DEVELOPMENT OF A SUSTAINABLE LIGNOCELLULOSIC BIODIESEL REFINERY

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ABSTRACT

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A novel co-hydrolysis process, which applies dilute acid pretreatment directly followed by enzymatic saccharification without detoxification and liquid-solid separation between these two steps was implemented to convert lignocellulosic biomass into monomeric sugars. A factorial experiment in a randomized block design was employed to optimize the co-hydrolysis process for several herbaceous crops (switchgrass, giant reed, and miscanthus) and corn stover, with corn stover showing the greatest overall sugar conversion. Under optimal reaction conditions, corn stover exhibited a total sugar yield (glucose + xylose) of 0.545 g/g dry biomass at 83.3% of the theoretical yield. The oleaginous fungus *Mortierella isabellina* was selected and applied to the co-hydrolysate medium to accumulate fungal lipids due to its capability of utilizing both hexose (C6) and pentose (C5) sugars. Mass balance data from the *M. isabellina* fermentation served as the basis for developing a theoretical biorefinery utilizing fungal lipid for biodiesel production. The unit operations identified for the biorefinery are (1) corn stover collection and transportation, (2) pretreatment and enzymatic co-hydrolysis, (3) lignin processing, (4) fungal lipid fermentation, (5) fungal biomass drying, (6) lipid extraction and transesterification, (7) anaerobic digestion and aerobic treatment of wastewater, and (8) solar-bio-power generation. Energy life cycle analysis results show the assumed biorefinery system has a net energy output of -113.79 MJ/kg biodiesel produced. Overall, aerobic fungal lipid fermentation is shown to be the most energy-demanding unit operation, accounting for nearly 50% of all energy inputs, with co-hydrolysis resulting in large water and energy savings.

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Introduction

Diesel fuels are pivotal components to both advanced and developing economies as they are necessary for the transport of industrial and agricultural goods, and are responsible for the operation of diesel tractors and other agricultural components such as pump sets (Meher, Sagar, & Naik, 2006). Economic growth in these economies necessitates an equivalent expansion in the transportation sector dependent on diesel fuel. Worldwide demand for petroleum-based transportation fuels has increased dramatically over the past decade. Fuel prices have also set record highs in recent years, affecting the cost of many consumer goods which track closely with the price of oil. This increased demand has brought renewed concerns over the sustainability and environmental impacts posed by these ultimately finite resources. While many alternative sources of energy exist from wind, solar, geothermal, and biomass; biofuels, particularly those produced from abundant and renewable biomass feedstocks, represent a near term solution for alleviating dependence on the petroleum based transportation economy while mitigating environmental impacts (Meher, Sagar, & Naik, 2006).

Biofuels are defined as liquid or gaseous transportation fuels that are principally produced using biomass as a feedstock (Yusuf, Kamarudin, & Yaakub, 2011). They have the ability to mitigate greenhouse gas (GHG) emissions by reducing the highly oxidized atmospheric carbon (CO_2), and storing it as soil organic carbon. Because plant biomass is used as the raw material for biofuel production, the combustion of said fuels theoretically adds no additional CO_2 into the atmosphere since it simply recycles the atmospheric CO_2 embodied in the plant matter (Zhu & Zhuang, 2012). Biodiesel is a biofuel produced as an alternative to petroleum-based diesel. It is composed of monoalkyl esters of long-chain fatty acids (fatty acid alkyl esters) produced from renewable oil feedstocks. Biodiesel is most commonly produced through a transesterification reaction in which triaclyglycerides (TAG) (long chain fatty acids bound to a glycerol backbone) found in vegetable oil or animal fat are reacted with a monohydric aliphatic alcohol (most commonly methanol) in the presence of a catalyst at high temperatures producing biodiesel and glycerol (Van Gerpen, 2005). The basic theoretical stoichiometric equation for the

transesterification reaction is such that 1 mole of triglyceride is reacted with 3 moles of methanol to produce 3 moles of fatty acid alkyl ester and 1 mole of glycerol byproduct. While the theoretical ratio of methanol to oil is 3/1, the actual ratio is closer to 6/1 in order to give an approximately 98% biodiesel conversion. The transesterification reaction is most commonly catalyzed with sodium hydroxide (NaOH), which allows for the reaction to occur at lower temperatures, thus lowering energy inputs during production.

Biodiesel has many properties that make it an attractive alternative to petroleum diesel. It is non-toxic, biodegradable, has a low emissions profile including reduced CO_2 emissions, a superior flash point to petro-diesel, negligible sulfur content, and is produced from a variety of renewable resources such as soybean, sunflower, palm oil, rapeseed, canola, jatropha, and waste oils and animal fats. Additionally, biodiesel blends have increased lubrication properties which reduce long term engine wear, and it is the only alternative fuel which can run on conventional unmodified compression-ignition (diesel) engines (Demirbas, 2009). Pure, unblended biodiesel can be directly inputted as engine fuel, or it can be blended in any number of ratios with petroleum diesel. The most common biodiesel blend in the United States is B20, which consists of 20% biodiesel and 80% petroleum diesel. Biodiesel standards exist in many countries in order to ensure quality control of the renewable fuel as it reaches consumers. Two of the most widely adopted biodiesel standards are the ASTM D6751 in the United States, and the EN 14214 standard in the European Union.

Worldwide biodiesel production stood at 431.259 thousand barrels/day in 2012, with Europe leading all regions with 170.923 thousand barrels/day, or 40% of the world's total. The United States accounted for 64 thousand barrels/day of biodiesel production in 2012, or 15% of the world's total (U.S. Energy Information Administration, 2014).

Rapeseed oil is the major feedstock used in biodiesel production accounting for 48% of all biodiesel produced in 2007 (Moser, 2009). Other major feedstock oil sources include soybean (22% of biodiesel produced), and palm (11%) oils, with the remaining (19%) inputs spread among a variety of

animal fats and other vegetable oil sources (Moser, 2009). Different oil feedstocks have different TAG chemical compositions, which in turn creates different fatty esters in the final biodiesel product. The composition of the fatty esters dictates the fuel properties of biodiesel. Fatty ester carbon chain lengths vary from C12 to C22 with chain lengths between those extremes predominating. As chain length increases the following properties typically increase: melting point, oxidative stability, kinematic viscosity, standard heat of combustion, and cetane number, while lubricity typically decreases. Likewise, as the number of double bonds in the carbon chain increases, the previously mentioned properties typically decrease. Saturated methyl esters, those with no carbon double bonds, have higher freezing points than unsaturated chains. Feedstocks with high free fatty acid (FFA) content (>3%) should be avoided as these substances will react with base catalysts such as NaOH, to form soap and water, ultimately retarding the transesterification process and leaving the TAG molecules unreacted.

One of the major barriers facing the biodiesel industry is the cost of feedstock acquisition. Feedstock costs account for over 80% of the total expense of biodiesel production (Moser, 2009), which has led the industry to look for alternative sources of oil. One strain of current research is focused on using lignocellulosic materials as sources of cheap sugars to grow fungal biomass for oil and biodiesel production.

The following chapters assess various lignocellulosic feedstock sources and pretreatment conditions for their conversion into fermentable media for fungal lipid production. A theoretical biodiesel refinery utilizing lignocellulosic pretreatment and fungal lipid fermentation as inputs is discussed, and its unit operations identified. Mass and energy balance data is collected on the system in order to identify which inputs have the largest impact on the overall system efficiency and sustainability.

1 Literature Review

1.1 Lignocellulose

Lignocellulosic biomass represents the most abundant source of renewable carbon with over 200 billion tons created annually (Chandel & Singh, 2011). Interest in its use as a feedstock for the production of advanced biofuels has gained momentum in recent years as shown by various government directives. The Renewable Fuels Standard 2 provision in the United States Energy Independence and Security Act of 2007 mandates the production of 36 billion gallons of biofuels by 2022, with 21 billion gallons coming from non-starch sources (i.e. lignocellulosic sources) while capping conventional biofuels (i.e. corn starch-based ethanol) at 15 billion gallons (Coyle, 2010). Support for lignocellulosic fuels is owed not only to its large supply, but also to its ability to mitigate greenhouse gas emissions, avoid competition with food resources, stimulate rural economies, and provide a stable and secure source of energy production (Coyle, 2010). Many sources of lignocellulose materials exist which can be readily converted into microbial biofuel production including municipal solid waste, pulp and paper wastes, forest and agricultural residues, and dedicated woody and herbaceous energy crops (Williams, Inman, Aden, & Heath, 2009). Agricultural residues, primarily corn stover, and herbaceous perennial energy crops have been the focus of much attention due to their positive environmental characteristics. Corn stover consists of the above ground biomass left once the kernels have been harvested, and the total amount produced annually is equal to the total weight of corn harvested. That is to say the corn grain to corn stover ratio is 1/1. However, a minimum corn stover amount of 50% must be left in the field to avoid erosion issues, and research has shown that the upper limit of stover that can be sustainably harvested while avoiding soil organic carbon losses is approximately 30% (Morey, Kaliyan, Tiffany, & Schmidt, 2010). Compared to their annual counterparts, perennial herbaceous energy crops, once established, do not need reseeding and require lower inputs for water, fertilizer, pesticides, and tillage. Additionally, they can often be grown on uncultivated or marginal lands where their deep root structures act to reduce soil

erosion, increase soil fertility, and accumulate greater soil organic carbon than their annual counterparts, including corn stover.

Lignocellulosic materials, despite their source, share similar biochemical characteristics. They are heterogeneous matrices of polysaccharides (cellulose and hemicellulose) and lignin, a phenolic polymer. Cellulose is a homopolymer of glucose subunits linked by β-1, 4 bonds. Individual cellulose chains are densely packed in microfibrils consisting of 36 individual cellulose chains forming a tight crystalline structure that is water insoluble and difficult to depolymerize (Mosier, et al., 2005). Hemicellulose is a branched heteropolymer consisting primarily of xylose (sometimes glucose) with subunits of galactose, fucose, mannose, glucose, or glucuronic acid substituted in the chain. It often contains side chains with acetate groups. Lignin is a polymer of phenyl propane (C9) which consists of a 6-carbon phenolic ring with a 3-carbon side chain. It provides a hydrophobic barrier to water penetration, and also protects plants from degradation by fungi, insects, and microbes. Hemicellulose hydrogen-bonds to cellulose microfibrils forming a complex network of polysaccharides, providing structure to the plant cell walls (Mosier, et al., 2005). Lignin fills in the spaces in the cell wall structure adding an additional degree of stability and protection to the polysaccharides. Cellulose and hemicellulose fractions of lignocellulose can potentially be broken down to their monomeric units and used as substrates for the microbial production of biofuels. While rich in polysaccharides, lignocelluloses have developed mechanisms to protect these sugars from degradation. Some key factors believed contributing to the resistant nature of lignocellulose include cellulose crystallinity and insolubility, the presence and abundance of lignin, sheathing of cellulose by hemicellulose, and the heterogeneous composition of the biomass itself (Mosier, et al., 2005). Though beneficial from an evolutionary standpoint, the natural recalcitrance of lignocellulose nonetheless negatively affects the conversion yield and processing costs associated with producing monomeric sugars for second generation biofuels, since expensive chemicals and enzymes must be applied in order to break down the carbohydrate fractions.

1.2 Pretreatment Methods and Microbial Inhibition Products

The goal of pretreatment is to disrupt the macromolecular structure of lignocellulose (lignin, cellulose, and hemicellulose) so that hydrolytic enzymes can penetrate and hydrolyze the polysaccharides into individual monomeric units. Effective pretreatment operations maximize the monosugar yield of both pentose and hexose sugars (from hemicellulose and cellulose, respectively) while minimizing the production of chemical by-products harmful to microbial growth. Various pretreatment technologies exist for monosugar extraction, and can be categorized as either physical or chemical in nature (Mosier, et al., 2005).

