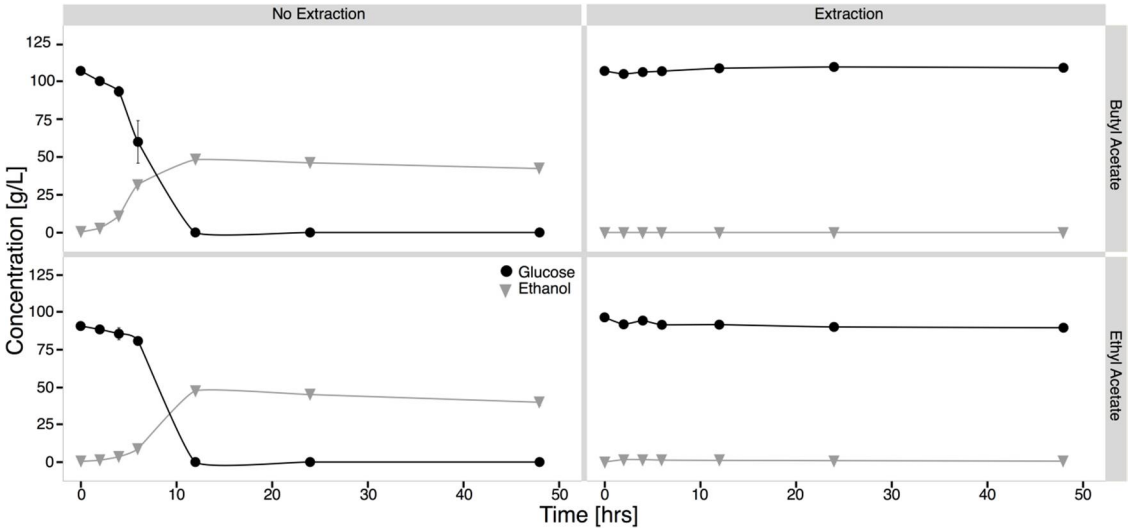

Figure 4-1: Glucose and ethanol concentrations in presence of the organic solvent ethyl acetate and butyl acetate in contrast to no inhibition fermentation. The graphs on the left show how the fermentation proceeds with no extraction applied. The graphs on the right show how the presence of the extraction solvent at 40 g/L ethyl acetate or 4 g/L butyl acetate in the fermentation dramatically inhibits the ethanol production. Butyl acetate content is much lower but has severe toxicity to the fermentation process; the same amount of ethyl acetate exhibits significantly lower toxicity and thus ethyl acetate selected for further experimentation. Error bars indicate the standard deviation.

The solubility of butyl acetate in water is 0.68 g/100 mL (at 20°C) (Haynes, 2012), while the solubility of ethyl acetate in water is 8 g/100 mL (at 20°C) (Wypych, 2000). Butyl acetate present in the fermentation broth had the same effect as if there were ten times the amount of ethyl acetate. Due to the greater toxicity of butyl acetate, ethyl acetate was selected for further study.

The model (Figure 4-2) proved that the final ethanol yield and its specific production rate are negatively influenced by acetic acid, ethyl acetate; while the ethanol yield is also significantly impacted by their interaction term (acetic acid × ethyl acetate). Figure 4-2 displays the fitted model in comparison with the collected data for the ethanol yield. The exact level of inhibition was determined using a Student's t- test pairwise comparison between each level (Figure 4-3). It is apparent from Figure 4-3 that the inhibition of ethyl acetate, on the ethanol yield and its production rate, is a strong function of the amount of acetic acid present in the fermentation broth. Acetic acid inhibition is more significant on the rate of the fermentation at concentrations 2 g/L and above; while ethyl acetate inhibition on both ethanol yield and its production rate is prominent at 10 g/L when in the presence of acetic acid and above 20 g/L concentration without acetic acid present in the media. The significant reduction in the specific ethanol production rate is noticeable at combinations (0 g/L ethyl acetate, 2 g/L acetic acid) and (10 g/L ethyl acetate, 2 g/L acetic acid). Therefore, acetic acid must be below 2 g/L if there is any ethyl acetate remaining the raffinate or that if there is acetic acid present in the media that the ethyl acetate concentration must be reduced to below 10 g/L.

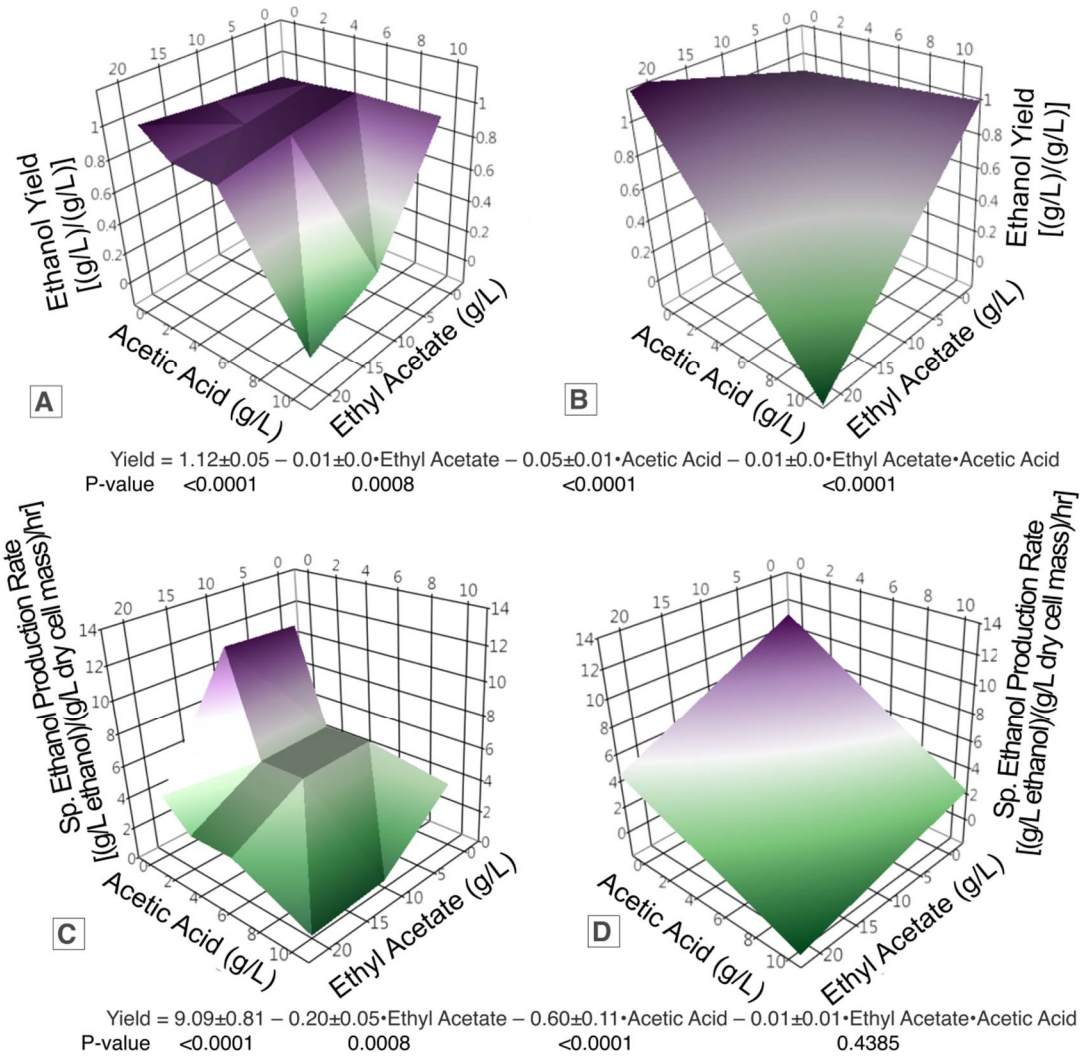

Figure 4-2: The effect of ethyl acetate and acetic acid on final ethanol yield and rate after 48 hours of fermentation and the ethanol specific production rate. A) Experimental data points of ethanol yield, B) the statistically fitted model using ANOVA results of the ethanol yield, C) experimental data points of ethanol specific production rate, and D) the statistically fitted model using ANOVA results of the ethanol specific production rate. The model for each type of inhibition is included below the respective figures.

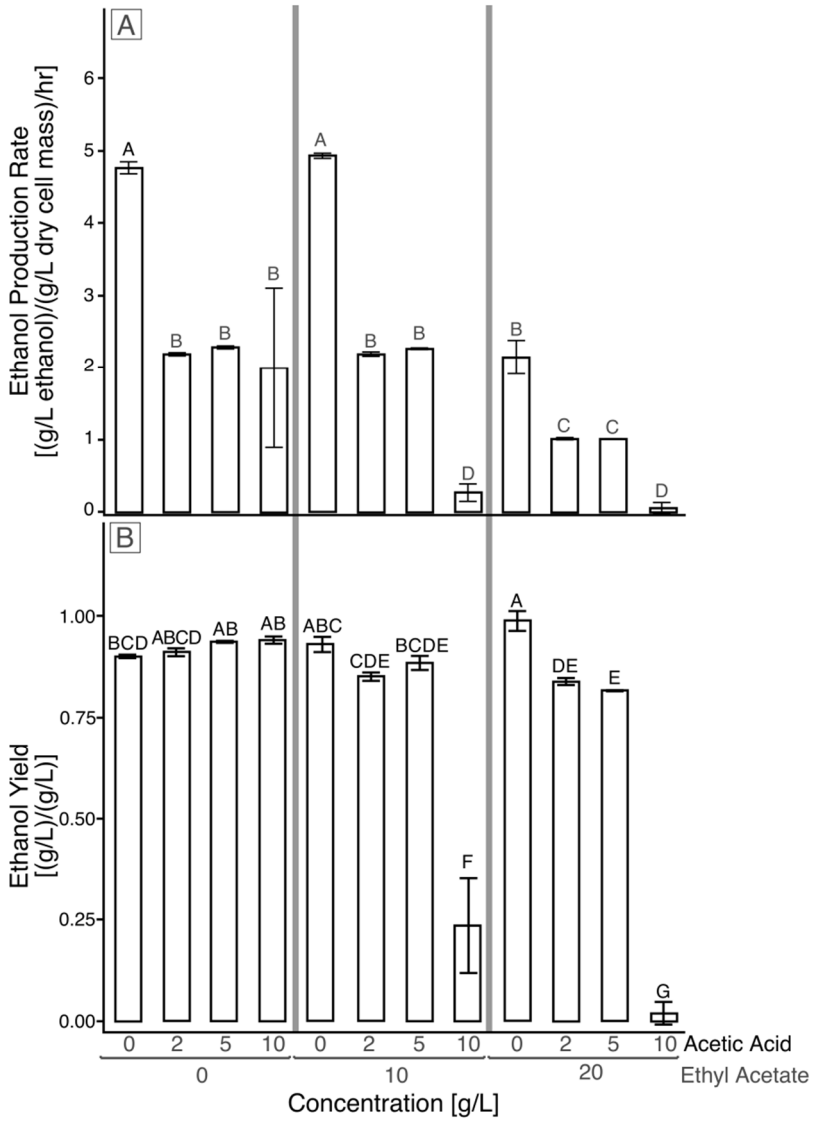

Figure 4-3: Student's t-test pairwise comparison of the twelve different combinations of ethyl acetate and acetic acid concentrations on A) the ethanol specific production rate and B) the ethanol yield. The letters above the bars show significant statistical difference between the means of the levels; the means are significantly different when they have no letter in common. Acetic acid inhibition on the ethanol specific production rate starts at 2 g/L + whereas this inhibition is not significant on the yield. Ethyl acetate inhibition on both specific production rate and yield is significant at 10 g/L in presence of acetic acid; while this number is 20 g/L + with no acetic acid in the media. The error bars indicate the standard deviation.