Physical pretreatment methods include comminution, steam explosion and hydrothermolysis, with steam explosion being the far more extensively studied of the three. Steam explosion involves rapidly heating lignocellulosic biomass with high-pressure steam without any added chemicals. Once the target temperature is reached, it is held there for a brief time in order to hydrolyze the hemicellulose fraction, with the process being terminated by an explosive decompression. The high temperature steam releases acetyl groups which are esterified to the hemicellulose chains. These acetyl groups form weak acetic acid which hydrolyzes and solubilizes the hemicellulose polymers into pentose sugars. Removal of the hemicellulose fraction allows for greater enzyme accessibility to the cellulose for hexose hydrolysis. Physical reduction and increased biomass surface area are considered less important with regards to the overall hydrolysis of the cellulose polymers (Brosse, El Hage, Sannigrahi, & Ragauskas, 2010).

Chemical pretreatments involve the addition of dilute acid or alkali to lignocellulose at elevated temperatures in order to remove either the hemicellulose or lignin fractions and improve cellulose hydrolysis. Dilute acid pretreatment involves the creation of a weak sulfuric acid (H_2SO_4) solution (1-3% weight/weight (w/w)) and adding lignocellulose at various solid loadings (5-25%), then heating the mixture at high heat (120-220˚C) for various times ranging from seconds to hours. The acid and heat solubilize the hemicellulose portion in the lignocellulose as well as a small amount of lignin, allowing for greater enzymatic access to the cellulose chains. After the initial pretreatment step, the pH of the biomass

slurry will be very low and must be neutralized with bases such as NaOH or $Ca(OH)_2$ in order to stop further degradation of the xylose monomers to inhibition products, and to create a pH more conducive to enzymatic hydrolysis (Lee, Iyer, & Torget, 2001) (Lee et al. 1999).

Alkali pretreatment methods are performed under lower temperatures and pressures than other pretreatment techniques. Two of the most common pretreatment methods involving alkali chemicals are lime pretreatment and ammonia fiber explosion (AFEX). Lime pretreatment involves spraying a lime/water solution over the biomass material, and storing it in a pile for a matter of hours to weeks depending on the temperatures involved, with higher temperatures reducing the total treatment time (3 hours (h) at 85˚C for wheat straw, and 13 h at 100˚C for corn stover) (Mosier, et al., 2005). This method solubilizes the lignin from biomass allowing for improved enzymatic access to the remaining polysaccharide fractions. It also removes acetyl and uronic acids on hemicellulose, further increasing enzyme accessibility.

AFEX pretreatment is an attractive method for releasing monosugars from herbaceous perennials and agricultural residues, garnering near theoretical yields at low enzyme loadings (< 5 filter paper unit (FPU)) (Mosier, et al., 2005). This treatment method involves trickling an ammonia solution (5-15%) through biomass that is tightly packed in a column reactor at high temperatures (160-180°C) and a low velocity of 1 $mL/cm^2/min$. Residence times are typically around 14 minutes. The aqueous ammonia delignifies the biomass and severs the linkages between lignin and the polysaccharide fractions while solubilizing some hemicellulose as well. Ammonia costs (and recovery) appear to be the limiting factor for this process. However, input costs may be outweighed by the high total sugar yield achieved by this process.

No matter the pretreatment process, the resulting hydrolysate (liquid containing the solubilized monosugars) will contain substances with properties that are inhibitory to the production of biofuels via microbial fermentation. The type and amount of these products is dependent on both the particular biomass being treated, as well as the reaction conditions it is subjected to. These inhibitory products can

often be the limiting factor to the viability of microbial biofuel production. The degradation products resulting from chemical pre-treatment of lignocellulosic biomass are divided into the following different categories: carboxylic acids, furans, phenols, and inorganic salts, with phenols showing the greatest inhibitory effect on fermentation (Pienkos & Zhang, 2009).

Carboxylic acids (primarily acetic acid) are ubiquitous in hemicellulose hydrolysate since that fraction of the biomass (as well as lignin to a smaller degree) is often highly acetylated. They are by-products of acid pretreatment, which releases carboxylic acids from their hemicellulose side chains (Benko, et al., 2007). Alkali degradation often creates hydroxycarboxylic acids such as glycolic acid and lactic acid. Formic acid is a product of sugar and lignin degradation, while levulinic acid is formed by solely by degrading sugar. Aromatic degradation products form primarily from the breakdown of sugars to furans and phenols formed by the cleavage of solubilized lignin. Furan aldehyde inhibitors are produced from the degradation of monosugars in the hydrolysate. The two most common furans include furfural which results from the breakdown of xylose, and hyrodxymethyl furfural (HMF), resulting from glucose degradation. Phenolic compounds result from the breakdown of polyphenolics in lignin. Inorganic salts are created by a variety of methods such as their release from both the lignocellulosic biomass itself and the walls of pretreatment vessels and pretreatment chemical reactions (Zhang, French, Hernandez, Alley, & Paraschivescu, 2011).

The manifestations of the toxic effects vary among the different inhibitors and the different organisms that are subject to them. Low molecular weight (MW) inorganic salts are able to penetrate cell membranes, whereas fermentation inhibitors with high MW influence the expression and activity of sugar and ion transporters in the cell membrane. Acetic acid can penetrate the microorganism cell wall and hinder cellular activity by acidifying the cytoplasm and disrupting the protein gradient across the cell membrane (Takahashi, Takahashi, Carvalhal, & Alterthum, 1999). Furfural and HMF interfere with glycolysis by disrupting dehydrogenase activity resulting in lower growth rates and yields. Phenolic compounds can pierce cellular membranes resulting in lower growth rates and inhibiting sugar transport

(Klinke, Thomsen, & Ahring, 2004). However, information regarding the specific effects these inhibitors have on oleaginous filamentous fungal lipid accumulation used for biodiesel production is quite limited and needs further study.

1.3 Enzymes

Enzymes are the catalysts for the depolymerization of the lignocellulosic polysaccharides. They have advantages of typically working at low temperatures and are highly specific reactions. However enzyme costs are typically very high, and the reaction rates can take days. Enzymes specific to glucose and xylose hydrolysis are termed glycoside hydrolases because they catalyze the hydrolysis of the glycoside linkages between monomeric units in the polysaccharides of lignocellulose. Because xylose is typically solubilized into monomeric units during many pretreatment processes, most research focuses on the cellulose enzymes, or those that depolymerize the cellulose chains of biomass. Cellulases must possess the dual ability to both bind a water soluble protein to the insoluble cellulose substrate, as well as depolymerize the highly crystalline structure of the sugar chain. These proteins are categorized as either complexed or noncomplexed systems. Noncomplexed cellulase enzymes work to degrade cellulose to monomeric glucose units under aerobic conditions. These enzymes include three subclasses: endoglucanases, exoglucanases, and β-glucosidases. Endoglucanases cleave glycosidic bonds in the amorphous central region of the cellulose molecules creating reducing and non-reducing ends. Exoglucanases release cellobiose (a disaccharide of glucose units) from the outside ends of the cellulose chain. Finally, β-glucosidases release glucose from cellobiose and soluble cellodextrins. Typically the endoglucanases and exoglucanases display both cooperatively and synergism, meaning that the combination of those enzymes improves overall hydrolysis yields. The endo-acting enzymes create new reducing and non-reducing ends for exo-acting enzymes to work on (Lynd, Weimer, van Zyl, & Pretorius, 2002).

1.4 Microbial Fermentation of Lignocellulose Hydrolysate

Because of the growing concerns over using food crops for the production of biofuels, many in the biodiesel industry are now looking to expand production away from rapeseed (canola) and soybean-based biodiesel. Attention has shifted to non-edible oil sources such as those produced from oleaginous microorganisms (those organisms with lipid contents in excess of 20%). Microbial lipids are viewed as a possible alternative for industrial biodiesel production because their triacylglycerol composition is similar to that of vegetable oils (Huang, Zong, Wu, & Liu, 2009). The major fatty acids present in the lipids produced by oleaginous microorganisms are myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3); all of which can be converted to biodiesel through a transesterification reaction (Chatzifragkou, et al., 2010). However, the limiting factor for microbial lipid production is often the organism's low productivity coupled with the high expense of its glucose substrate (Hui, Wan, Hai-tao, Xue-jiao, & Yu-hua, 2010). In order to overcome cost barriers, a less expensive carbon source is desired as well as utilizing microorganisms exhibiting high biomass and lipid yields. Lignocellulosic biomass is of great interest as a sugar substrate source for microbial lipid accumulation due to its abundance and potential to serve as an inexpensive feedstock. Both glucose and xylose can be extracted from such sources, with glucose being the more abundant and easier metabolized of the two (Hu, et al., 2011). Organisms that can utilize both pentose and hexose sugars for fermentation are thus highly desired in order to increase the efficiency of lipid production from lignocellulosic materials (Gong, et al., 2012).

In general, lipids are accumulated in oleaginous organisms due to the presence of the ATP-dependent citrate lyase. Under nutrient limited conditions, especially nitrogen limitation with an abundant carbon source (high carbon/nitrogen (C/N) ratio), AMP deaminase is up-regulated eventually leading to the presence of citrate in the cell cytosol. Citrate is cleaved by the ATP-dependent citrate lyase producing acetyl-CoA, which is used in fatty acid biosynthesis. It is by this pathway that oleaginous microorganisms shift their carbon sources away from growth and toward lipid production (Ratledge, 2004).

Though glucose is the more easily assimilated of the two major lignocellulosic carbon sources, studies have investigated oleaginous fungal lipid production using xylose as the sole carbon source as well as using both glucose and xylose simultaneously. Xylose (80 g/L, C/N ratio of 285) was used as the sole carbon source to cultivate the oleaginous molds *Mortierella isabellina* ATHUM 2935 and *Cunninghamella echinulata* ATHUM 4411 resulting in lipid concentrations of 6.1 g/L and 6.7 g/L, respectively at 360 h, however, more than 25 g/L of unconsumed xylose remained in the media (Fakas, et al., 2009). Studies have reported lipid accumulations from xylose of 2.2 g/L for the oleaginous mold Colletotrichum sp.DM06, and 4.3 g/L for Alternaria sp.DM09 under nitrogen stressed conditions respectively (Dey, Banerjee, & Maiti, 2011). The oleaginous yeast *Trichosporon cutaneum* AS 2.571 assimilated glucose and xylose simultaneously, and accumulated intracellular lipid up to 59% (w/w) with a lipid coefficient up to 0.17 g lipid/g sugar, upon cultivation on a 2/1 glucose/xylose mixture (Hu, et al., 2011). Additionally, a variety of research has been conducted on oleaginous mold lipid accumulation from pretreated lignocellulosic biomass enzymatic hydrolysate. When using a mixture of wheat straw and bran as feedstock with solid culture, 80 mg lipid/g dry solids cell mass (mg/gds) was accumulated by the oleaginous mold *Microsphaeropsis sp* (Peng & Chen, 2008). For semi-solid culture, 11 g oil/100 g dry sweet sorghum was produced (Economou, Aggelis, Pavlour, & Vayenas, 2011). Researchers using rice straw and wheat bran under solid culture produced 68.2 mg/gds and 60.32 mg/gds lipid using *Colletotrichum sp.* (DM06) and *Alternaria sp.* (DM09). The mutant strain *Mortierella alpina* (MAI502-8) accumulated 27.4 and 10.05 g/L cell mass and lipid respectively from a mixture of glucose and xylose media (5/3 w/w) over 11 days of total culture time (Peng, et al., 2011). Under varying C/N ratios (35, 44 and 57), lipid accumulated in *M. isabellina* ATHUM 2935 was 36%, 51.2% and 64.3%, respectively using rice hull hydrolysate (Economou, Aggelis, Pavlour, & Vayenas, 2011). Ruan demonstrated that *M. isabellina* grown on un-detoxified corn stover hydrolysate was able to produce similar biomass and lipid content when grown on substrate with identical glucose and xylose concentrations and C/N ratios without any added inhibitors (Ruan & Zanotti, 2012). Additionally it was shown that this oleaginous mold was able to utilize acetic acid for fungal growth and lipid accumulation further demonstrating the utility of

using molds capable of metabolizing both glucose and xylose in the presence of inhibitory compounds (Ruan & Zanotti, 2012).