4.4.4 Solvent Recycle

The LLE results in Table 4-4 indicate that ethyl acetate concentration is 35.8 g/L in the aqueous phase after stage II which is higher than its inhibition threshold; therefore, it is necessary to lower the concentration of ethyl acetate below its inhibition level. The solvent reduction step will also serve as the recovery and recycle process for the extraction solvent. Evaporation under vacuum was selected to remove the ethyl acetate from the raffinate stream because of the high relative volatility of ethyl acetate. Using UNIF-LL property method within Aspen Plus™ to estimate the vapor-liquid equilibrium of water-ethyl acetate system at different pressures, indicates that the relative volatility at the evaporation condition is about 600 (at 8 KPa) (Magnussen, Rasmussen, & Fredenslund, 1981). The azeotropic point of ethyl acetate and water, 8.5% wt. of water at 70.3°C (Sorensen, 1980) is not close to the composition of this mixture from the beginning to the end of the evaporation step. Figure 4-4 shows how evaporation readily reduces the ethyl acetate concentration in the extracted biomass hydrolysate. The ethyl acetate concentration was below the level of inhibition within ten minutes of evaporation. Glucose, xylose, and acetic acid concentrations do not significantly change with evaporation; sugars are not volatile and acetic acid concentration is very low, around 1.0 g/L, after two stages of LLE.

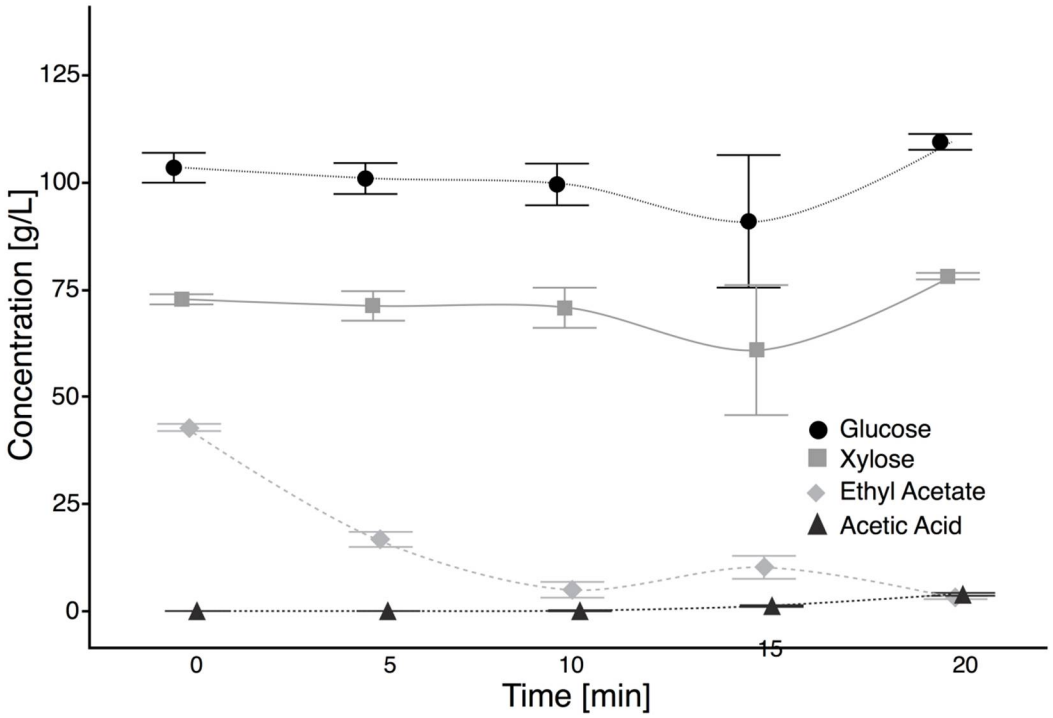

Figure 4-4: Rotary evaporation effect on the samples collected after two-stage LLE with ethyl acetate, glucose, xylose, acetic acid, and ethyl acetate concentrations versus time. Concentration of glucose, xylose, and acetic acid were not significantly altered during evaporation; while ethyl acetate concentration decreases to its non-inhibitory level (5 g/L) after 10 minutes of evaporation. The error bars indicate the standard deviation.

4.4.5 Impact on Fermentation

The samples collected after the rotary evaporation were used for fermentation. Figure 4-5 compares the four fermentation environments; 1) a reference broth consisting of the YEPD media with adjusted sugars content to 100 g/L of glucose, 2) a control broth consisting of the YEPD media with adjusted sugars content to 100 g/L of glucose and 10 g/L ethyl acetate, 3) liquid biomass hydrolysate after two stages of LLE with ethyl acetate followed by rotary evaporation, and 4) the liquid biomass

hydrolysate sans manipulation. The results, shown in Figure 4-5, indicate that while the extraction does not modify the hydrolysate to behave like the reference broth of pure glucose, it does improve the performance from the hydrolysate that has not been subjected to LLE.

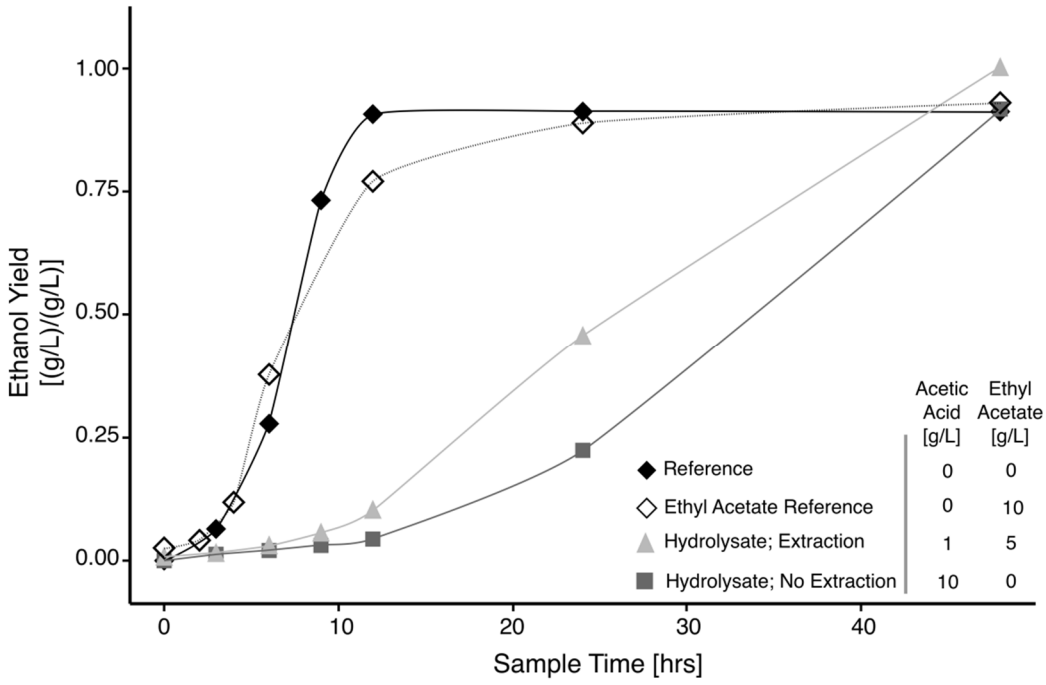

Figure 4-5: The ethanol production yield of the reference and control samples, YEPD media with adjusted sugar to 100 g/L glucose with 0 and 10 g/L ethyl acetate respectively, in contrast to the ethanol production from the liquid biomass hydrolysate and liquid biomass hydrolysate after LLE and solvent recovery steps. LLE with ethyl acetate followed by vacuum evaporation enhances the performance of liquid biomass hydrolysate fermentation through yield and specific production rate improvement. The error bars indicate the standard deviation.

Statistical analysis was performed to compare the final ethanol concentration, ethanol production rate, and the ethanol yield between the fermentation products of liquid biomass hydrolysate that was subjected to LLE and the liquid biomass hydrolysate with no extraction. The ethanol specific production rate was calculated

assuming linear production rate of ethanol after the lag phase (0-6 hours) and before the plateau. The ethanol yield in these results is the final concentration of ethanol per maximum theoretical ethanol concentration. The Student's t-test revealed significant difference between the two extracted and non-extracted groups; when comparing the final ethanol content, ethanol specific production rate, and the ethanol yield. The extraction on the hydrolysate resulted in a final ethanol concentration increase of 13.3% ± 1.5, ethanol specific production rate of 13.8% ± 1.3, and ethanol production yield of 10.6% ± 0.9.

4.5 Conclusion

There is a need for a scalable technology to increase the efficiency of the commercial scale second-generation bioethanol. The approach for this study was to use liquid-liquid extraction to remove the acetic acid, followed by evaporation to recover the extraction solvent and to decrease it toxic effect on the yeast. A two-stage LLE was sufficient to reduce the acetic acid content below the inhibition level by extracting 90% of the acid. The interactive and synergistic inhibition of the acetic acid and ethyl acetate on yeast fermentation was demonstrated and was found to a significant factor. The designed process, including LLE and evaporation, resulted in significant increase in ethanol specific production rate, ethanol yield, and final ethanol concentration.

Nomenclature

γ: Activity coefficient

μ: Chemical potential

f: Fugacity

f^*: Fugacity of the pure compound

G: Gibbs free energy

K: Partition coefficient

N: Molar amount

P: Pressure

R: Universal gas constant

T: Temperature

x: Molar fraction

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CHAPTER 5. TECHNO-ECONOMIC ANALYSIS FOR INCORPORATION OF LIQUID-LIQUID EXTRACTION SYSTEM TO REMOVE ACETIC ACID INTO A COMMERCIAL SCALE BIOREFINERY[3]

5.1 Abstract

Mitigating the effect of fermentation inhibitors in bioethanol plants can have a great positive impact on the economy of this industry. Liquid-liquid extraction (LLE) using ethyl acetate is known for its ability to remove acetic acid from an aqueous solution. Extraction using ethyl acetate as the organic solvent can also remove fermentation inhibitors in a bioethanol production facility. The fermentation broth resulting from LLE has higher performance for ethanol yield and its production rate.