1.5 Energy-Neutral Lignocellulosic Biodiesel Production

In order for lignocellulosic biodiesel to be considered a viable alternative to petroleum-based fuels it must demonstrate that it can be produced in a sustainable manner. This means that an ideal biorefinery system should have a positive net energy contribution from the feedstock source; otherwise it is no different than a traditionally energy-intensive pulp mill which creates fiber as its primary product and uses residual lignin as a supplemental power source through combustion (Zhu & Zhuang, 2012). Evaluating the energy output of a biofuel system requires a detailed evaluation of all mass and energy flows. Energy life cycle analysis (ELCA) is a method for determining the cumulative energy inputs from different sources as well as the total efficiency of the production process (Pradhan, et al., 2011). Energy balance involves accounting for the amount of energy used during the production and comparing it to the amount contained in the biofuel and other co-products. The results obtained from the energy balance analysis can be expressed as the ratio of energy produced to energy consumed by a production system, otherwise known as the net energy ratio (NER). Increase of NER for biofuel production is key to establishing a sustainable biorefining industry. If the calculated NER is greater than unity, then the biofuel could be utilized to replace all the energy needed in its production, and thus it would be considered infinitely renewable (Pradhan, et al., 2011).

Improving NER can be achieved by supplementation with renewable energy sources, such as solar thermal, wind, geothermal, and biomass. Among these sources, solar thermal energy, with the advantages of abundance and availability, represents an excellent candidate to be combined with a lignocellulosic refinery to produce biofuels. Several solar thermal conversion technologies have been developed in the past several decades, such as, flat plate thermal collector, evacuated-tube solar thermal collector, parabolic trough system, power tower system, dish solar system, and Fresnel reflectors (Mills, 2001). Among these sources, solar energy with the advantages of abundance and availability represents an

excellent candidate to be combined with a lignocellulosic refinery to produce biofuels. Many solar technologies have been developed in the past several decades such as silicon wafer PVs, thin-film PV, low-temperature solar thermal systems, and CSP systems (Mills, 2001) (Shukla, Sumathy, Erickson, & Gong, 2013) (Price H. , et al., 2002) (Green, 2007). According to their solar conversion mechanisms, these solar technologies are mainly divided into two categories: PV and CSP. PV uses semiconducting materials to directly covert photons into electrons and generate electricity, while CSP uses heat absorbers or reflective surfaces to collect solar thermal energy for both low temperature (hot water heater) and high temperature (CSP technologies) applications. Integrating such solar technology with lignocellulosic biofuel production could lead to a sustainable biorefining platform capable of replacing fossil fuel consumption, while maximizing its NER value.

1.6 Objective and Hypothesis

While research has established the fact that microbial lipid production from lignocellulosic sources is possible, improvements are still needed. According to Papanikolaou et al., biomass yields of at least 30 g/L with reserve lipid content near 30% are required in order to make microbial oil production commercially feasible (Papanikolaou, Komaitis, & Aggelis, 2005). While lab-scale experiments have come close to reaching such lipid levels, their productivities are often very low due to inhibition products present in the fermentation broth, requiring costly detoxification steps to remove. Little is known about the specific type or concentration of inhibitors produced under different pretreatment conditions for various lignocellulosic sources, as well as the individual and synergistic effects those inhibitors may have on oleaginous fungal growth and lipid production. Additionally, an energy neutral production process is also critical to sustain lignocellulosic biodiesel production.

Therefore the goal of the study is to develop a sustainable lignocellulosic biodiesel production system that has better biodiesel yield, low water consumption, and a neutral energy balance. The specific objectives of the research include the following:

1. Identify a microorganism capable of fermenting substrates derived from lignocellulosic biomass for bio-lipid production, and determine the pretreatment method best-suited for its fermentation.

2. Optimize biomass pretreatment conditions for total sugar yield with regards to the following parameters: chemical concentration, treatment time, temperature, and solid loading.

3. Identify specific inhibition products resulting from the pretreatment of lignocellulosic biomass, as well as conduct fermentation experiments in order to determine the microbial growth and lipid production on said lignocellulosic-derived substrates.

4. Perform a broad mass and energy balance, along with a life cycle energy analysis in order to determine a rough estimate of the net energy required, as well as the feasibility of commercially scaling up a solar-bio-powered microbial biodiesel production process.

2 Chapter 2

2.1 Abstract

The herbaceous perennial energy crops miscanthus, giant reed, and switchgrass, along with the annual crop residue corn stover, were evaluated for their bioconversion potential. A co-hydrolysis process, which applied dilute acid pretreatment, directly followed by enzymatic saccharification without detoxification and liquid-solid separation between these two steps was implemented to convert lignocellulose into monomeric sugars (glucose and xylose). A factorial experiment in a randomized block design was employed to optimize the co-hydrolysis process. Under the optimal reaction conditions, corn stover exhibited the greatest total sugar yield (glucose + xylose) at 0.545 g/g dry biomass at 83.3% of the theoretical yield, followed by switch grass (0.44 g/g dry biomass, 65.8% of theoretical yield), giant reed (0.355 g/g dry biomass, 64.7% of theoretical yield) and miscanthus (0.349 g/g dry biomass, 58.1% of theoretical yield). The influence of combined severity factor on the susceptibility of pretreated substrates to enzymatic hydrolysis was clearly discernible, showing that co-hydrolysis is a technically feasible approach to release sugars from lignocellulosic biomass. The oleaginous fungus *M. isabellina* was selected and applied to the co-hydrolysate mediums to accumulate fungal lipids due to its capability of utilizing both C5 and C6 sugars. Fungal cultivations grown on the co-hydrolysates exhibited comparable cell mass and lipid production to the synthetic medium with pure glucose and xylose. These results show the potential for combining fungal fermentation with a co-hydrolysis pretreatment process for lignocellulosic lipid accumulation, and could enhance the utilization efficiency of lignocellulosic biomass for advanced biofuels production.

Keywords: co-hydrolysis, lignocellulosic biomass, oleaginous fungus, lipid accumulation

2.2 Introduction

Lignocellulosic biomass represents the most abundant natural polymer in the biosphere, and interest in its use as a feedstock for the production of advanced biofuels has gained momentum in recent years as

shown by various government directives. The Renewable Fuels Standard 2 provision in the United States Energy Independence and Security Act of 2007 mandates the production of 36 billion gallons of biofuels by 2022, with 16 billion gallons coming from lignocellulosic sources while capping conventional biofuels (i.e. corn starch-based ethanol) at 15 billion gallons (Coyle, 2010). Support for lignocellulosic fuels is owed not only to its large supply, but also its ability to mitigate greenhouse gas emissions, avoid competition with food resources, stimulate rural economies, and provide a stable and secure source of energy production (Coyle, 2010). Many sources of lignocellulosic biomass exist, including municipal solid wastes, pulp and paper wastes, forest and agricultural residues, and dedicated woody and herbaceous perennial energy crops. Herbaceous perennial energy crops in particular, have been the focus of much attention due to their positive environmental characteristics. Compared to annual crops, perennials, once established, do not need reseeding, and require lower inputs (i.e., water, fertilizer, pesticide, tillage). Additionally they can often be grown on uncultivated or marginal lands where their deep root systems act to reduce soil erosion, increase soil fertility, and accumulate greater soil organic carbon than their annual counterparts (i.e. corn or canola) (Williams, Inman, Aden, & Heath, 2009). Among the twenty perennial grasses studied by the European Union, the most promising in terms of biofuel production were: miscanthus, giant reed, switchgrass and reed canarygrass (Lewandowski, Scurlock, Lindvall, & Christou, 2003). Miscanthus, giant reed, switchgrass, as well as corn stover will be the primary focus of this investigation.

Many studies have been devoted to the pretreatment and enzymatic hydrolysis of the herbaceous perennial energy crop switchgrass, however, information about the recalcitrance and bioconversion potential of miscanthus and giant reed is still limited. Wet explosion (Sorensen, Teller, Hilstrom, & Ahring, 2008), ammonia fiber expansion (Murnen, et al., 2007), one-step extrusion/NaOH (de Vrije, de Haas, Tan, Keijsers, & Claassen, 2002), and aqueous-ethanol organosolvent treatments were applied for the bio-conversion of miscanthus to fermentable sugars (Brosse, El Hage, Sannigrahi, & Ragauskas, 2010). Dilute acid pretreatment and ethanol-alkaline treatment were carried out for giant reed

bioconversion. All such studies employed common methods of biomass processing where, after pretreatment, the solids are separated from the liquid stream, washed to neutralize and detoxify the remaining solids, then subjected to enzymatic hydrolysis in order to extract glucose monomers (Decker, Brunecky, Tucker, Himmel, & Selig, 2009). Depending on the particular pretreatment method, the discarded liquid stream will often contain a large percentage of the solubilized xylose, and to a lesser extent, glucose monomers and soluble lignin. Since xylose can compose a large fraction of the lignocellulosic biomass, it is critical from an economic standpoint to retain this fraction in order to improve pretreatment efficiency. Additionally, such a process requires large amounts of water to neutralize and detoxify the solid biomass, and separation of the solid and liquid streams results in significant turnaround times, and introduces the possibility of contamination (Studer, Brethauer, DeMartini, McKenzie, & Wyman, 2011). Therefore, development of a novel process (co-hydrolysis), which eliminates liquid-solid separation, detoxification or washing of the pretreated solids, and directly carries out enzymatic hydrolysis after pretreatment would make a significant contribution to the production of advanced biofuels. To date, comprehensive evaluations of co-hydrolysis of lignocellulosic biomass are sparse. A few researchers have studied the effects of higher solids loading for un-detoxified pretreated wheat straw (Georgieva, Hou, Hilstrom, & Ahring, 2008), the effects of increasing enzyme dosage in comparing washed-solids of wheat straw versus whole-slurry hydrolysis (Felby, Klinke, Olsen, & Thomsen, 2003), and the combined effects of severity, and enzyme and solid loadings on co-hydrolysis performance of populous (Studer, Brethauer, DeMartini, McKenzie, & Wyman, 2011).

Realizing the potential benefits of co-hydrolysis comes with its own challenges, such as utilizing microbes that are robust enough to withstand elevated levels of toxins specific to each biomass source and pretreatment method, while containing the ability to ferment both hexose and pentose sugars into biofuels. There are few studies on the bioconversion of both the hexose and pentose fractions of herbaceous perennial energy crops like switchgrass, miscanthus, or giant reed. The extreme thermophilic bacterium *Thermotoga elfii* metabolized undetoxified miscanthus hydrolysate containing glucose and xylose for

hydrogen production, while the yeast *Scheffersomyces stipitis* CBS6054 was able to convert giant reed hydrolysate to ethanol (Scordia D. , Cosentino, Lee, & Jeffries, 2012). Our previous study found that the oleaginous mold *M. isabellina*, when grown on corn stover co-hydrolysate, could produce lipid yields comparable to those from synthetic hydrolysate without toxins (Ruan & Zanotti, 2012). In addition, there is still limited information regarding its lipid production from herbaceous perennial energy crops, even though lipid accumulation by *M. isabellina* from lignocellulosic biomass has been studied (Economou, Makri, Aggelis, Pavlou, & Vayenas, 2010). Thus, the aim of this study is to provide a comparative evaluation of *M. isabellina* lipid accumulation on the co-hydrolysates of four different lignocellulosic feedstocks: switchgrass, giant reed, miscanthus, and corn stover, using a co-hydrolysis process for bioconversion.

2.3 Materials and Methods

2.3.1 Lignocellulosic Biomass

Corn stover and switchgrass were collected from the Michigan State University Crop and Soil Science Teaching and Research Field Facility. Miscanthus and giant reed were obtained from Werks Management, LLC (Fishers, IN). Each feedstock was air-dried and ground using a mill (Willey Mill, Standard Model No. 3, Arthur H. Thomas, Philadelphia, PA) with a 2 mm size screen. All materials were then sieved to make a particle size distribution of less than 30 mesh (< 1.6 mm) but greater than 80 mesh (> 1 mm). The biomass samples were analyzed for cellulose, xylan, and lignin content according to the National Renewable Energy Laboratory's (NREL) analytical procedure for determination of structural carbohydrates and lignin in biomass.

2.3.2 Dilute Acid Pretreatment

To evaluate the combined glucose and xylose recovery from cellulose and xylan in each biomass sample, dilute acid pretreatment was performed with a factorial randomized block design. Dilute acid concentration, pretreatment time, and temperature were the factors investigated. Eighteen treatments with two replicates were applied to each lignocellulosic biomass for a total of thirty-six individual samples per

feedstock. Each sample was treated in a screw cap 125 mL serum bottle and placed in an autoclave (Brinkmann 2540M, Tuttnauer USA Co. Ltd, Hauppauge, NY). Dilute acid pretreatments were carried out at sulfuric acid concentrations of 1%, 2% and 3% (w/w), respectively. Retention times of 1, 2, and 3 h were applied once the reaction temperature reached its predetermined level of 110, 120, or 130˚C, respectively. Biomass concentration for pretreatment was fixed at 10% dry matter. After dilute acid pretreatment, biomass slurries were titrated to a pH of 4.5-5 with 5 mol/L sodium hydroxide.

2.3.3 Enzymatic Saccharification

After dilute acid pretreatment and pH adjustment of the biomass slurry, various amounts of 0.1 mol·L-1 citrate buffer (pH 4.8) and an enzyme mixture consisting of 34.85 mg cellulase (Accellerase 1500®, protein content 69.7 mg/mL, lot number 3016295230; Genencor, Palo Alto, CA) and 4.31 mg xylanase (Accellerase XY, protein content 43.1 mg/mL, lot number 4900667792; Genencor, Palo Alto, CA) per gram of initial dry biomass were added to the slurry to achieve a final dry matter concentration of 8% for all pretreated solutions. All of the resulting samples were incubated at 50°C in a shaking incubator at 150 rpm for 72 h. All enzymatic hydrolysis experiments were carried out in duplicates. After hydrolysis, the samples were removed from the shaker and put on ice to stop the reaction; the hydrolysate was separated by centrifugation at 7025 x g for 5 minutes to obtain a clear sugar solution, which was then filtered through a 0.22 μm polyethersulfone membrane filter for HPLC analysis. The clear enzymatic hydrolysate solutions were stored in a 4°C refrigerator for further use.

2.3.4 Microorganisms and Culture Conditions

Mortierella isabellina ATCC 42613 was obtained from the American Type Culture Collection (Manassas, VA). This strain was first cultured on potato dextrose agar (Sigma-Aldrich, St. Louis, MO) to produce spores at 30˚C. After 14 days cultivation, the spores were washed with sterilized distilled water to obtain a spore suspension (stored at 4°C). Seed cultures were grown with 24 g/L potato dextrose broth (Sigma-Aldrich, St. Louis, MO) with 8 g/L yeast extract at 25°C and 180 rpm for 2 days in a rotary shaker (Thermal Scientific, Odessa, Texas) with a spore concentration of 1-2×107 spore/mL. The salt

medium of submerged batch cultures contained: KH_2PO_4 (1 g/L) (Mallinckrodt Bakker), $MgCl_2*6H_2O$ (0.5 g/L) (Mallinckrodt Bakker, St. Thomas, US virgin islands), $ZnSO_4*7H_2O$ (0.0014 g/L) (Sigma-Aldrich), $MnSO_4*H_2O$ (0.0016 g/L) (Sigma-Aldrich), $CoCl_2*6H_2O$ (0.0036 g/L) (Sigma-Aldrich), and $FeSO_4*7H_2O$ (0.00275 g/L) (Sigma-Aldrich, St. Louis, MO). 2.74 g/L yeast extract (DOT Scientific Inc., Burton, MI) was used as the sole nitrogen source. The carbon sources were synthetic sugars (composed of a mixture of glucose and xylose using similar concentrations found in the co-hydrolysates), and lignocellulosic biomass co-hydrolysates. Corn stover and switchgrass co-hydrolysates were diluted to obtain similar sugar concentrations to those found in miscanthus and giant reed co-hydrolysates. The pH of the medium was adjusted to 6.0 ± 0.1 before autoclaving. 250 mL Erlenmeyer flasks were filled with 50 mL of growth medium and sterilized at 121°C for 15 minutes. The growth medium was inoculated with a 10% (volume/volume (v/v)) seed culture and cultivated at 25 ± 0.5°C on a rotary shaker (Thermal Scientific, Odessa, Texas) with an agitation speed of 180 rpm.

2.4 Mass Balance

Mass balance analysis was based on the co-hydrolysis and fermentation data. Lipid productivity was calculated by the amount of lipid accumulated during fermentation divided by the dry weight of initial lignocellulosic biomass per day.

2.5 Analytical Methods

The sugar yield from combined dilute acid pretreatment and enzymatic hydrolysis was calculated to evaluate the performance of co-hydrolysis and conduct the mass balance analysis. The sugar yield was determined by the ratio of the measured amount of sugars (glucose and xylose) in co-hydrolysates to the dry weight of initial biomass and also calculated as the percent of theoretical glucose and xylose yields.

The combined severity factor (Log CS), which couples reaction conditions of time, temperature, and acid concentration into a single variable, was used to compare sugar yields. Combined severity is calculated as follows: Log CS = t * exp [(TH-TR)/14.75] – pH, where t is reaction time in minutes, TR is

the hydrolysis temperature in °C, and TH is the reference temperature of 100°C (Lloyd & Wyman, 2005); after pretreatment, the pH measurement was determined by a pH meter (Fisher Scientific, PA).

Mycelia were collected by filtration and washed twice with distilled water. Cell mass was determined by drying in an oven at 105 ± 1°C to obtain a consistent weight. Glucose, xylose, acetic acid, formic acid, furfural and HMF in the co-hydrolysates and fermentation broths were determined by High Performance Liquid Chromatography (Shimadzu prominence) equipped with a Bio-rad Aminex HPX-87H analytical column and a refractive index detector. The mobile phase was 0.005 mol/L sulfuric acid with a flow rate of 0.6 mL/min. The column temperature was set at 65°C. HPLC standards including glucose (Catalog Number: 49158), xylose (Catalog Number: 95729), sodium acetate (Catalog Number: S8750), sodium formate (Catalog Number: 17841), HMF (Catalog Number: 53407), and furfural (Catalog Number: 185914) were purchased from Sigma-Aldrich, St. Louis, MO. Dried mycelia were ground in a mortar and used for lipid extraction. Total lipid was determined gravimetrically (Ruan & Zanotti, 2012).

2.6 Statistical Analysis

A general linear model using R software (R Version 2.15.0, Vienna, Austria) was applied to the experimental data in order to perform an analysis of variance (ANOVA) and multiple comparisons. Tukey's test, using a comparison-wise type I error rate of 0.05, was adopted to compute honestly significant differences among different feedstocks regarding sugar yield and inhibitor generation. Within each feedstock, the significance of individual factors, as well as the interactions between factors on sugar conversion and inhibitor generation, was identified using ANOVA analysis. Bonferroni's test was carried out at an experimental type I error rate of 0.05 to conduct multiple comparisons of both sugar and inhibitor production under different co-hydrolysis conditions.

2.7 Results and Discussion

2.7.1 Degradation of Carbohydrates into Sugars and Inhibitors

The composition of lignocellulosic biomass indicated that cellulose was the most abundant fraction in each feedstock (Table 2-1). Corn stover and switchgrass exhibited similar but higher cellulose content (36.3% and 37.4%, respectively) compared to miscanthus (34.2%) and giant reed (29.7%), respectively. The hemicellulose fraction in each feedstock was of a xylan type as indicated by the relatively high amounts of xylose in the polysaccharide. The xylan content for corn stover (22.0%) and switchgrass (22.1%) were equivalent; while miscanthus (19.0%) and giant reed (19.2%) had comparatively lower levels. In addition, miscanthus and giant reed contained more lignin (22.9% and 22.1%, respectively), compared to switchgrass (20.9%) and corn stover (18.6%).

Dilute acid pretreatment and enzymatic hydrolysis using both the conventional and co-hydrolysis processes were represented in Figure 2-1. Results from the co-hydrolysis process at various pretreatment conditions for the different feedstocks are listed in Tables 2-2, 2-3, 2-4, and 2-5 and summarized in Figures 2-2, 2-3, 2-4, 2-5, 2-6, 2-7, 2-8, and 2-9, respectively. ANOVA and Tukey's HSD analysis indicated that there were significant ($p < 0.0001$) mean differences on sugar yields between feedstocks, with the exception of miscanthus and giant reed ($p > 0.05$).

The glucose and xylose yield for corn stover co-hydrolysis was dependent on the main factors of pretreatment temperature and sulfuric acid concentration ($p < 0.05$), but independent of treatment time ($p > 0.05$). There were also significant two-way interactions between time and temperature ($p < 0.05$), time and sulfuric acid concentration ($p < 0.05$), and temperature and sulfuric acid concentration ($p < 0.05$). A pretreatment condition of 130°C, 2% H_2SO_4 for 1 h led to the greatest sugar yield of 0.545 g/g dry initial biomass and 83.3% of theoretical glucose and xylose yield for corn stover, producing a total of 52.09 ± 0.13 g/L of fermentable sugars, including 31.1 ± 0.38 g/L glucose and 21.0 ± 0.51 g/L xylose (Table 2-2, Figure 2-2).

ANOVA analysis indicated that the glucose and xylose yield of switchgrass co-hydrolysis was independent of pretreatment temperature, time and sulfuric acid concentration. The effects of two-factor interactions between time and sulfuric acid concentration ($p < 0.05$), as well as temperature and sulfuric acid concentration ($p < 0.05$) were significant. The treatments of 2% H_2SO_4 at 130°C for 2 h had the highest glucose and xylose yields of 0.44 g/g dry initial biomass and 65.8% of theoretical glucose and xylose yield (Table 2-3). It generated 22.8 ± 1.22 g/L glucose and 19.23 ± 0.16 g/L xylose (Figure 2-3).

The glucose and xylose yield for giant reed co-hydrolysis was shown to be dependent on the main effects of pretreatment temperature ($p < 0.0001$), sulfuric acid concentration ($p < 0.05$), and pretreatment time ($p < 0.05$). Two-way interactions of time and sulfuric acid concentration ($p < 0.0025$), and temperature and time ($p < 0.0001$)) were also significant. Two different pretreatment conditions (3% H_2SO_4 at 130°C for 1 h, and 2% H_2SO_4 at 130°C for 1 h) were both shown to have the highest glucose and xylose yield of 0.355 g/g dry initial biomass at 64.7% of the theoretical glucose and xylose yield (Table 2-4). However, the concentrations of individual sugars from these two pretreatment conditions are significantly different ($p < 0.05$). The treatment condition of 3% H_2SO_4, 130°C, and 1h generated 18.35 ± 0.67 g/L glucose and 15.56 ± 0.46 g/L xylose, while the treatment condition of 2% H_2SO_4 at 130°C for 1 h generated 16.71 ± 0.76 g/L glucose and 17.2 ± 0.14 g/L xylose, respectively (Figure 2-4).

For the miscanthus co-hydrolysis glucose and xylose yield, ANOVA analysis showed that the main pretreatment factors of temperature ($p < 0.0001$), time ($p < 0.0001$), and sulfuric acid concentration ($p < 0.05$) had significant impacts, while the effects of two-factor interaction terms: temperature and time ($p < 0.0001$), temperature and sulfuric acid concentration ($p < 0.05$), and time and sulfuric acid concentration ($p < 0.05$) as well as the three-factor interaction of temperature, time and sulfuric acid concentration ($p < 0.05$) were also considered statistically significant. The highest glucose and xylose yield was 0.349 g/g dry initial biomass at 58.1% of the theoretical glucose and xylose yield from the treatment of 2% H_2SO_4 at 130°C for 2 h (Table 2-5), corresponding to 17.76 ± 0.94 g/L glucose and 15.55 ± 0.08 g/L xylose, respectively (Figure 2-5).

Comparison of co-hydrolysis of the four feedstocks demonstrates that combined glucose and xylose yields of miscanthus and giant reed were significantly ($p < 0.0001$) lower than corn stover and switchgrass. The lower conversion yield was possibly due to those feedstocks having relatively higher lignin contents which likely hindered the enzyme action due to steric actions or absorbing active enzymes during the enzymatic hydrolysis step in the co-hydrolysis process (Liao, et al., 2005).