The techno-economic analyses that have studied the second-generation biofuel have not yet addressed the impact of removing the fermentation inhibitors on the economic performance of an industrial plant. This manuscript attempts to fill the knowledge gap in fully analyzing the application of a separation system to mitigate the fermentation inhibition effect and to provide an analysis on the economic impact of removal of acetic acid from corn stover hydrolysate on the overall revenue of the biorefinery. This study examines the pros and cons associated with implementing liquid-liquid extraction column along with the solvent recovery system into a

[3] Chapter 5 is adapted from the manuscript "Techno-economic Analysis for Incorporation of Liquid-Liquid Extraction System to Remove Acetic Acid into a Commercial Scale Biorefinery". Which is in preparation for submission to Journal of Biomass and Bioenergy

commercial scale bioethanol plant. Using the necessary details from the NREL-developed model of corn stover biorefinery, the capital costs associated with the equipment and the operating cost for the use of solvent were estimated and the results were compared with the profit gain due to higher ethanol production. The results suggest that the additional capital and manufacturing cost were about 1 and 5.9 % of the total capital and manufacturing costs of the plant respectively whereas the higher ethanol production rate and yield resulted in $0.35 lower MESP per gallon of bioethanol.

5.2 Introduction

Current challenges facing commercialization of second-generation bioethanol production from sustainable lignocellulosic resources include but are not limited to: the biomass transport and its liquidation and the enzyme and microorganism inhibitors that increase the bioethanol production cost (Balan, 2014; Ladisch, Ximenes, Engelberth, & Mosier, 2014). A thriving bioethanol industry is possible if higher yields of bioethanol can be achieved in every step of the production process (Sassner, 2008). Cost estimation studies have shown that the most promising cost reductions were achievable through enhanced fermentation kinetics to increase the reaction rates and reduce residence time (Stephen, Mabee, & Saddler, 2012).

It was previously demonstrated that the use of liquid-liquid extraction (LLE) to remove acetic acid, a ubiquitous fermentation inhibitor, has no negative impact on the fermentable sugars (Chapter 3). When ethyl acetate is used as the organic solvent, it

can be removed from the fermentation broth and therefore has no lasting toxicity on downstream fermentation. LLE can enhance bioethanol fermentation performance by increasing the reaction rate and final specific ethanol yield by 14 and 11 %, respectively.

Numerous techno-economic analyses have been published that evaluate the economic aspects for moving forward with production of second-generation bioethanol at an industrial scale (Aden et al., 2002; D. Humbird, Mohagheghi, Dowe, & Schell, 2010; Kazi, 2010; Klein-marcuschamer, Simmons, & Blanch, 2011; Mussatto, 2010; Petter & Tyner, 2014; Sievers, Tao, & Schell, 2014; Wang, Ou, Brown, & Brown, 2015). Each of the published techno-economic analyses has focused on different aspects of the second-generation bioethanol production facilities and how to optimize independent stages of the process. Table 5-1 summarizes the stages of the corn stover biorefinery that have been considered for their impact on the overall economic feasibility of the bioethanol plant and how each can be improved.

Table 5-1: Breakdown of the areas that have been the focus of different studies for their techno-economic impact on an industrial scale biorefinery

Bioethanol production aspect	Areas that were studied	Impact or measured outcome
Biomass/feedstock	Compositional variations	Corn stover has the least ethanol production cost compared to woods and switchgrass. (Huang, Ramaswamy, Al-Dajani, Tschirner, & Cairncross, 2009)
		An optimized unit configuration has a more significant impact on the economy than the biomass characterization differences. (Sassner, 2008)
		Composition variations in corn stover can lead up to 10 % of variation in MESP. (Ling Tao, Templeton, Humbird, & Aden, 2013)
	Handling, storage, distribution, and harvest	An MESP of above $2.27/gal makes this industry unattractive for farmers and investors. (Alex Marvin, Schmidt, Benjaafar, Tiffany, & Daoutidis)
Pretreatment	Different methods optimization	Hot water pretreatment has the lowest capital cost but lime pretreatment has the lowest total fixed cost compared to other pretreatment methods. (Eggeman & Elander, 2005)
		Ionic liquid pretreatment is not economically viable compared to the other common practices. (Klein-marcuschamer et al., 2011)
		To separate the solid and liquor after the acid pretreatment, vacuum filtration has the lowest capital cost. (Sievers et al., 2014)
		Deacetylation and mechanic refining combined with dilute acid pretreatment can reduce the MESP by $0.23-$0.30/gal. (L. Tao et al., 2012)

Table 5-1 continued

Enzymatic Hydrolysis	Solids loading	Solid loading of up to 30 % can significantly reduce the MESP. (D. Humbird et al., 2010)
	Enzyme production and resources	At maximum ethanol yield the enzyme cost accounts for about $0.60 of the total MESP. (Klein-marcuschamer, Oleskowicz-popiel, Simmons, & Blanch, 2012)
		Enzyme is the second largest contributor to the ethanol cost with $0.30-$0.50 / gal. (McMillan, Jennings, Mohagheghi, & Zuccarello, 2011)
Fermentation	Microorganism strain	Optimum fermentation configuration can decrease MESP up to $0.27/ gal. (Dutta, Dowe, Ibsen, Schell, & Aden, 2010)
	Fermentation unit configuration	*S. cerevisiae* can provide the most economically attractive bioethanol. (Meyer, 2013)
By-product integration	DDGS	Integrating DDGS as a co-product increases MESP from $2.18 to $2.27. (Wang et al., 2015)
	Lignin	Utilization of lignin in biorefinery can greatly enhances the biorefinery performance. (Holladay, White, Bozell, & Johnson, 2007)
	Acetic acid	Acetic acid can be produced for $2.51/gal at a pulp mill biorefinery with 550 tonne/day capacity. (Mao, Genco, van Heiningen, & Pendse, 2010)
	Ethyl acetate	Cost effective production of ethyl acetate from bioethanol and bio-acetic acid is feasible through different technologies. (Hong Thuy, Kikuchi, Sugiyama, Noda, & Hirao, 2011)

Table 5-1 clearly indicates that the focus of the techno-economic studies thus far has been on specific configuration of a single unit – simultaneous saccharification, continuous process, etc. – and not on optimization of units surrounding fermentation.

It has been shown that *S. cerevisiae* in a separate saccharification and fermentation unit along with the option of organic acid production, as co-products, was the most favorable option (Meyer, 2013). The impact of varying fermentation configuration, implemented in an earlier NREL-developed model (Wooley & Putsche, 1996), demonstrated that at the highest achievable ethanol concentration but under the lowest performance setting there is a noticeable reduction in MESP – as much as $0.27/gallon ethanol (Dutta et al., 2010). On the other hand integrating the bioethanol production with co-products, such as acetic acid along with ethanol, has shown to increases the sustainability and economic stability of biorefinery (Mao et al., 2010; Van Heiningen, 2006).

There remains a need for a more thorough understanding of the impact of inhibitor removal from the biomass hydrolysate on the economic aspects of a commercial scale second-generation bioethanol plant.

The novelty of this manuscript lies within the techno-economic analysis of using an eco-friendly, non-toxic solvent to increase bioethanol production through both rate and yield enhancement. The recyclability of the extraction solvent promotes its sustainability which falls within the overall long-time goal of embracing second-generation bioethanol as viable energy source. As previous study suggested (in Chapter 4), adopting a liquid-liquid extraction system is an efficient technique to remove known fermentation inhibitors regardless of the biomass type and the species of fermenting microorganism. Though exact kinetics of the inhibition of acetic acid and other known inhibitors on the ethanol producing microorganisms requires

additional study (Athmanathan, Sedlak, Mosier, & Ho, 2010; David Humbird, National Renewable Energy, & Harris Group, 2011; Mohagheghi et al., 2014), the straight-forward, linear equations that have been used in these calculations are expandable for use at various flowrates, with different materials of constructions, a range of years of operation, and different compositions of feedstocks. The comparative analysis that has been incorporated into this systematic study clarifies the most important parameters that determine whether or not liquid-liquid extraction can be a viable addition to an existing biorefinery.

5.3 Materials and Methods

The basis for this study is derived from the NREL model (David Humbird et al., 2011) which simulates a 61 million gallon per year ethanol production plant from corn stover. The parameters related to the total capacity of the plant the flowrates and the revenue that were used in this set of calculations were extracted from this model.

The economic analysis of the proposed fermentation inhibitors removal system is performed using the cost breakdown algorithm (Turton, 2012). Cost estimations associated with chemical processing facilities are categorized into capital and operational or manufacturing costs. Incorporating a liquid-liquid extraction unit to remove the fermentation inhibitors, would alter the economy of the biorefinery mainly due to purchasing cost of new equipment (extraction column and the solvent recovery unit) and the extracting solvent. On the other hand, enhanced production of the bioethanol – through yield and rate– would increase the sale and overall flowrate of the plant. A six-tenth model was incorporated when necessary to adjust the

equipment pricing to the sizing relevant to the capacity of this biorefinery (Turton, 2012).

The sizing of the flash drum was estimated using the Aspen Process Economic Analyzer (Aspen Tech Inc., Burlington, MA) linked to the Aspen Plus ™ simulation file that was created to model the extraction and solvent recovery unit. Furthermore the Aspen Process Economic Analyzer was incorporated to use the data and predict the flash drum purchasing cost as well as the rest of the costs related to capital (installing, piping, etc.) and manufacturing (labor, maintenance, etc.) for this system. The detailed economic report is provided in Appendix D.

Flooding velocities calculations (Seader, Henley, & Roper, 1998) were used along with the known flowrates in the extraction column to estimate the cross sectional area of the extraction column. These sizing parameters and the assumed retention time were used in the tray column cost analysis (Peters, Timmerhaus, & West, 2003) for purchasing cost estimation.

To understand the impact of the ethanol specific production rate on the overall flowrate of the products, the fermentation rates were implemented in the reactor design kinetics equations. Cell growth, glucose consumption, and ethanol production rates were extracted from Pearl (1927) using the assumptions and coefficients from later studies (Athmanathan et al., 2010; Ghose & Tyagi, 1979).

The assumptions in this study are mentioned in the appropriate sections of the manuscript; detailed discussion of the assumptions can be found in Appendix C.

5.4 Results and Discussion

Economic evaluation of chemical process plants involve considering both capital and manufacturing costs associated with each step of the plant. Therefore, it was necessary to assume that incorporating a liquid-liquid extraction system into an existing biorefinery would alter the economic dynamic of the process in terms of column and solvent recovery units as the fixed and the solvent purchasing as the manufacturing cost, while profiting from the sale of additional ethanol produced from an increased throughput due to a higher fermentation rate. As a result, this study aims to investigate the following: purchasing cost of the extraction column, solvent initial cost and its recovery procedure, excess sale of ethanol, and impact of higher fermentation rate. Figure 5-1 shows the schematic diagram of a corn stover biorefinery and where the proposed system (in green) as implemented in the NREL system.