The co-hydrolysis of corn stover generated the lowest mean concentration of acetic acid (3.92 ± 0.29 g/L) (Table 2-2), followed by switchgrass (4.04 ± 0.32 g/L) (Table 2-3), miscanthus (5.12 ± 0.65 g/L) (Table 2-5) and giant reed (5.66 ± 0.86 g/L) (Table 2-4). Tukey's HSD analysis showed that corn stover and switchgrass had no significant ($p > 0.05$) differences for acetate generation at the comparison-wise type I error rate of 0.05, while miscanthus and giant reed were significantly different from corn stover and switchgrass ($p < 0.0001$). Under the best co-hydrolysis conditions of sugar production, the acetic acid concentrations reached 6.43 g/L in miscanthus hydrolysate (Table 2-5) and 6.79 or 6.31 g/L for giant reed (Table 2-4), respectively. In contrast, an acetate concentration of 4.08 g/L was obtained from co-hydrolysis of corn stover (Table 2-2) and 4.2 g/L from switchgrass, respectively (Table 2-3).

Furfural and HMF are generated by thermal conversion of pentose and hexose sugars. The experimental results (Tables 2-2, 2-3, 2-4, and 2-5) demonstrated that, among the four feedstocks, corn stover hydrolysate exhibited the lowest mean concentrations of furfural (0.54 ± 0.31 g/L) and HMF (0.0918± 0.018 g/L), switchgrass hydrolysate had the highest mean furfural concentration (0.77 ± 0.41 g/L), and giant reed hydrolysate had the highest mean HMF concentration (0.24 ± 0.12 g/L). Tukey's HSD analysis demonstrated that there were significant differences in HMF concentrations between corn stover and giant reed ($p < 0.0001$), miscanthus and giant reed ($p < 0.0001$), as well as switchgrass and giant reed ($p < 0.0001$). However, miscanthus and corn stover ($p > 0.05$), switchgrass and corn stover ($p > 0.05$), switchgrass and miscanthus ($p > 0.05$) were not significantly different in regards to HMF concentrations. Tukey's HSD also indicated that there were no significant differences on furfural concentrations among the four feedstocks ($p > 0.05$).

2.7.2 Combined Severity Factor Analysis

The effects of combined severity factor on the co-hydrolysis of the four feedstocks are presented in Figures 2-6, 2-7, 2-8, and 2-9. The concentrations of glucose, xylose, acetate, HMF and furfural were chosen as the responses in order to evaluate the effects of the combined severity factor. The general trend for glucose production in the feedstocks was an initial increase with greater combined severity factor followed by a decrease at higher severity factor levels. Corn stover was an exception to this trend showing no decrease in glucose production even at higher severity levels. It was clear that xylose release was negatively associated with the combined severity factor for all materials due to the degradation of xylose into byproducts, mainly furfural, under harsh conditions. This corresponded well to the fact that furfural generation was positively correlated with the combined severity factor in all samples. Acetic acid likewise showed a positive correlation with combined severity factor, particularly for miscanthus and giant reed. However, HMF production appeared to be independent of the combined severity factors for the range being studied.

The maximum overall sugar concentrations for corn stover, switchgrass, giant reed and miscanthus were obtained in the combined severity factor ranges of 1.94, 2.14, 1.98 and 2.18, or 2.25, respectively (Tables 2-2, 2-3, 2-4, and 2-5). This indicated that the three herbaceous perennial energy crops required harsher pretreatment conditions compared to corn stover. The combined severity analysis effectively allowed for the observation of sugar and inhibitor trends for co-hydrolysis with regards to the dilute acid pretreatment parameters.

2.7.3 Microbial Lipid Accumulation from Lignocellulosic Hydrolysates

The hydrolysates of corn stover, switchgrass, miscanthus and giant reed obtained from the most effective co-hydrolysis conditions were evaluated for their potential uses as carbon sources to cultivate *M. isabellina* for lipid accumulation. The cell mass, lipid, and lipid productivity of *M. isabellina* cultivation, along with sugar concentrations and C/N ratios in the culture medium are shown in Table 2-6.

The deleterious effects of acetic acid, furfural, and HMF on cell growth and lipid accumulation of yeasts has been studied (Chen, Zhao, & Xia, 2009) (Hu, Zhao, Zhao, Wu, & Zhao, 2009) (Huang, Wu, Liu, Li, & Zong, 2012), however, information regarding their effects on oleaginous fungal lipid accumulation is quite limited. The experimental results from this study demonstrated that *M. isabellina* cultured on the different co-hydrolysates, with the exception of giant reed co-hydrolysate, exhibited comparable lipid accumulation compared to synthetic hydrolysate (Table 2-6). The switchgrass co-hydrolysates accumulated 4.4 ± 0.44 g/L lipid at a culture time of 118 h with initial acetic acid, HMF and furfural concentrations at 3.25 ± 0.088, 0.07 ± 0.002 and 0.97 ± 0.02 g/L, respectively, in the fermentation broth. Miscanthus co-hydrolysate produced 3.71 ± 0.45 g/L lipid at the same culture time with initial concentrations of acetic acid, HMF and furfural in the fermentation broth of 5.01 ± 0.039, 0.07 ± 0.0009 and 0.92 ± 0.02 g/L, respectively. The corn stover co-hydrolysate produced 3.18 ± 0.02 g/L lipid at 88 h with initial acetic acid, HMF and furfural concentrations of 2.4 ± 0.004, 0.02 ± 0.0004 and 0.19 ± 0.0097 g/L, respectively, in the culture broth. The synthetic hydrolysate produced 3.15 ± 1.13 g/L lipid at 68 h. While giant reed co-hydrolysate generated 3.02 ± 0.31 g/L lipid at 136 h accompanied by initial acetic acid, HMF and furfural concentrations of 6.2 ± 0.0735, 0.19 ± 0.0004 and 0.51 ± 0.019 g/L in culture media, respectively. Compared to the culture on synthetic hydrolysate, the increased cell mass from co-hydrolysates could be attributed to the strain's capability of utilizing acetic acid and minor sugars such as galactose and arabinose (Chen, Zhao, & Xia, 2009) (Fei, et al., 2011) (Ruan & Zanotti, 2012) found in the co-hydrolysate. Experimental data also indicate that there is a correlation between the period of lag phase and inhibitor concentrations which lead to the difference of lipid productivity among various co-hydrolysates. It was found that the higher acetic acid, HMF and furfural concentrations in the fermentation broth, such as giant reed, the longer the lag phase and vice versa.

2.7.4 Material Balances

Mass balance analysis demonstrated the effects of co-hydrolysates on utilization efficiency of different lignocellulosic biomass for microbial lipid production (2-10). One kg of dry corn stover contains

0.363 kg cellulose, 0.22 kg xylan, and 0.186 kg lignin. After co-hydrolysis, 0.33 kg of glucose and 0.20 kg of xylose were obtained resulting in the production of 0.0628 kg lipid from fungal fermentation (0.0628 g lipid/g corn stover). One kilogram of dry switchgrass contains 0.374 kg cellulose, 0.221 kg xylan and 0.205 kg lignin. Cellulose and xylan were converted to 0.25 kg glucose and 0.20 kg xylose enabling *M. isabellina* to accumulate 0.0722 kg lipid (0.0722 g lipid/g switchgrass). One kg of dry miscanthus and giant reed were able to produce 0.0499 kg and 0.0421 kg lipid, respectively (0.0499 and 0.04211 g lipid/g biomass).

The mass balance analysis demonstrated that the *M. isabellina* lipid accumulation on co-hydrolysates were superior to other widely studied lipid producing strains such as yeasts grown on similar feedstocks. It has been reported that the oleaginous yeast *Trichosporon fermentans* grown on un-detoxified sulfuric acid pretreated rice straw hydrolysate containing glucose (5.1 g/L), xylose (25.5 g/L), arabinose (4.6 g/L), acetic acid (1.4 g/L), furfural (0.5 g/L), and HMF (0.08 g/L) resulted in a lipid yield of 0.017 g lipid/g dry biomass (Huang, Zong, Wu, & Liu, 2009). The comparison of lipid yield showed filamentous fungi could be a better microbial option to accumulate lipid from lignocellulosic biomass.

2.8 Conclusion

This study indicates that the co-hydrolysis process is a technically feasible method to maximize biomass conversion (generate C5 sugars, C6 sugars and acetic acid), and eliminate the need of a large amounts of water for washing and detoxification. The oleaginous fungus *M. isabellina* ATCC 42613 further demonstrated its unique capacity to not only utilize glucose, xylose and acetic acid in the hydrolysates to accumulate fungal lipids, but also tolerate relatively high concentrations of inhibition products. These results conclude that combining co-hydrolysis of lignocellulosic feedstocks and *M. isabellina* cultivation for lipid accumulation could be a promising solution for advanced lignocellulosic fuel production.

3 Chapter 3

3.1 Abstract

Energy balance data is presented for a proposed solar-bio-powered lignocellulosic biodiesel refinery utilizing aerobic filamentous fungal fermentation grown on corn stover co-hydrolysis. The biorefinery assumes eight different unit operations including (1) corn stover collection and transportation, (2) corn stover pretreatment and enzymatic hydrolysis, (3) lignin processing, (4) fungal lipid fermentation, (5) fungal biomass drying, (6) lipid extraction and transesterification, (7) anaerobic digestion and aerobic treatment of wastewater, and (8) solar-bio-power generation. Results demonstrate that aerobic fungal lipid fermentation is the most energy-demanding unit operations, accounting for nearly 49% of the total energy input. Integration of solar-bio-power generation and fungal lipid accumulation is shown to be an effective approach for creating a sustainable lignocellulosic biodiesel production with a positive energy balance. Compared to concentrated solar power (CSP), photovoltaics (PV) technology is more suited to satisfy the energy needs of the lignocellulosic biodiesel production. The PV-bio-powered lignocellulosic biodiesel production has a net energy output of 28.83 MJ/kg biodiesel produced, and a net energy ratio (NER) of 1.11.

Keywords: Anaerobic digestion, Co-hydrolysis Net energy ratio (NER), Energy balance

3.2 Introduction

The world economy today is virtually petroleum-based, however, concerns over its sustainability and environmental impact have led to calls for alternative forms of energy production. While many alternative sources of energy exist such as wind, solar, geothermal, and biomass; biofuels represent a near term solution to alleviating dependence on the petroleum based economy while mitigating environmental impacts (Meher, Sagar, & Naik, 2006).

Biofuels are defined as liquid or gaseous transportation fuels that are principally produced using biomass as a feedstock (Yusuf, Kamarudin, & Yaakub, 2011). Currently, most biofuels on the market are

those termed “first-generation” which are produced from various food crops such as corn, soybean, canola, or other vegetable oils. First-generation fuels face their own drawbacks. Any increased demand for first-generation fuel crops may result in land use changes on a domestic or international level. If areas containing a significant amount of stored carbon such as forests or peat bogs are converted to agriculture production (which stores comparatively less soil-organic carbon compared to forests and bogs), then a net release of sequestered carbon into the atmosphere may occur, creating a carbon debt that could possibly take the biofuel production system decades to pay off (Mani, Sokhansanj, Tagore, & Turhollow, 2010). Increased production of first-generation biofuels may also lead to competition with food resources, and possibly lead to shortages and or price increases of feed commodities. One possible solution is the use of lignocellulosic materials as biofuel feedstock, termed second-generation biofuels, which avoid competition with food resources, and may provide greater environmental benefits than first-generation biofuels. Lignocellulosic materials include municipal solid waste, pulp and paper waste, forest and agricultural residues, and dedicated woody and herbaceous energy crops, and represent the most abundant source of renewable carbon on earth with over 200 billion tons created annually (Chandel & Singh, 2011). Current U.S. policy favors the displacement of petroleum-based fuels with lignocellulosic derived sources as seen by the Renewable Fuels 2 Standard in the U.S. Energy and Independence Act of 2007. This law mandates the production of 36 billion gallons of biofuels by 2022, with 21 billion gallons coming from lignocellulosic sources while capping first-generation biofuels at 15 billion gallons (Coyle, 2010).

In order for biofuels to be a viable alternative to petroleum-based fuels they must demonstrate that they can be produced in a sustainable manner. This means that an ideal biorefinery system should have a positive net energy contribution from the feedstock source; otherwise it is no different than a traditionally energy-intensive pulp mill which creates fiber as its primary product and uses residual lignin as a supplemental power source through combustion (Zhu & Zhuang, 2012). Evaluating the energy output of a biofuel system requires a detailed evaluation of all input and output values. Energy life cycle analysis (ELCA) is a method for determining the cumulative energy inputs from different sources as well as the

total efficiency of the production process (Pradhan, et al., 2011). Energy balance methodology involves accounting for the amount of energy used during the production and comparing it to the amount contained in the biofuel. The results obtained from the energy balance analysis can be expressed as the ratio of energy produced to energy consumed by a production system, otherwise known as the net energy ratio (NER). Increase of NER for biofuel production is key to establishing a sustainable biorefining industry.