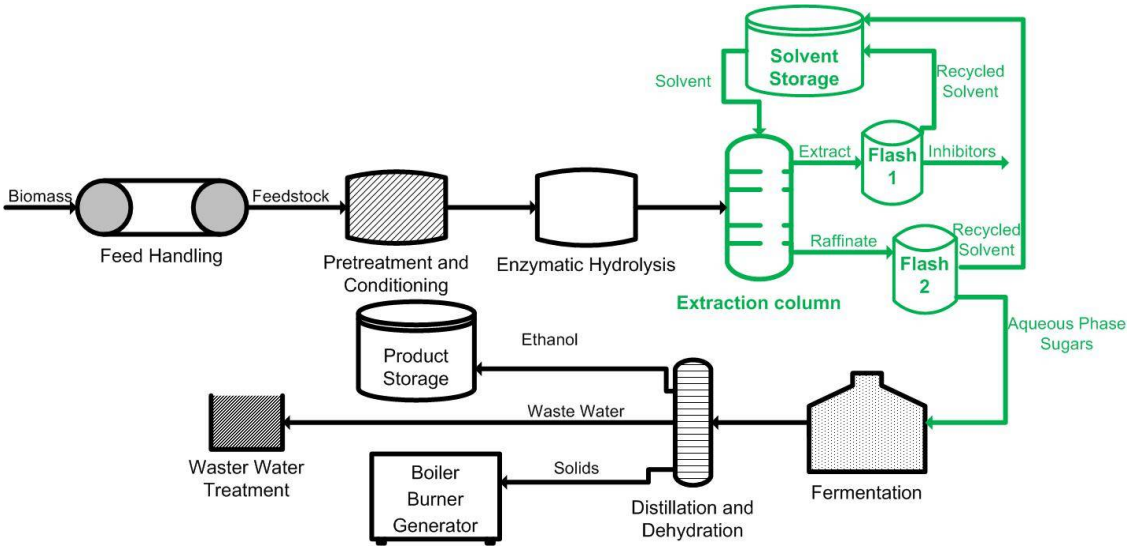

Figure 5-1: Schematic diagram of the main stages of corn stover biorefinery and where the solvent removal system is being incorporated. The unit operations and streams in green were the focus of this study. The unit operations in black were already present in the NREL Aspen Plus™ simulation files.

5.4.1 Cost of the extraction column

The major equipment for the LLE addition includes the flash drum and a tray column. At the industrial scale, the vacuum evaporation unit can be modeled as a flash drum. More detailed analysis includes piping and installation costs as well.

Flooding calculations (Seader et al., 1998) were performed using the aqueous and organic phase flowrates given by the NREL model. The cross sectional area of the LLE column was determined from the flooding calculations and preferred column type (sieve plate tray column). By varying the retention time and the flowrates, the length of the column was also approximated. The sizing characteristics implemented in the empirical models for column cost estimations (Turton, 2012), adjusting for the

cost year index, and the materials of construction suggested by the NREL study are used for column cost calculation. Figure 5-2 depicts the linear relationship between the flowrate of the organic solvent and the cost of the extraction column. The line on the plot specifies the desirable partition coefficient (K_{AA}=the ratio of the acetic acid concentration in organic phase over the aqueous phase) of 3.5. At solvent to feed volume ratio of 1, purchasing cost for the extraction column is $970,000 which accounts for less than 1 % of the total equipment cost of the biorefinery plant.

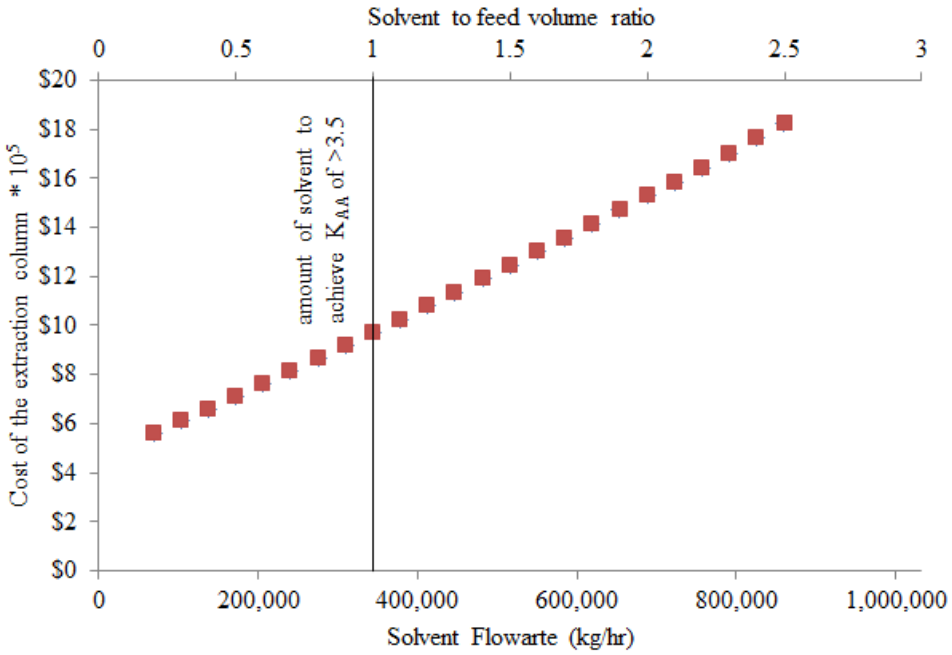

Figure 5-2: The purchasing cost of the extraction column as a function of the flowrate and the volume ratio of the solvent stream. The line on the plot shows the minimum solvent flowrate needed to achieve acetic acid partition coefficient (K_{AA}) of 3.5 which makes the LLE process efficient to remove the acetic acid from biomass hydrolysate.

5.4.2 Initial and recovery cost of the solvent

Previous study suggested that simple vacuum evaporation would recover ethyl acetate (Chapter 4). Two flash drums were implemented following the extraction column connected to the two exiting streams from the extraction column. The purpose of the first flash drum, connected to the extract stream, was to separate the acetic acid from ethyl acetate to enable the recycle of the solvent within the extraction column. A second flash drum was connected to the raffinate stream and was used to evaporate ethyl acetate from the aqueous phase – being pumped to the fermentation reactor – below its inhibition point.

The Aspen Process Economic Analyzer, along with ASME design code, was used to estimate the total cost of these flash drum at $490,202 for the conditions specified in the simulation. The total cost for the evaporation module was estimated to be $980,404.

Initial purchasing cost of the solvent is a function of the flowrate of the needed solvent as well as the retention time in the column. Figure 5-3 shows the effect of solvent flowrate (and the solvent to feed ratio) on partition coefficient of acetic acid. Conclusively more than 300,000 kg/hr flow of solvent is essential to obtain satisfactory extraction performance (K_{AA} greater than 1) to remove enough of acetic acid below its inhibition threshold (2 g/L of acetic acid significantly reduces the fermentation rate.

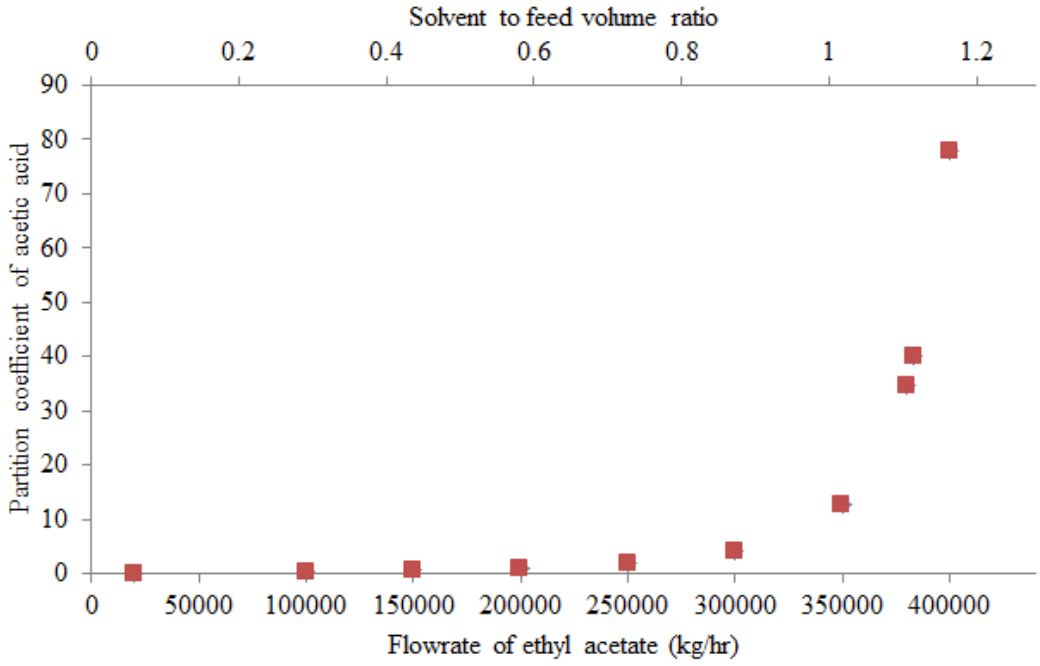

Figure 5-3: Solvent flowrate and solvent to feed volume ratio effects on the partition coefficient of acetic acid. At flowrates above 300,000 kg/hr (=0.87 solvent to feed volume ratio) K_{AA} starts to increase exponentially (starting from K_{AA}=4). A flowrate of 350,000 kg/hr (corresponding to solvent to feed volume ratio of 1) is the point where K_{AA} equals 12.8 and makes the liquid-liquid extraction efficient to remove the acetic acid.

The extent of the importance of the purity of the solvent on its performance in extracting the acetic acid is demonstrated in Figure 5-4. Ethyl acetate will dissolve acetic acid in each run through the extraction column and if recycled without removing acetic acid, its extractability drops significantly. Figure 5-4 shows the significant impact of acetic acid present in the solvent stream on the acetic acid partition coefficient in the extraction column; any amount more than 1.2% wt. of acetic acid drops the partition coefficient below one which makes the extraction

column completely inefficient. Only at 0.2 wt. % and below of acetic acid the partition coefficient gets above 3.6.

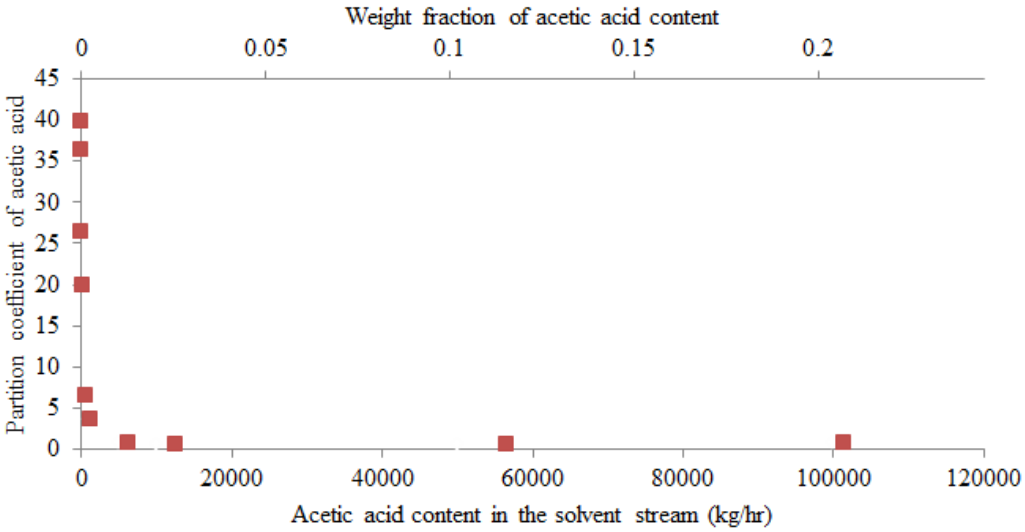

Figure 5-4: Acetic acid content and its corresponding weight fraction in the solvent stream impact on the partition coefficient of acetic acid through the extraction column. Acetic acid content in the solvent stream has a very strong negative effect on K_{AA}. At 0.2 wt. % the acetic acid partition coefficient is 3.6 and at 1.2 wt. % (5000 kg/hr) the partition coefficient drops to less than 1 (0.8).

Figures 5-3 and 5-4 clarify that at sufficient flowrate and purity, ethyl acetate can remove the necessary amount of acetic acid from the biomass hydrolysate. The solvent stream entering the extraction column should have a flowrate above 300,000 kg/hr with less than 0.2 % wt. acetic acid content to achieve an acetic acid partition coefficient of ≥ 3.6 during the extraction process.

Vapor-liquid equilibrium predicted using the UNIFAC property methods set – was used to determine the optimum temperature for the flash drum at 10 % vacuum (8KPa) to obtain reasonable relative volatility of acetic acid over ethyl acetate for the

first flash drum and ethyl acetate over water for the second flash drum. The relative volatilities acetic acid and water were found to be 6.15 and 250, respectively at 14.8 °C and 41 °C. At the optimized conditions in flash 1, 100 wt. % of ethyl acetate is collected in the vapor phase exiting the unit, gets liquefied at ambient pressure, and then recycled to solvent storage. In flash 2, 99.6 wt. % of ethyl acetate, which was dissolved in the aqueous phase, evaporates. This decreases the ethyl acetate concentration below its inhibition in the stream that is entering the fermentation reactor (as shown in Chapter 4); while the vapor is being recycled to the solvent storage tank. These two flash drums collect over 99.9 wt. % of ethyl acetate in each run (3499000kg/hr from a total of 350000 kg/hr). Figure 5-5 illustrates the recovery of ethyl acetate from flash drums 1 and 2.

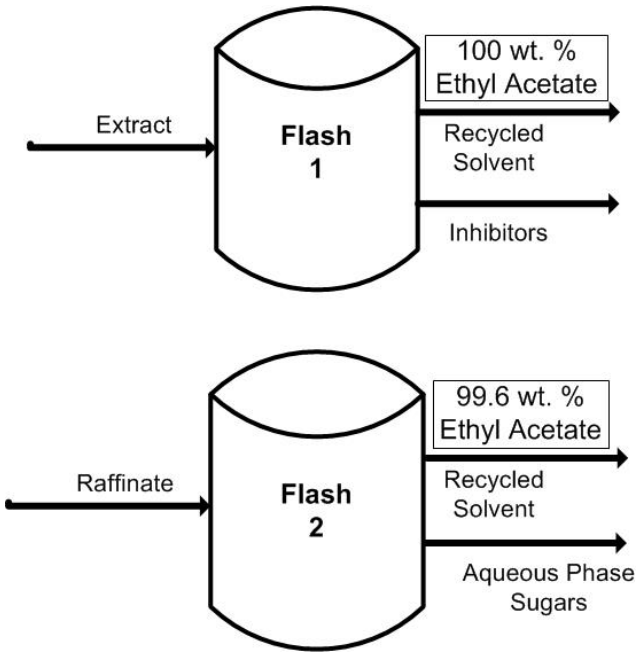

Figure 5-5: The recovery of the solvent from the two flash drums. The percentages on the recycled solvent streams indicate the percentages of ethyl acetate recovered in the vapor stream compared to the amount that entered the drum.

The annual purchasing cost of the solvent is a linear function of solvent recyclability. 99.9 % recovery in each run means that 0.1 wt. % (350 kg/hr) of ethyl acetate from the solvent storage has to be added to the solvent stream at each run and this amount keeps accumulating over the course of the year that the plant is continuously running. The amount of the solvent that has to be purchased annually to replace the lost solvent was added up to $7,800,000. When 99.9 % of the solvent is being recovered at each run of the extraction, the annual purchasing cost of the solvent ($7,800,000) adds 5.9 % to the total estimated manufacturing cost of the plant calculated by the NREL model. Figure 5-6 further demonstrates the annual cost of the solvent increases as percent of the recovered solvent decreases and clarifies the importance of achieving high recovery of ethyl acetate followed by the extraction unit.

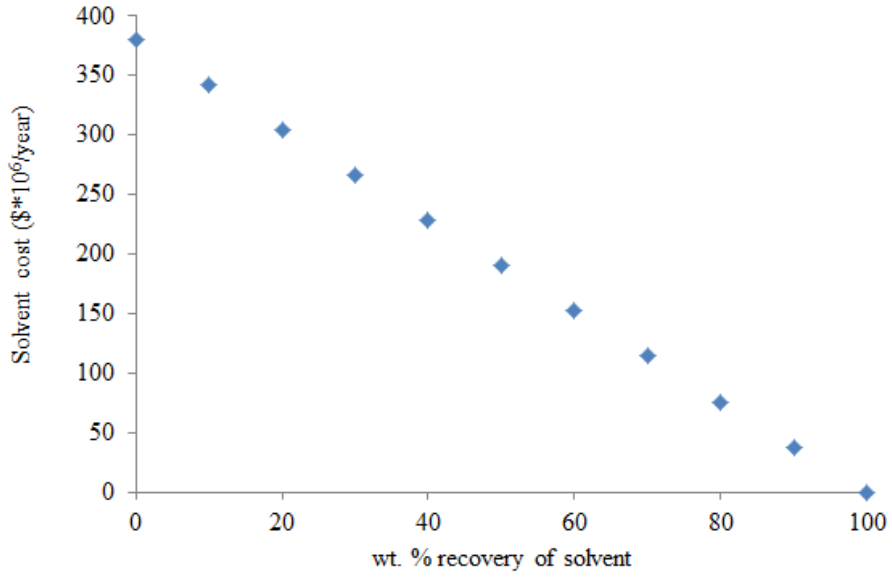

Figure 5-6: The purchasing cost of the solvent per year as a function of the recovery percentage of the solvent. The purchasing cost of the solvent increases linearly with decrease in ethyl acetate recovery.

5.4.3 Excess sale of ethanol

Various studies have been conducted to recover ethanol from fermentation broth. Pervaporation is a well-studied method (Bolto, Hoang, & Xie, 2011; Jin et al., 2011) with optimized operational conditions (Peng, Shi, & Lan, 2011). Sorption (Kim, Hendrickson, Mosier, Ladisch, & Hilaly, 2011) and liquid-liquid extraction (Egan, Lee, & McWhirter, 1988) were also viable for ethanol dehydration techniques. The NREL model incorporated a distillation column for initial product recovery, so for the sake of comparison, same was applied in this study.

Energy requirements to recover and purify ethanol increase as more ethanol is produced. The distillation process to recover ethanol produced in a sugar fermentation unit was modeled and it was determined that the steam required per unit of ethanol is a function of the ethanol content present in the broth (Zacchi & Axelsson, 1989).

Using the steam tables (Harvey, 1998), to find the enthalpy (kJ/kg) of the steam at the conditions of the steam generator of the NREL model – 125 psig and 164 °C –, and the unit price of the steam (Turton, 2012) the dollar amount of steam needed for ethanol distillation can be obtained. The net changes in ethanol sale was estimated based on the beer flowrate leaving the fermentation reactor and the change in its ethanol content times the MESP (Minimum Ethanol Selling Price) minus the steam cost to recover that amount of ethanol. It was assumed that the other costs associated with distillation, such as reboiler, condenser, and pump sizes, as well as the corrosion rate, were not going to change in the range of 0.2 to 12 % wt. of ethanol content.

Figure 5-7 displays the steam price and the ethanol sale – MESP of $2.15 – in one plot and it illustrates that separation costs were negligible when compared to the sale increase from higher bioethanol concentration.

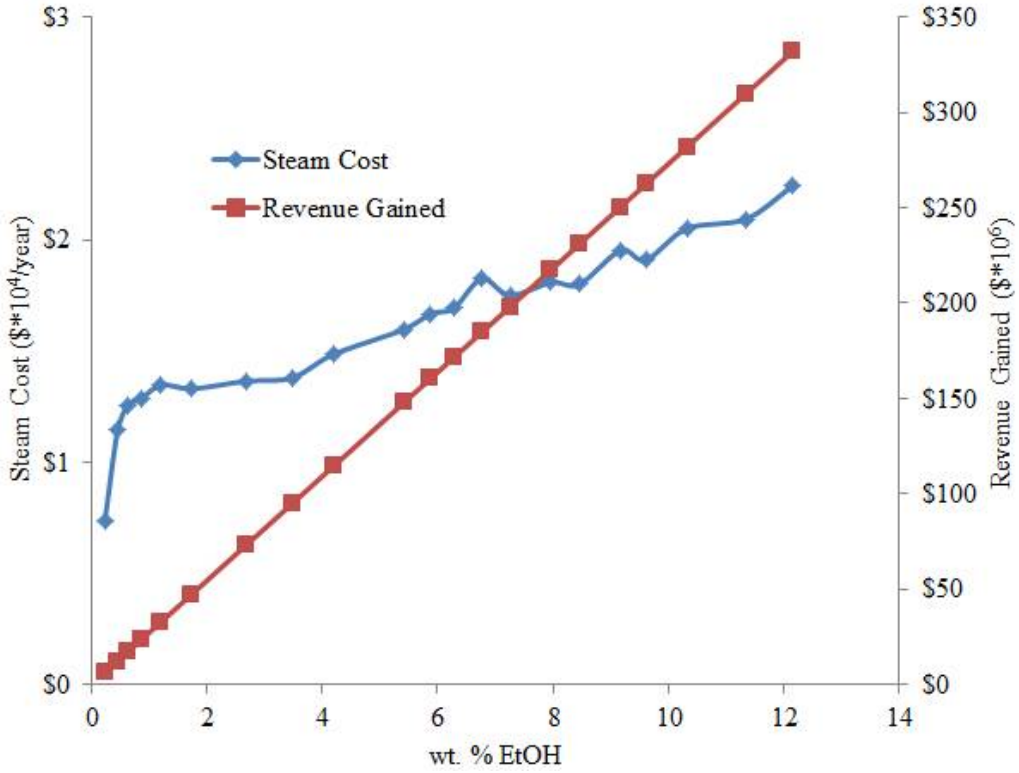

Figure 5-7: The effect of the ethanol content in the fermentation broth on the cost of steam to recover the ethanol as well as its impact on the net changes in the revenue of the biorefinery. The cost of the steam used to recover the ethanol from the fermentation broth was negligible compared to the profit gained by the excess ethanol sale as the net change in revenue line has a linear relationship with the wt. % of ethanol despite increasing amount of steam needed to recover the ethanol.