Renewable energy sources such as solar, wind, geothermal, and biomass could be applied to supplement power to biorefineries and improve their NER. Among these sources, solar energy with the advantages of abundance and availability represents an excellent candidate to be combined with a lignocellulosic refinery to produce biofuels. Many solar technologies have been developed in the past several decades such as silicon wafer PVs, thin-film PV, low-temperature solar thermal systems, and CSP systems (Mills, 2001) (Shukla, Sumathy, Erickson, & Gong, 2013) (Price H. , et al., 2002) (Green, 2007). According to their solar conversion mechanisms, these solar technologies are mainly divided into two categories: PV and CSP. PV uses semiconducting materials to directly covert photons into electrons and generate electricity, while CSP uses heat absorbers or reflective surfaces to collect solar thermal energy for both low temperature (hot water heater) and high temperature (CSP technologies) applications. Hydrogenated amorphous silicon (a-Si:H) thin film PV and parabolic trough power generation (CSP technology) are selected as the solar power units to be studied for the lignocellulosic biofuel system. Integrating these solar technologies with lignocellulosic biofuel production could lead to a novel biorefining concept that replaces fossil fuel consumption during the process and maximizes NER for next-generation biofuel production.

Much of the literature on lignocellulosic biofuels has focused on the ethanol production process, where a pretreatment step is first utilized to disrupt the macromolecular structure of the material, and then the solid portion is separated and washed so that hydrolytic enzymes can penetrate and hydrolyze the glucose polymer chain into its monomeric glucose constituents for ethanol fermentation. No general analyses have been conducted on the production of biodiesel from lignocellulosic feedstocks. Our

previous studies developed a new lignocellulosic biodiesel production method to significantly improve process efficiency via removing the solid and liquid separation step after feedstock pretreatment, though the process energy consumption without using other renewable energy is still high, and corresponding energy balance is not favorable for commercial lignocellulosic biodiesel production.

Therefore, the goal of this chapter is to develop a lignocellulosic biodiesel refining system using the combination of biological and solar technologies to create a neutral energy balance, and thus a sustainable biofuel product. The mass and energy balance was first conducted based on individual unit operations, and then a system analysis was carried on to conclude an energy neutral lignocellulosic biodiesel refinery.

3.3 Methodology

In keeping with LCA principles, two main steps were identified to carry out the analysis. First, we determined the major unit operations in a lignocellulosic biodiesel refinery capable of producing 20 million gallons of biodiesel a year, including (1) corn stover collection and transportation to the biorefinery, (2) corn stover pretreatment and enzymatic hydrolysis, (3) lignin processing, (4) fungal lipid fermentation, (5) fungal biomass drying, (6) lipid extraction and transesterification, (7) anaerobic digestion and aerobic treatment of wastewater, and (8) solar-bio-power generation. The boundary for the process is shown below Figure 3-1, which is described in detail in the following sections. Second, we aimed to conduct an energy inventory in order to calculate the net energy production and thus determine the amount of solar energy input needed to create a sustainable biofuel product. Individual mass inputs to the biorefinery system were converted to common energy units based on their life-cycle energy content (when known), which is defined as the energy needed to produce and transport the final product (Pradhan, et al., 2011). The energy equivalent for fuel sources combusted for energy production in the refinery were taken to be their low heating value (LHV) (Pradhan, et al., 2011). Based on the mass and energy balance, the net energy analysis (NER) for the system was determined. A sensitivity analysis was likewise conducted in order to identify those factors which could be adjusted in a reasonable manner based on

current literature knowledge. A functional unit of 1 kg of biodiesel produced will serve to quantify the mass and energy products throughout the biorefinery system.

Environmental considerations are not explored as this study is focused on the net energy production of a conceptualized stand-alone biorefinery. Facility construction, and the production of capital equipment are likewise excluded from the scope of this study, since these impacts are typically considered negligible when weighed against the total amount of product manufactured over their useful lifetimes (International 2010). Several co-products were identified during this biorefinery operation including lignin and glycerol. A mass-based system expansion approach is used to account for these co-products. Energy inputs associated with seed cultivation during fermentation were ignored in this study. Likewise, the electricity usage associated with the downstream recovery and pumping of products was ignored as those technologies vary between plants and the electricity consumed by said processes is likely to be relatively small compared to upstream processes like fermentation, which can account for approximately 67% of a plants electricity usage (Vogel & Todaro, 1997). Finally, it should be noted that the when converting electricity consumption to a common energy unit, the theoretical conversion factor of 3.61 MJ/kWh was used. This value does not include inefficiencies or transmission line losses that are often included in other lifecycle analysis such as Pradhan, which used an energy equivalent of 7.4 MJ/kWh (Pradhan, et al., 2011).

Since fungal-based biodiesel production from lignocellulosic sources is still at the research stage, there are no current operating facilities to serve as a foundation for the analysis. However, many of the unit operations identified are currently used in other industries, and it is reasonable to assume that if and when such a process is developed on an industrial scale it will resemble existing practices as closely as possible (Kim & Parker, 2008). Thus, much of the data is based on current peer-reviewed resources and industry-derived reports. Thermodynamic calculations were utilized for certain process steps where existing data was deemed incomplete or inadequate, such as drying of biomass after fermentation, or the heat energy required for pretreatment reactions.

3.3.1 Corn Stover Collection and Transportation

The agriculture residue corn stover will be the lignocellulosic feedstock considered as the substrate for fungal-lipid fermentation in this study. It should be made clear that we do not view corn stover to be the only suitable source for fungal-lipid production, rather we consider it as a readily available and attainable resource which can bridge the gap until dedicated energy crop production develops. In our scenario corn stover is treated as an agriculture waste from corn production, and as such, only energies related to its collection, transport, and fertilizer replacement are examined.

The energy input of corn stover production is summarized from harvesting of the corn stover all the way to its delivery to the biorefinery gate (Morey, Kaliyan, Tiffany, & Schmidt, 2010). The following values were used in this study: (1) collection/transport to local storage of 196.9 MJ/ton corn stover, (2) local storage inputs of 30.5 MJ/ton corn stover, (3) corn stover compaction for transport of 233.7 MJ/ton corn stover, (4) transport of compacted corn stover to end users 62.4 MJ/ton corn. We also assume that harvesting corn stover likewise removes nutrients which must be replaced by adding additional fertilizer to what is typically applied during regular corn production. The amount of Nitrogen (N), Phosphorus (P), and Potassium (K) fertilizer replacements were 8.8, 0.6, and 7.2 kg/ton dry matter, respectively (Morey, Kaliyan, Tiffany, & Schmidt, 2010) (Sheehan, et al., 2004). Energy inputs for N, P, and K fertilizer production were 47.7, 13.35, and 8.09 MJ/kg, respectively (Krohn & Fripp, 2012).

3.3.2 Corn Stover Pretreatment and Enzymatic Hydrolysis

Corn stover received by the biorefinery must undergo both physical and thermochemical treatment steps in order to disrupt the macromolecular structure of lignocellulose (lignin, cellulose, hemicellulose). This allows hydrolytic enzymes to later penetrate and hydrolyze the carbohydrate polymers to their monomeric sugar constituents, which can readily be metabolized for fungal lipid production. The approximate composition of corn stover is 36.3% (w/w) cellulose, 22.0% (w/w) xylan, and 18.6% (w/w) lignin. Physical treatment is the initial size reduction of the biomass. This process is

assumed to be accomplished using a hammer mill, which has energy inputs of approximately 180 MJ/ton of herbaceous biomass (Zhu & Zhuang, 2012).

A novel co-hydrolysis process for lignocellulosic pretreatment and enzymatic saccharification is integrated into this analysis, while co-products are utilized for additional energy production. Co-hydrolysis refers to an initial pretreatment, directly followed by enzymatic saccharification without detoxification and liquid–solid separation between these two steps in order to convert lignocellulose into monomeric sugars (glucose and xylose).The dilute sulfuric acid pretreatment was the thermochemical treatment utilized in this case, where milled corn stover is mixed with water and sulfuric acid, and heated at a particular temperature for a predetermined amount of time. Dilute acid pretreatment is efficient at solubilizing much of the hemicellulose fraction as well as small amounts of the lignin. Our previous study found optimal corn stover pretreatment conditions to be 10% solid loading (w/w), 2% acid (w/w) at 130°C for 1 h (Ruan Z. , Zanotti, Zhong, Ducey, & Liu, 2013). Energy consumption to heat the pretreatment slurry to its desired temperature is calculated using the following equation.

$$E_{slurry} = M_{slurry} \times C_{p-slurry} \times \Delta T \quad (1)$$

Here E_{slurry} (kJ) is the energy consumption for heating the pretreatment slurry, M_{slurry} is the total mass of the slurry (kg), $C_{p\text{-}slurry}$, is the specific heat capacity of the slurry (3.964 kJ/kg/°C), and ΔT is the temperature difference between the initial slurry temperature (13°C) and final desired final temperature (130°C) (Kim & Parker, 2008). Latent heat of vaporization is not factored into the equation due to the low reaction temperature and high system pressure. Energy to heat the reaction vessel up to the desired temperature is ignored in this study. The pretreatment step is also assumed to replace any additional sterilization steps since the process occurs at a high enough temperature, time, and at an adequately low pH.

After thermochemical pretreatment is complete, the hydrolysate is cooled to 50°C. Regenerative heat exchange is assumed to occur between the hot pretreatment slurry at 130°C and the incoming water

used for the next pretreatment batch. This unit operation has some uncertainty since the 10% solids loading may cause issues with recovering heat in a heat exchanger system which requires further exploration. The total heat recovered is assumed to follow equation 1, with the exception of ΔT being 80°C. It is assumed that 50% of the recovered heat is used to maintain the enzymatic reaction temperature of 50°C, and other 50% is for preheating the pretreatment slurry. Hydrolytic enzymes are added to the cooled slurry at 50°C in order to cleave the hydrolytic bonds in the remaining cellulose and hemicellulose chains. An enzyme loading of 47 kg protein/ton biomass was used in our previous study resulting in a total sugar yield of 83.3% (Ruan Z. , Zanotti, Zhong, Ducey, & Liu, 2013). A lignin solubility of 13% at our pretreatment conditions is assumed, which is consistent with past studies (Chen, Zhao, & Xia, 2009).

3.3.3 Lignin Processing

Biomass residues remaining after enzymatic hydrolysis consist primarily of the insoluble lignin fraction at 87% of its original value. Studies show that net system energy can be improved by either combusting the residual lignin for process uses, or refining it into value-added products. This study will utilize lignin for combustion due its relatively high low heating value (LHV) of 24.4 MJ/kg (Tomani, 2009). Lignin is typically dewatered to 65% moisture content before undergoing a drying process to further reduce its moisture content before combustion. The energy required for lignin drying must consider not only the total lignin mass, but also its water content to account for the heat of vaporization, as shown by the following equation.

$$E_{lignin} = M_{wl} \times W \times [(C_{p-water} \times \Delta T) + \Delta H_v] + [M_{wl} \times (1 - W)] \times (C_{p-lignin} \times \Delta T) \quad (2)$$

Here, E_{lignin} is the total energy to dry the dewatered lignin, M_{wl} is the total mass of wet lignin after dewatering (kg), W is the percent of water in lignin, ΔH_v is the latent heat of water at 75°C (2321.37 kJ/kg), $C_{p\text{-}water}$ is the specific heat capacity of water (4.187 kJ/kg/°C), $C_{p\text{-}lignin}$ is the specific heat capacity of solid lignin (1.1 kJ/kg/°C), and ΔT is the temperature difference between the initial lignin temperature (40°C) and the drying temperature (75°C) (Kim and Parker 2008) (Voitkevich, Kabo, Blokhin,

Paulechka, & Shishonok, 2012). A boiler efficiency (the percentage of feedstock heating value converted to steam) of 80% is assumed (Humbird, et al., 2011).