5.4.4 Impact of higher fermentation rate

An improved fermentation rate results in shorter residence times in the bioreactor which translates into either smaller bioreactor size or higher flowrate of the pre-fermentation broth. Higher total flowrate of the fermenting streams will cause higher annual production.

Material balance on a batch reactor leads to Equation 1 (Levenspiel, 1979) where t_R is the residence time at the reactor, N_{i0} is the starting molar content of the i compound in the mixture, X_i is fraction of the i compound, V_R is the reacting volume of the vessel, and r_i is the kinetic rate of compound i.

$$t_R = N_{i0} \int_0^{X_{ie}} dX_i \Big/ -V_R r_i \qquad (1)$$

For ethanol anaerobic fermentation reaction, the glucose consumption rate and ethanol production rate were estimated with Equations 2 and 3 respectively. In these equations, G is the glucose, P the product (ethanol), and C the cell mass concentrations. V_{max} is the maximum rate of the reaction, $P_{max,g}$ is the maximum tolerable concentration of ethanol by the microorganism, $Y_{P/G}$ is ratio of production over consumption, and n is an arbitrary number to fit the inhibition term (Ghose & Tyagi, 1979).

$$dG/dt = -v_{max} C\left(1 - P/P_{max,g}\right)^n \qquad (2)$$

$$dP/dt = -Y_{P/G}\, dG/dt \qquad (3)$$

Under the designed NREL conditions for fermentation, the inhibition effect of ethanol was negligible and therefore the ethanol production rate equation was not a function of ethanol concentration. Substituting r_i in Equation 1 with Equation 3, shows that the retention time has an inverse linear relationship with the rate of the reaction. Hence it is safe to conclude that since the retention time decreases with higher production rate, the flowrate will increase linearly with higher production rate.

Therefore 14 % of higher ethanol production rate (as shown in Chapter 4) will increase the fermentation broth flowrate by about 14 % as well and this will translate to 514,203 kg/hr of beer exiting the bioreactor instead of 451,055 kg/hr and 20 M$ /year of excess ethanol (about 12 % increase) at 5.4 % wt. of ethanol content.

5.4.5 Sensitivity Analysis

Breakeven calculations (Sen, 2012) to obtain MESP showed the significant impact of solvent recovery percentage. The results indicate that the at the conditions of this study, 11% higher ethanol yield lowers the MESP by $0.09, however the rate increase of 14% reduces the MESP by $0.16 per gallon. Combining the effect of yield and rate increase has even a more significant drop in the MESP of $0.35 compared to MESP of the NREL model.

Table 5-2 summarizes the costs and profits associated with the LLE system and it shows that the most pronounced cost imposed by liquid-liquid extraction system on the overall economic balance of the plant is introduced by the purchasing cost of the

solvent. Therefore sensitivity analyses were performed on the impact of the solvent cost on the MESP.

Table 5-2: The costs and profits associated with inserting a liquid-liquid extraction system in a corn stover biorefinery to remove acetic acid from pre-fermentation broth

	Sources		Amount
Costs	Fixed	Column	$ 970,000
		Flash drums	$ 980,404
	Variable	Solvent	$/year 7,800,000
Profits	Improved ethanol yield		$/year 18,200,000
	Improved flowrate		$/year 21,000,000

Sensitivity analysis is performed to show how MESP changes with yield and/or rate increase in the fermentation process with respect to the cost of purchasing the solvent per year. The results in Figure 5-8 indicate that solvent recovery has a prominent impact on the MESP at all of the three different scenarios. In scenarios 1 and 2, the fermentation improves by 11 % yield and 14 % production rate increase respectively and the results were very close with the first scenario being marginally competitive with NREL-calculated MESP at lower than 38×10^6 $/year solvent cost. Scenario 3 however combines the 11 % yield and 14 % production rate improvements to breakeven point MESP calculation and it shows significant drop in the MESP. The three scenarios tend to merge at lower solvent annual cost and diverge at higher solvent annual cost. Adapting liquid-liquid extraction system lowers the MESP from $2.15/gallon when the annual purchasing cost of the solvent is 38×10^6 and lower.

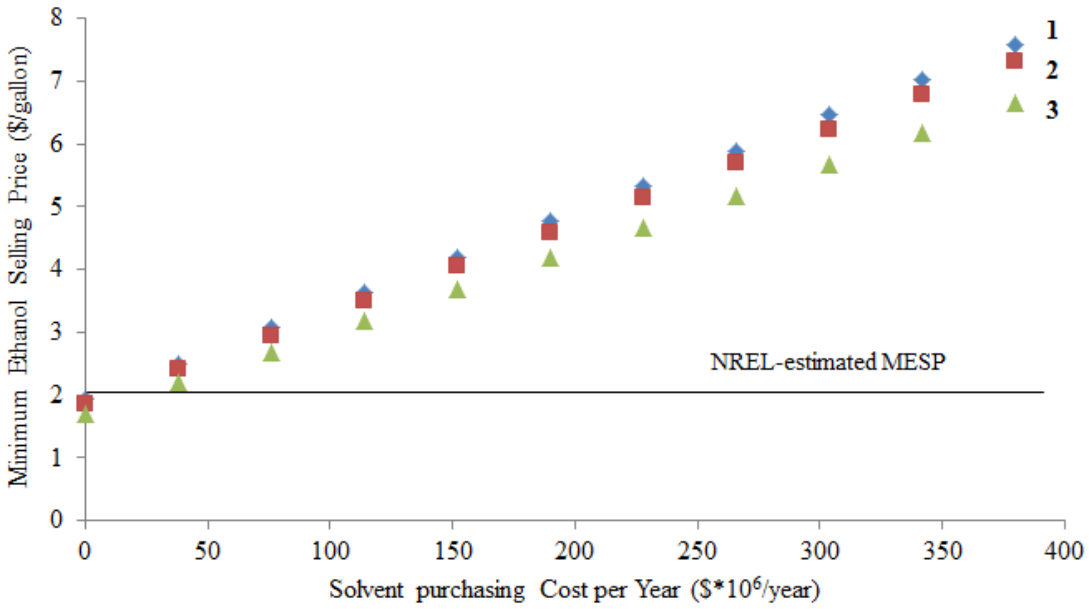

Figure 5-8: Sensitivity analysis on MESP with varying solvent annual purchasing cost for three scenarios, 1) 11 % increase in the ethanol yield through the fermentation, 2) the 14 % increase in the ethanol production rate, and 3) both ethanol yield and production rate improvement implemented together. The line on the graph indicates the MESP estimated by NREL-developed model.

5.5 Conclusion

Side-by-side comparison of the two major costs with two major sources of excess profit leads to concluding that solvent cost is primary concern for adapting this fermentation inhibitors removal system into an existing corn stover biorefinery.

The fixed costs that were being added to the capital cost of the plant was less than 1 % and the purchasing cost of the solvent added 5.9% to the total manufacturing cost, the profit gained by the excess ethanol sale outweighs the manufacturing and capital cost.

In this study, major fixed expenses (i.e. land acquisition) and variable expenses (i.e. piping and labor costs) were assumed to be included in the existing biorefinery. It can be concluded that adaptation of this system as fermentation inhibitors removal technology is greatly depended on the recovery stage of the solvent. When high percentage of solvent can be recovered at low cost then liquid-liquid extraction to remove acetic acid is an economically viable choice to add to a corn stover biorefinery plant. Sensitivity analysis indicated that MESP greatly decreases when the effects of ethanol yield and production rate enhancements, through liquid-liquid extraction, are combined in the system.

List of Abbreviations

NREL: National Renewable Energy Laboratory

MESP: Minimum Ethanol Selling Price

LLE: Liquid-Liquid Extraction

UNIFAC: UNIQAC (Universal Quasi-Chemical) Functional-group Activity Coefficient

AA: Acetic Acid

DDGS: Distiller's Dried Grain with Solubles

CHAPTER 6. SUMMARY AND CONCLUSION

6.1 Restatement of the objectives

The objectives stated in Chapter 1 were addressed throughout this dissertation. The three main areas that were studied include:

6.1.1 Solvent selection studies

This study demonstrated that shorter chain esters, namely ethyl acetate and butyl acetate, were performing well in extracting acetic acid from corn stover hydrolysate.

6.1.2 Fermentation performance

The tests proved that solvent recovery is a necessary tool to eliminate the toxicity effect of the organic solvent. It was shown that when acetic acid is removed from biomass hydrolysate, fermentation performance enhances in terms of yield, final concentration, and specific production rate of ethanol.

6.1.3 Techno-economic analysis

Through this analysis it was clear that the purchasing cost of the solvent is the biggest contributor to the cost estimation of this system. Achieving high solvent recovery is essential to lower the MESP in the process.

6.2 Chapter summaries

Chapter 1 introduced bioethanol as the sustainable resource for liquid transportation fuel and stated the importance to improve the technology to produce bioethanol from lignocellulosic biomass. This background and the challenges for overcoming the inhibition effect of the pretreatment degradation products were discussed in this chapter and led to the objectives that motivated this research.

Chapter 2 gave an overview of the state of the art of the methods and techniques that have shown to mitigate the inhibition effect of acetic acid on different strains of *Saccharomyces cerevisiae*. The pros and cons associated with each of the separation, gene modification, and adaptation methods were evaluated. Literature review lead to discovering the gap of knowledge that exists in optimizing and systematically assessing liquid-liquid extraction for the purpose of fermentation inhibitors removal.

Solvent selection results were discussed in Chapter 3. The criteria that were implemented to narrow down the selection of organic solvents in the Aspen Plus simulation model seemed to be in good agreement with the laboratory experiments that were conducted to further test the selected solvents. The nine solvents that were broadly studied have very low miscibility with water and low affinity to the fermenting sugars.

Fermentation studies in Chapter 4 revealed the significant impact of liquid-liquid extraction on the performance of *S. cerevisiae* NRRL Y-1546. In order to pinpoint the level of toxicity of ethyl acetate in presence of acetic acid, synergistic inhibition

experiments were conducted. Simple vacuum evaporation was shown to be effective in removing the organic solvent from the broth. Improvements in yield, ethanol content, and ethanol production rate proved the significant positive effect of liquid-liquid extraction on bioethanol production process.

Techno-economic analysis in Chapter 5 gave a prospective on the economic aspect of incorporating the liquid-liquid extraction system in a corn stover biorefinery plant. The specifics for this study were extracted from an earlier Aspen Plus™ model of a 61Mgal/ year bioethanol production plant with corn stover as feedstock. Capital and manufacturing costs that are associated with this system are mainly related to the extraction column and the purchasing cost of the solvent. Sensitivity analysis of the impact of the fermentation enhancement and solvent recovery on MESP (Minimum Ethanol Selling Price) indicated that high percentage of the solvent recovered and making use of both yield and rate improvements are necessary to significantly decrease the MESP.