3.3.4 Fungal Lipid Fermentation

Energy consumption is a major consideration in aerobic fermentation systems. Many components can affect the final power consumption such as agitation, air compression, refrigeration, pasteurization, and downstream processing. Identifying the operating conditions and unit operations is a key step towards accurately accounting for all energy inputs. Aerobic fungal fermentation for biodiesel production has no current known commercial applications in which to base this analysis on. However, a viable process for producing and extracting gamma-linolenic acid (GLA) from the aerobic culture of the filamentous fungus *Mucor circinelloides* has been previously implemented and serves as the process basis for the analysis (Cohen & Ratledge, 2010). The fermentation and downstream processing steps considered in this study include (1) fermentation, (2) pasteurization, (3) filtration/drying, and (4) lipid extraction. Electricity data for the filtration/drying step is ignored in this study.

Our previous experimental data for the filamentous fungus *M.* isabellina were used to carry out the mass and energy balance of the fungal-lipid fermentation (Ruan Z. , Zanotti, Zhong, Ducey, & Liu, 2013). A fungal cell mass concentration of 12.84 g cell/L, comprised of 24.82% lipid was reported at 96 h of batch culture. This equates to a lipid yield of 0.0624 g lipid/g corn stover (Ruan Z. , Zanotti, Zhong, Ducey, & Liu, 2013).

Due to the fact that the *M. isabellina* fermentation is exothermic, the reaction heat needs to be removed to maintain the desired fermentation temperature of 27°C. The oxygen uptake rate for the fungus is 1.0 mol/kg dry fungal biomass/h (unpublished data). Since the amount of energy released from consumption of one mol oxygen for aerobic cultures is approximately 460 kJ, the reaction heat generated during the fermentation can be calculated by multiplying oxygen uptake and energy released per mol oxygen consumption (Doran, 2013). Heat added due to agitation and the cooling effects of evaporation were ignored in this study. Centrifugal water-cooled chillers are selected to cool the fermentor. The

chiller efficiency (defined as kW electricity needed to remove 12,661 kJ in 1 h) is assumed at 0.6 kW/ton. The electricity demand for cooling the fermenters can then be calculated using heat of the fermentation and chiller efficiency.

The fermentors for this process are assumed to contain 100 m^3 of broth, continuously stirred, with air sparged through the fermentation liquid. The aeration rate is 0.5 m^3 air/m^3 broth/min. The culture time is 96 h. Energy input for the process, largely electricity for agitation and air compression, is calculated as follows (Alves & Vasconcelos, 1996).

$$P = \frac{P_g}{\eta_g} + \frac{P_c}{\eta_C} \quad (3)$$

Here, P_g, P_c, and P are agitation power (W), compressed power (W), and total electrical power (W), respectively. The parameters η_g and η_C are the global efficiencies for agitation and compressor, respectively. Air compressor power is described by the following equations.

$$P_c = \alpha_1 Q \quad (4)$$

$$\alpha_1 = \frac{\gamma}{\gamma-1} P_0 \left[\left(\frac{P_1}{P_0}\right)^{\frac{\gamma-1}{\gamma}} - 1\right] \quad (5)$$

Here Q is the volumetric air flow rate (m^3/s), α_1 is the coefficient described by equation 5, P_0 is the atmospheric pressure (N/m^2), P_1 is the compressor outlet pressure (N/m^2), and $\gamma = 1.4$ for air compression. Agitation power (P_g) is described by equation 6 below.

$$P_g = \left(0.90 + 2.1\, e^{-\frac{7.32 Q P_0}{P_2}}\right) N_P \rho N^3 D^5 \quad (6)$$

Here N_p is the un-aerated power number, ρ is the fluid density (kg/m^3), N is the rotation speed (revolutions/s), P_2 is the pressure at the bottom of the vessel (N/m^2), and D is the agitator diameter (m) (Alves & Vasconcelos, 1996). Assumed values for each parameter are listed in Table 3-1.

Fungal cells remain metabolically active after substrate consumption, and may begin to consume their oil reserves. As previously detailed, a pasteurization step is necessary to inactivate fungal lipases

and phospholipases in order to prevent any unwanted loss in oil content. It has been reported that heating the fermentor to 60°C for approximately 30 minutes prior to harvesting can alleviate these issues. Since fermentation is an exothermic reaction, the cooling process water can simply be shut off, allowing the fermentor to reach the desired temperature without any additional heating or cooling input (Cohen & Ratledge, 2010). Therefore, the energy requirement for pasteurization step is not accounted for in this case.

3.3.5 Fungal Biomass Drying

Harvesting the fungal biomass generally includes mechanical dewatering followed by a final thermal drying step. Following mechanical dewatering, the biomass is assumed to have a water content of 80%. The drying temperature is assumed to be 105°C, while the incoming biomass temperature is assumed to be 55°C, slightly lower than the broth temperature after pasteurization. Energy to dry the remaining fungal biomass can similarly be calculated using equation 2 with the following changes. M_{wl} is replaced by the total weight of wet fungal mass (M_f), $C_{p\text{-}lignin}$ is replaced with specific heat capacity of dry fungal cells ($C_{p\text{-}f}$), H_v is 2066.16 kJ/kg at 105°C, and ΔT is 50°C. The specific heat capacity for fungal biomass ($C_{p\text{-}f}$) was calculated based on the composition of the dry cell mass of *M. isabellina* shown in equation 7 (Singh & Heldman, 2009).

$$C_{p_f} = 1.424 \times X_h + 1.549 \times X_p + 1.675 \times X_f + 0.837 \times X_a \quad (7)$$

Here, X is the mass fraction, and the subscripts h, p, f, and a, represent carbohydrate (0.48), protein (0.22), fat (0.25), and ash content (0.05), respectively. The coefficients represent the specific heat capacity for each mass fraction and are expressed in units of kJ/kg/°C.

3.3.6 Lipid Extraction and Transesterification

Lipid extraction from dried fungal cells can be modeled using a soybean oil extraction processes without any additional or specialized equipment (Cohen & Ratledge, 2010). Mass and energy data regarding fungal cell crushing, lipid extraction, crude oil refining, and transesterification were collected

from the U.S. Soybean Board's 2009 life-cycle analysis study (Pradhan, et al., Energy Life Cycle Assessment of Soybean Biodiesel, 2009). Consistent with industry-wide practices for soybean biodiesel production, fungal cells will undergo hexane extraction followed by crude oil refining to remove impurities. Refined oil is converted to biodiesel through an acid catalyzed transesterification process. 0.12 kg of glycerol co-product is produced for every 1 kg of biodiesel. Glycerol possesses a relatively large low heating value (LHV) of 16 MJ/kg and is combusted to produce heat in this study (Emami, Tabil, & Adapa, 2015).

3.3.7 Wastewater Treatment

Fermentation effluent after fungal cell harvest is treated by a combined anaerobic digestion, aerobic treatment, and reverse osmosis (RO) process to extract more energy out of the remaining organic matter (OM), reclaim the water, and recycle it back to the process. Anaerobic digestion (AD) first utilizes the OM in the effluent to produce methane biogas as an energy by-product, and prepare the effluent with fewer nutrients for the following aerobic treatment. A continuously stirred mesophilic digester operating at 35°C is assumed to carry out the anaerobic digestion. The fermentation effluent has a chemical oxygen demand (COD) of 35 g/L. The COD reduction of 80% is set for the digestion. Energy usage by the AD system includes electricity for mixing the digestate, as well as heat necessary for raising the waste to the desired temperature. Parasitic electricity cost is assumed to be 2% of the total heat produced by methane combustion, and parasitic heat demand for heating the reactor will follow equation 1, with C_p being that for water (4.18 kJ/kg/°C), and ΔT being 15°C. Total methane production is based on an assumed yield of 0.25 kg CH_4/kg COD destroyed (Khanal, 2009). The effluent waste after anaerobic treatment will have a COD of 7 g/L and will then undergo further aerobic treatment. The required aerobic energy input is calculated based on an energy consumption of 0.317 kWh/m^3 for the effluent with a COD concentration of 500 mg/L (Water Environment Federation, 2009). A reverse osmosis (RO) and hydrated lime treatment is recommended to be implemented, which could simultaneously produce reclaimed water and concentrated NaOH solution for the pretreatment uses, and improve the process efficiency. The reclaimed

water from aerobic wastewater treatment process still has 2% Na_2SO_4. A reverse osmosis unit with 80% recovery of the feed water can convert 1 kg reclaimed water into 0.8 kg pure water and 0.2 kg brine solution. Hydrated lime is then applied on the brine solution to generate $CaSO_4$ and 10% NaOH solution. $CaSO_4$ is settled and removed from the solution. 10% NaOH could be re-used as alkali in the pretreatment. The energy consumption for the RO and lime treatment is 3.35 kWh/m^3 reclaimed water (von Gottberg, Pang, & Talavera, 2012).

3.3.8 Combined Solar and Biological Power Generation

Two solar technologies, PV and CSP, were investigated to be combined with lignin and methane combustion to generate the power to satisfy the energy needs of the system. Since the power generation principles of PV and CSP are different, PV-biological power and CSP-biological power were compared to compare the effects of different solar technologies on the energy balance of the sustainable lignocellulosic biodiesel production system.

The PV-biological power generation includes an amorphous silicon (a-Si:H) thin film PV unit and lignin/methane combustion unit (Figure 3-2). The PV module is used solely for electricity generation. The a-Si:H thin film PV is selected because of its low temperature coefficient (0.1%/°C) that allows the PV unit to be operated at a wide range of temperatures without substantial power loss (Pathak, Pearce, & Harrison, 2012). The electricity generated from the PV module is used to power the unit operations in the lignocellulosic biodiesel production. The lignin/methane combustion unit (boiler) is dedicated to generate thermal energy for the heat demand of the lignocellulosic biodiesel production. The PV-biological power generation has advantages of direct electricity generation and high utilization efficiencies of electricity and heat. The PV panels need to be 37^0 tilted at Meade County, Kansas to obtain maximal solar collection. The average solar radiation available to be extracted by PV is 18 MJ/m^2/day at Meade County (Marion & Wilcox, 1994). The electricity conversion efficiency of the thin film PV is 12%. The thermal efficiencies of boilers for lignin/methane combustion are set at 95%.

Parabolic trough technology is currently a proven commercial CSP technology on the market today. The parabolic trough solar system is capable of concentrating solar energy to generate steam up to 400°C with a solar thermal efficiency between 30-40% (Price H. , et al., 2002). Therefore, the CSP-biological power generation uses parabolic solar trough power technology to be integrated with lignin/methane combustion to provide power to the lignocellulosic biodiesel production system (Figure 3-3). Both parabolic solar trough and lignin/methane combustion technologies generate thermal energy to produce electricity and heat for process uses. The CSP-biological power generation includes parabolic solar trough receiver, boilers, and steam turbine cogeneration. Combining solar thermal energy with lignin/methane combustion has advantages of solving unsteady energy flow issues of solar radiation (using lignin/methane combustion during the period without solar radiation) and alleviating the demand of large solar thermal storage. Meade, KS was again the location for the studied system. The parabolic trough receivers were one-axis tracing parabolic troughs with horizontal north-south axis. The mirror facet uses aluminum skins with a cardboard honeycomb core and 3M's EPC-305+ polymeric reflector (Price H. , et al., 2002). The average solar radiation available to be extracted by the parabolic trough receiver is 18 MJ/m^2/day at Meade County, Kansas (Price H. , et al., 2002). Solar-radiation-to-steam thermal efficiency (considering radiation and convection receiver losses, piping and storage thermal losses, and heat-medium to steam thermal losses) is assumed to be 78%. The thermal efficiency of the boilers was set at 95%. The electricity and thermal efficiency of the steam turbine cogeneration are assumed at 25% and 60%, respectively.