6.3 Recommendations for future work

This work can be extended in two major categories:

1. Exploring science behind organic solvent inhibition:

It was shown that ethyl acetate and butyl acetate have severe toxicity impact on NRRL Y-1546, however the inhibition and toxicity mechanism of these esters at different pH is not known. Complete genomic studies on acetic acid effect on many different types of ethanol producing microorganisms at different condition have

already been published; however the exact inhibition effect of the other organic solvent is an area that needs further investigation. This study will be helpful to have a more detailed and scientific understanding on the impact of liquid-liquid extraction with organic solvents on the bioethanol fermenting yeasts.

2. Engineering studies on scale-up and detailed economic and energy balance of the liquid-liquid extraction and solvent recovery system:

The fermentation performance at the pilot scale must be verified improve the accuracy of the assumptions for commercial scale outcome. Implementing pilot scale fermentation results in a detailed techno-economic study can result in a better understanding of the changes in fermentation parameters after removing acetic acid with ethyl acetate. As examples: if glucose and xylose consumption rates change, what is the effect on total production rate? Is ethanol production a linear function of the rate changes? How will the co-products increase in volume? How does the un-recycled solvent change the waste treatment of the facility?

Life cycle assessment through energy balance and carbon footprint studies of the system within the boundaries of biorefinery is other latitude of this project that can be further developed in later studies. Both pilot scale and life cycle assessment results will help move forward the developing second-generation biofuel industry.

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APPENDICES

Appendix A Schematic Diagram of the Parr Reactor

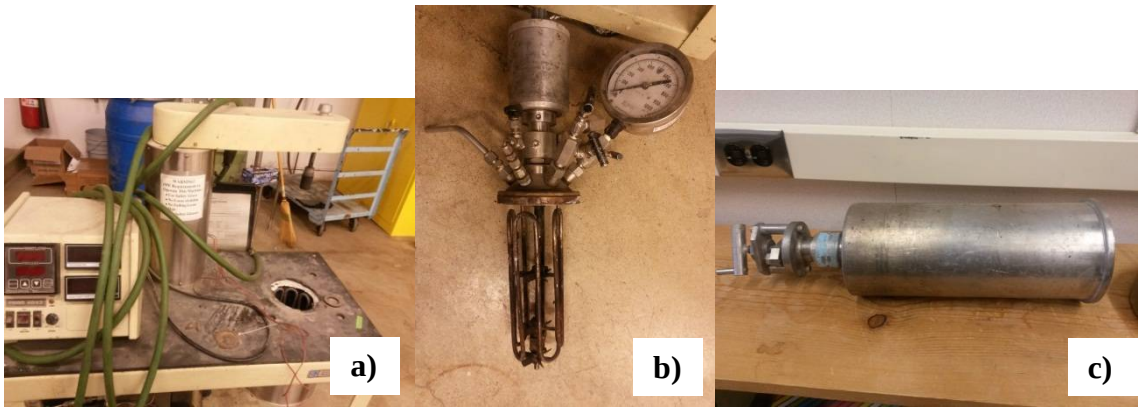

Figure A-1: The components are the Parr reactor (Model 4530, Parr Instrument Company, Moline, IL) a) the controller, the stand, and the heating element, b) the coil and magnetic stirrer, and c) the 2 liter vessel

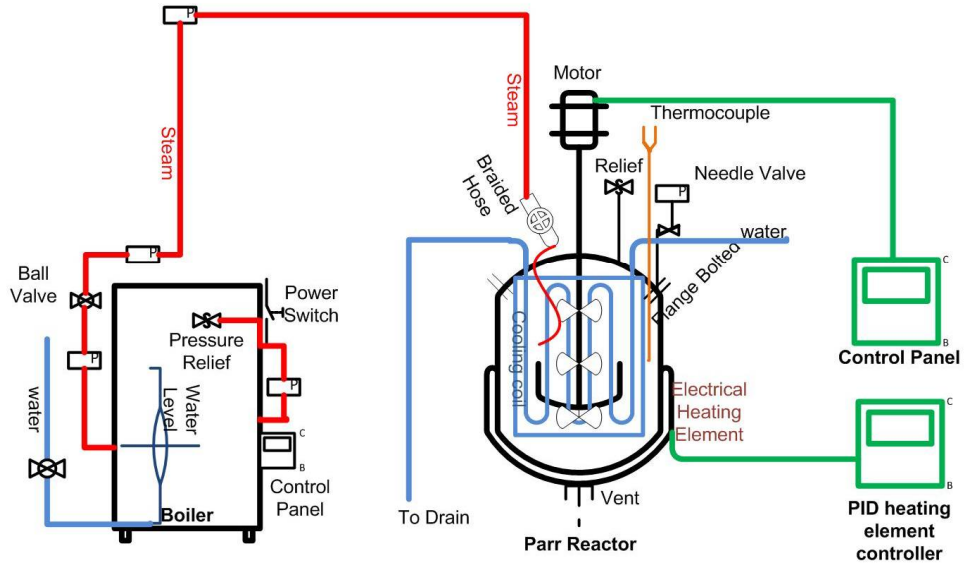

Figure A-2: The schematic diagram of the Parr reactor and the Sussman boiler

Appendix B Inhibition Effect of Ethyl Acetate on glucose consumption by NRRL Y-1546 in Bioethanol Fermentation

The growth of cell yeast follows Equation 1(Pearl, 1927):

$$dC/dt = \mu C(1 - C/C_{max})(1 - P/P_{max,grow})^{n} \quad \text{(B-1)}$$

Where C is the cell mass concentration, C_{max} is the maximum concentration of the cell, P is the product concentration, $P_{max,grow}$ is the maximum concentration of product in which the cell growth is possible, n is the Levenspiel constant of the inhibition term, and μ is the specific growth rate of the cell.

Ghose et al. (1979) estimated n as 1 and $P_{max,grow}$ as 87 g/L for the bioethanol fermentation.

The substrate consumption rate therefore can be studied with Equation 2.

$$dS/dt = -(v_{max}S/K_m + S)(1 - P/P_{max})^{n}C \quad \text{(B-2)}$$

Where S is the substrate concentration, v_{max} is the maximum consumption rate, and K_m is the Monod constant which represents the rate when the substrate consumption is half of the initial value.

In bioethanol fermentation the substrate is glucose. In this case n is estimated to be 1 (Brown, Oliver, Harrison, & Righelato, 1981) and P_{max} to be 140 g/L (Athmanathan,

Sedlak, Mosier, & Ho, 2010). Considering the low K_m in glucose consumption, 0.315, (Maiorella, Blanch, & Wilke, 1983) compared to the high initial amount of glucose (100 g/L in this work), Equation 2 can be replaced by Equation 3 because $K_m << S$.

$$dG/dt = -v_{max} C(1 - P/P_{max,G})^n \quad \text{(B-3)}$$

In this case the production rate of ethanol is linearly related to the glucose consumption rate with yield constant ($Y_{P/G}$) (Equation 4).

$$dP/dt = -Y_{P/G} \, dG/dt \quad \text{(B-4)}$$

In this work the ethanol final concentration reaches 50 g/L at 100 % yield, therefore it is safe to assume that the ethanol inhibition term in Equation 3 is negligible. The fermentation experiments were started with an initial cell mass concentration of 1 g/L which makes for minor the cell mass concentration gradient over time. Therefore for this study the glucose consumption rate can be modeled with Equation 5.

$$1/C \, dG/dt = -v_{max,G} \times (\text{Inhibition term of ethyl acetate}) \quad \text{(B-5)}$$

The fermentation data with no inhibition was used in the built-in solver tool in Microsoft Excel to estimate $v_{max,G}$ for this yeast strain, and it was calculated at 4.003 g/g/h (data not shown).

In order to find the inhibition term in Equation 5, the specific glucose consumption rate of each fermentation experiment was calculated by fitting a first order linear equation to the data after the lag phase and before the plateau. The slope of this linear equation

divided by the cell mass concentration equals the specific consumption rate of glucose at each condition. Plotting the natural logarithm of the rates versus the ethyl acetate (Figure B-1) concentration shows an apparent correlation.

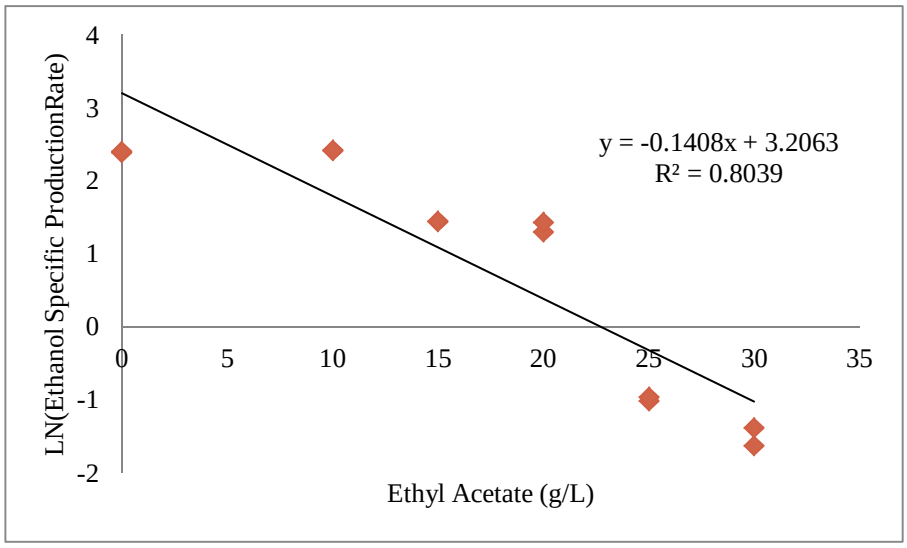

Figure B-1: The linear relationship between the natural logarithm of specific rate of glucose consumption and ethyl acetate concentration present in the media with no other inhibition

This linear equation can be incorporated in Equation 5 to showcase the ethyl acetate impact on the specific rate of glucose consumption in bioethanol production by NRRL y-1546.

$$1/C \; dG/dt = 98.82 \times e^{-0.1408 \times \text{Ethyl acetate concentration}(\frac{g}{L})} \qquad \text{(B-6)}$$

Appendix C Equations and Tables Used in the Cost Estimation and Analysis

Part I: Mass balance and unit conversion factors

Initial cost of the solvent = unit cost of the solvent ($/kg) ×volume ration of the solvent to feed ×flowrate of the stream to the fermenter (kg/hr) ×retention time of the extraction column (hr)

The cost of the solvent per year = (initial cost of the solvent/years of operation of the plant) + initial cost of the solvent ×(1-fraction of the recovered solvent per run) ×runs per year

The steam price to recover the ethanol ($/year) = unit steam price ($/kJ) × steam needed per unit ethanol (kJ/kg) (Zacchi & Axelsson, 1989)×ethanol production (kg/year)

Part II: Constants, equations and tables used from the engineering textbooks

Unit steam price (Turton, 2012) = 14.05 $/GJ

- Extraction Column Cross Sectional Area Estimation (Seader, Henley, & Roper, 1998)
- Unlike vapor-liquid columns, designing liquid-liquid extraction columns are complex and not as straightforward. One way to estimate the cross sectional area of an extraction column is by estimating the sum of flooding conditions as 50 % of the total actual superficial phase velocities.

Table C-1 is reconstructed based on table 8.6 from Seader *et al.* and is a useful tool to select the appropriate total superficial phase velocity depending on the column type.

Table C-1: The range of characteristics for different extraction columns (Seader et al., 1998), HETP refers to the "Height Equivalent to Theoretical Plate" and U_D+U_C refers to total superficial phase velocity

Extractor Type	1/HETP, m^{-1}	U_D+U_C, m/h
Packed column	1.5-2.5	12-3
Pulsed packed column	3.5-6	17-23
Sieve-plate column	0.8-1.2	27-60
Pulsed-plate column	0.8-1.2	25-35
Schiebel column	5-9	10-14
RDC	2.5-3.5	15-30
Kuhni column	5-8	8-12
Karr column	3.5-7	30-40
RTL contactor	6-12	1-2

- Cost of the extraction column

The model fitted to the equipment costs by Turton (2012) was used to estimate the purchasing cost of the extraction column based on its size. In Equation C-1, C_p^0 is the cost of the equipment at ambient pressure and carbon steel as constructing material, A is the size of the equipment, and K_1, K_2, and K_3 are empirical constants fitted to specific equipment type and its description.

$$\text{Log}_{10}C_p^0=K_1+K_2\log_{10}(A)+K_3[\log_{10}(A)]^2 \qquad \text{(C-1)}$$

The constants for the tray and packed towers are K_1=3.4974, K_2=0.4485, and K_3=0.1074.

Equation C-1 is normalized for 2001 prices therefore the cost indices of 2011 and 2001 (from table 7.4 of Turton) was used to adjust the calculated price with equation C-2, since the NREL model was also developed in 2011.

$$C_2=C_1(I_2/I_1) \qquad \text{(C-2)}$$

In Equation C-2, C_2 and C_1 are the cost of the equipment in year 2 and 1, and I_2 and I_1 are the cost indices of these years respectively.

Part III: Properties that were extracted from the NREL model (Humbird, National Renewable Energy, & Harris Group, 2011)

- Years of the operation of the plant = 30 years
- Operating hours in year = 8410 hours
- MESP (Minimum Ethanol Selling Price)= $2.15
- Flowrates

Stream 501 (the beer leaving the fermentation unit) = 451055 kg/hr

Stream 301(the stream leaving the pretreatment unit to the bioreactor) = 383574 kg/hr

- Materials of construction

The fermenters are assumed to be made of 304SS.

- Physical conditions of the streams, steams, and blocks

The steam is produced at the steam generator at 900 psig and 850 °F; 35 % of this steam is being used for distillation at 125 psig and 164 °F. Using the steam table (Harvey, 1998) the enthalpy of this steam is 2770 kJ/kg.

There are five fermenters in this simulation each 950,000 gallon capacity with 36 hours of residence time.

- Capital and manufacturing cost breakdown

Total installed equipment cost of this plant is $232,000,000; Table C-2 gives the detailed list of the equipment cost. Total capital investment is estimated to be $422,500,000. Total manufacturing cost per year of operating the biorefinery is $131,500,000 per year and Table C-3 lists the sources of these costs.

Table C-2: The capital cost of the corn stover biorefinery plant ($)

Parameter	Value
Pretreatment	29900000
Naturalization/conditioning	3000000
Saccharification & fermentation	31200000
On-site enzyme production	18300000
Distillation and solid recovery	22300000
Wastewater treatment	49400000
Storage	5000000
Boiler/turbogenerator	66000000
Utilities	6900000

Table C-3: The manufacturing cost of the corn stover biorefinery plant ($/year)

Parameter	Value
Feedstocks + Handling	45200000
Sulfuric Acid	1500000
Ammonia	4000000
Glucose	11800000
Other raw materials	7900000
Waste disposal	1500000
Net electricity	-6600000
Fixed costs	10700000
Capital depreciation	13400000
Average income tax	7500000
Average return on investment	34600000

Table C-4: The economic parameters used in MESP calculations

Parameter	Value	Unit
Total operating cost	95,064,356	$/year
Electricity revenue	6,600,000	$/year
Discount Rate	10%	-
Total project investment	422,500,00 0	$
Tax Rate	35%	-
Equipment life span	30	year
Depreciation period	7	years
Depreciation cost	13,400,000	$/year
Return on investment	34,600,000	$

Part IV: The rate of reactions in batch reactors

Using the material balance in a batch reactor and assuming well-mixing and uniformity at all time results in Equation C-3 and C-4, which at constant volume they can be further rearranged to Equation C-5. (Levenspiel, 1979)

Input-output=accumulation + disappearance (C-3)

$$0 = -dN_A/dt = (-r_A)V \quad \text{(C-4)}$$

$$t = -\int_{C_{A0}}^{C_A} dC_A/-r_A \quad \text{(C-5)}$$

In these equations, r_A is the reaction rate of compound A, N_A is the molar amount of A, C_A is the concentration of A, C_{A0} is the initial concentration of A, V is the total reactor volume, and t is time. Appendix B includes the fermentation reaction rate derivations and assumptions.

Part V: The Aspen Plus ™ model

Figure C-1 shows the PFD (Process Flow Diagram) of the Aspen Plus ™ simulation that modeled the acetic acid extraction and solvent recovery system. This model was also used to perform the sensitivity analyses that were reported in Chapter 5.

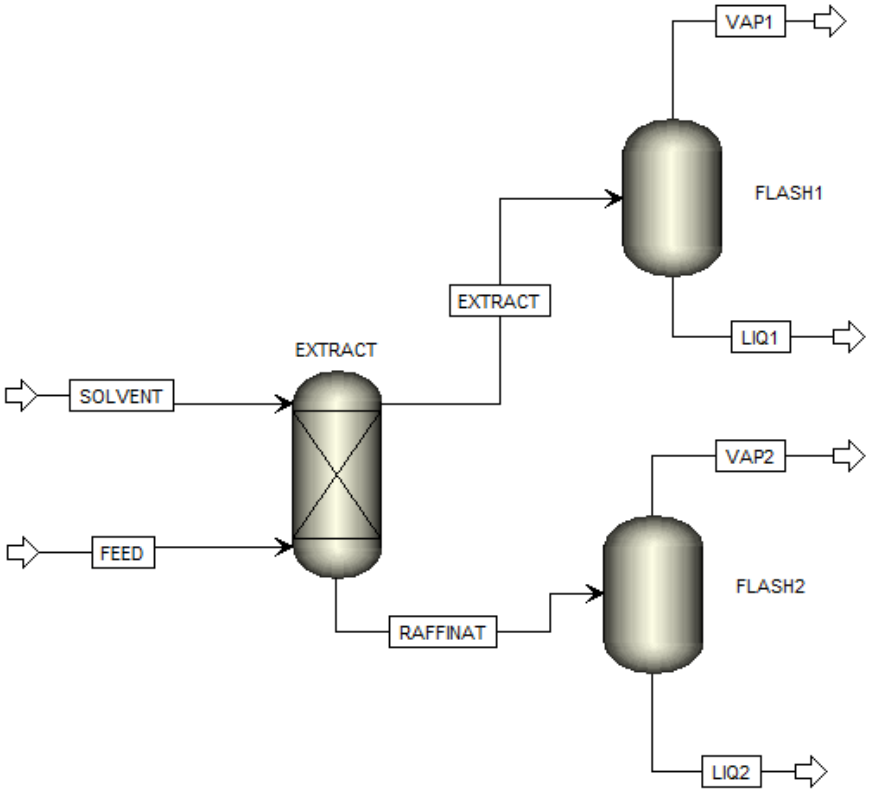

Figure C-1: PFD of the liquid-liquid extraction and flash solvent recovery system

In this model the "Extract" block is the extraction column with 30 trays and operating at ambient pressure and 25 °C. "Flash1 and 2" refer to the flash drum used to recover the solvent from both raffinate and extract streams at 40 °C and 60 mmHg – Chapter 4 –. "Solvent" and "Feed" streams enter the column at the first and last tray at 25 °C. The

property method of choice for this simulation was UNIF-LL. For the purpose of vapor-liquid equilibrium data generation the property method was changed to UNIFAC.

Part VI: Minimum Selling Price of Ethanol (MESP) calculation

These equations are derived from Sen et al. (2012) selling price calculations. At a breakeven point the total revenue and total cost are equal. We can break down the total revenue to biofuel revenue (BR) and the revenue from electricity sale (ER). This total equals the summation of operating cost (OC), return on investment (ROI), and income tax (IT) as shown in Equation C-6.

$$BR+ER=OC+ROI+IT \qquad \text{(C-6)}$$

In this equation the income tax is defined as the multiplication of the tax rate (TR) on the total revenue minus the total cost. Equation C-7 is showing this relationship.

$$IT=TR\times(BR+ER-OC-DC) \qquad \text{(C-7)}$$

These equations make it possible to estimate the amount of the biofuel revenue, using the total capacity of the biorefinery it is possible to estimate the per gallon price of ethanol.

The data that were extracted from the NREL-developed model for MESP calculations are presented in Table C-4.

Appendix D Labor and Maintenance Costs

The labor and maintenance cost for different sections of the separation system as estimated by the Aspen Process Economic Analyzer are presented in Table D-1.

Table D-1: The breakdown cost of the separation system in US dollars

Prime Contractor	Labor Cost	Maintenance Cost	Total Cost
Equipment	11,602	478,600	490,202
Piping	28,191	101,301	129,491
Civil	66,488	85,199	151,687
Steel	5,282	28,696	33,978
Instrument	11,283	322,847	334,130
Electrical	57,618	388,720	446,339
Paint	8,976	5,604	14,580
Direct Subtotals	189,439	1,410,968	1,600,407
Construction Equipment and Indirect			203,500
Construction Management, Staff, and Supervisor			208,000
Fright			56,400
Taxes and Permits			88,200
Engineering			499,700
Other Project Costs			210,306
Contingency			515,972
Indirect Subtotals			1,782,078
Contract Totals	189,439	1,410,968	3,382,485

VITA

VITA

Mahdieh Aghazadeh was born and raised in Qazvin, Iran. She attended Sharif University of Technology in Tehran for her undergraduate study and received her Bachelors of Science in Chemical Engineering from the Chemical and Petrochemical Engineering Department in 2008. After a year of working in an educational/engineering services company in Tehran she moved to United States to start her graduate degrees. She earned her Masters of Science degree in Chemical Engineering from the Chemical and Biochemical Engineering Department at University of Maine in Orono, ME in 2011. Having met Dr. Abigail Engelberth at University of Maine, she decided to move to West Lafayette, Indiana to pursue her doctorate degree in Agricultural and Biological Engineering. She aims to continue her research and scientific career in the region.