3.4 Results and Discussion

Mass and energy data for each unit operation in the biorefinery system are listed below in Tables 3-2, and 3-3. The net energy production for the entire system is found to be -113.79 MJ/kg biodiesel produced. This equates to an NER value of 0.58. The sensitivity analysis indicates that the lipid yield is the most important parameter to significantly improve the net energy output (reducing a large amount of the energy demand).The sensitivity analysis indicates that lipid yield is the most important parameter to

improve the net energy output (reducing a large amount of the energy demand). The net energy output for the system without combined solar-bio-power generation is changed by 50% responding to a 20% change of lipid yield (Table 3-4) With combined solar-bio-power generation, the net energy outputs are improved to 28.83 and 357.59 MJ/kg biodiesel for the systems with PV- and CSP-bio-power units, respectively; the corresponding NER values are changed to 1.11 and 2.32 (Table 3-5). The detailed interpretation for individual unit operations and integrated system are discussed as follows.

3.4.1 Corn Stover Collection and Transportation

Mass balance data in Table 3-2 reveals that nitrogen fertilizer replacement is the largest individual input for this operation stage at 0.15 kg/kg biodiesel, followed by potassium fertilizer replacement (0.12 kg/kg biodiesel), diesel fuel for harvesting stover (0.10 kg/kg biodiesel), and phosphorus fertilizer replacement (0.01 kg/kg biodiesel), respectively.

Total energy consumption for corn stover collection and transportation amounted to 13.23 MJ/kg biodiesel. Paralleling its mass input, nitrogen fertilizer replacement is shown to be the greatest energy consumer at 6.97 MJ/kg biodiesel, followed by diesel fuel for harvest (5.15 MJ/kg biodiesel), and phosphorus fertilizer replacement (0.13 MJ/kg biodiesel), respectively. Energy demands for harvesting and fertilizer replacement significantly outweigh those for transportation to the biorefinery, indicating that focusing efforts on reducing travel distance to the biorefinery have less of an impact than upstream processes. This is largely owed to the energy-intense production of agricultural chemicals, particularly nitrogen fertilizer. Advances by the fertilizer industry have greatly increased the efficiency of nitrogen production over the last several decades, and may continue to play a role, however, near-term energy reductions will most likely be achieved through optimizing fertilizer application rates. This is poses its own issues as there is little agreement in the literature as to the proper nutrient replacement scheme after stover harvest. Petrolia indicates that in a corn-soybean rotation, nitrogen supplementation would be unnecessary after stover harvest (Petrolia, 2008), while Brechbill and Tyner show nitrogen replacement values nearly identical to those assumed in this study (Brechbill & Tyner, 2009). Sensitivity analysis data

on fertilizer application rates shown in Table 3-4 indicate that slight reductions in nitrogen fertilizer input can lead to significant energy savings. Future work in this area should seek to minimize nutrient inputs while maximizing stover removal. Ways to address this issue could include researching crop rotation schemes to minimize nitrogen replacement and maximize stover output, or developing dedicated stover harvesters to reduce fuel usage. Due to the high uncertainty with this stage of the biorefinery, continued efforts are needed in order to study the effects of a fully-developed con stover harvesting supply system.

3.4.2 Corn Stover Pretreatment

Mass balance data for corn stover pretreatment shows significant inputs for both corn stover (16.59 kg/kg biodiesel) and water (147.67 kg/kg biodiesel) (Table 3-2). These two components represent the largest single mass contributors in the entire system. The water input during pretreatment accounts for just over 96% of all water consumption. Though water usage is significant, the co-hydrolysis process actually serves to conserve process water compared to traditional pretreatment methods. This is due to the fact that, once pretreatment is complete, the liquid portion of the slurry is retained and further processed. Without co-hydrolysis, water consumption would be double its current value, or 295.34 kg/kg biodiesel.

Total energy consumption during this processing stage amounted to 42.68 MJ/kg biodiesel, or roughly 16% of the total energy input. The bulk of this energy is comes from the thermal heat input required to raise the pretreatment slurry to its final temperature. Due to the water conservation step during co-hydrolysis, significant energy savings are realized, as any additional water input would likewise require heat energy for sterilization. This means that any reduction in water usage leads to a proportionate drop in energy consumption. Again, without co-hydrolysis the thermal energy input would be nearly double its current value, increasing it to 85.26 MJ/kg biodiesel.

Thermal energy demand is largely dependent on the reaction temperature, time and solids loading of the pretreatment steps. Therefore, any adjustments to the pretreatment process should focus efforts on increasing solids loading. Adjusting the corn stover solids input from 10% to 12% results in a decrease of the net energy deficit from -42.68 to -34.74 MJ/kg biodiesel, respectively. However, it should be noted

that at higher solids loadings, additional water must be added in order to match the dilution requirements imposed by the *M. isabellina* fermentation, thus the energy savings may not be as great as suggested. Though co-hydrolysis comes with certain drawbacks, namely increased toxin concentrations leading to longer fermentation times and lower lipid productivity, it is shown to have great utility in water and energy savings compared to more common methods (Ruan & Zanotti, 2012). Enzymatic hydrolysis requires a significant input of enzymes (0.65 kg protein/kg biodiesel) (Figure 3-4). The enzyme loading (65.2 g protein/kg corn stover) is approximately four times higher than what is reported by other literature studies with similar pretreatment conditions (Zhu & Zhuang, 2012). Unpublished data from our lab indicates that enzymatic loading can be further dropped by nearly quarter with little loss in saccharification efficiency, thus halving the energy input for this step could be achieved (the result is not used for the mass and energy balance analysis in this study). Reducing enzyme loading, without sacrificing total sugar yield may require more severe pretreatment conditions in regards to temperature and time, invariably increasing the concentration of compounds toxic to fermentation.

3.4.3 Lignin Processing

Utilizing residual lignin as a fuel source is critical to improving the overall energy balance of the biorefinery system. After enzymatic hydrolysis, 3.85 kg lignin rich residue/kg biodiesel remains, along with 15.44 kg water/kg biodiesel, which is lost to evaporation (Figure 3-4). Lignin residues provide approximately 75.30 MJ/kg biodiesel of energy, more than enough to offset the energy demand associated with lignin drying (38.98 MJ/kg biodiesel) (Table 3-3). The sensitivity analysis shows that changing drying temperature of the lignin process has a relatively large impact on the net energy output (Table 3-4). A 20% reduction in the drying temperature leads to a 6.7% saving on the net energy output.

3.4.4 Fungal Lipid Fermentation

Aerobic fungal fermentation proved to be by far the largest energy consumer of all unit operations at 132.42 MJ/kg biodiesel (Table 3-3), approximately 49% of all energy input. Much of the energy needs associated with this process are due to the electricity consumption required to agitate, aerate

and cool the fermentation broth, and as a result of the long fermentation time. Large reductions in energy input are possible with modest adjustments to mixing speed and culture time as shown in the sensitivity analysis (Table 3-4). When agitation speed and aeration rate are reduced from 90 rpm and 0.5 volume gas flow/volume liquid/minute (vvm) to 72 rpm and 0.4 vvm respectively, the energy demand of agitation and aeration is cut from 111.83 to 91.03 MJ/kg biodiesel. If fermentation time is shortened from 96 h to 76.8 h, energy consumption is cut to 107.79 MJ/kg biodiesel. It should be noted that the total kWh calculated for this study based on the assumed parameter values may be on the low side. For instance, the calculated air electric power consumption for the air compressor component of fermentation is approximately 10,500 kWh using Alves' assumptions. However, looking at a fully loaded centrifugal air compressor operating at 3 barg air pressure and supplying 45 m^3/min (equivalent to the 0.5 vvm used in this study) the total power draw is 260 kW, or nearly 25,000 kWh usage at 96 h of fermentation time (Ingersoll Rand, 2015). This is an approximately 2.5 times greater power draw than assumed in this study, meaning that the already very high electricity usage may be even higher.

Decreasing fermentation time is likely best addressed to maximize lipid yields by maximizing lipid productivity. Ruan demonstrate that once the initial lag phase is overcome in dilute-acid co-hydrolysis, the lipid productivity is similar to that when grown on synthetic sugar substrate (Ruan & Zanotti, 2012). Aeration rate and agitation speed are both critical parameters as demonstrated in this study. Any proposed large-scale culture should thus strive to decrease agitation and aeration rates by all possible means meanings to reasonable levels while still maintaining sufficient lipid yields.

3.4.5 Fungal Biomass Drying

Once fungal fermentation is complete, a total of 13.67 kg wet biomass/kg biodiesel is produced for harvest. Drying the fungal biomass evaporates 10.94 kg water/ kg biodiesel requiring an energy input of 27.56 MJ/kg biodiesel, in order to reduce the moisture content to near 0%. Much of this energy input is due to the latent heat of vaporization of water. Decreasing energy consumption for this unit process is likely to come from upstream optimization of the enzymatic hydrolysis or fermentation parameters in

order to increase biomass yield. The sensitivity analysis also demonstrates that reducing the drying temperature to 84°C can reduce the net energy output by 4.84%.

3.4.6 Lipid Extraction and Transesterification

Extraction and downstream processing of fungal oil follows well-established industrial practices from the soybean biodiesel industry including lipid extraction, oil refinement, and transesterification. Microbial lipid extraction converts 2.73 kg of fungal biomass into 1.04 kg of fungal lipid, and is the most energy intense step in this unit operation, accounting for 7.64 MJ/kg biodiesel, mainly due to thermal energy requirements. The extraction also requires 2.65 kg of water/kg biodiesel. The final transesterification step producing 1 kg of biodiesel requires 0.14 MJ, and produces 0.120 kg of glycerol co-product (Figure 3-4). Combustion of waste glycerol produces enough energy (2.27 MJ/kg) to power the transesterification process. The whole lipid extraction and transesterification requires 7.78 MJ/kg biodiesel to power the operation (Table 3-3).

3.4.7 Wastewater Treatment

Applying anaerobic digestion to treat fungal fermentation wastewater significantly improves the overall energy balance of the biodiesel refinery by adding 47.79 MJ/kg biodiesel (Table 3-3). The methane production of 0.96 kg/kg biodiesel is from the digestion on the fermentation and lipid extraction wastewater (Figure 3-4). After the anaerobic digestion, the AD effluent of 134.82 kg/kg biodiesel is treated by the aerobic treatment process that consumes 0.154 MJ energy. 132.12 kg reclaimed water from the aerobic treatment is further treated by a combined RO and hydrated lime treatment. 1.793 MJ/kg biodiesel and 2.44 kg lime/kg biodiesel are used by the treatment to convert the reclaimed water into 105.70 kg clean water and 22.46 kg wet $CaSO_4$ (Figure 3-4).

3.4.8 Solar-Bio-Power Generation

The total energy demand for a 20 million gallon per year corn stover biodiesel production is 270.39 MJ/kg biodiesel (Table 3-6). The process co-products of lignin, glycerol, and fermentation

wastewater are used to generate energy via combustion and anaerobic digestion in order to off-set the energy demand of the production process. The lignin and glycerol combustion (with 95% boiler thermal efficiency) can generate 71.54 and 2.16 MJ/kg biodiesel of thermal energy, respectively (Table 3-5) The methane from the anaerobic digestion of fermentation effluent can be combusted (with 95% boiler thermal efficiency) and produces 45.40 MJ/kg biodiesel of thermal energy (Table 3-5). The total energy output of the stand-alone lignocellulosic biodiesel production system is 156.60 MJ/kg biodiesel including both biodiesel and thermal energy of lignin/methane/glycerol combustion (Table 3-6). The net energy output is -113.79 MJ/kg biodiesel, and the corresponding NER is only 0.58, which means that the energy output from the utilization of these co-products is not enough to cover energy the demands of biodiesel production (Table 3-6, Figure 3-5). Thus, a renewable energy source, solar energy in this case, is integrated into the onsite power generation system to realize a self-sustaining lignocellulosic biodiesel production.

PV and parabolic trough CSP are combined with biological power generation to evaluate their impacts on the energy efficiency of the solar-bio-powered lignocellulosic biodiesel production facility and determine the preferred system configuration. Since the electricity demand of the lignocellulosic biodiesel production is the biggest portion of the overall energy uses usage (Figure 3-5), 147.62 MJ electricity/kg biodiesel is used as the baseline to for the analysis. According to the yearly average solar radiation at Meade KS, the PV-biological power system uses 0.19 m^2/kg biodiesel thin-film PV panel (12,416,705 m^2 PV panel for a 20 million gallon lignocellulosic biodiesel process) to generate 147.62 MJ electricity/kg biodiesel, and combusts glycerol, lignin and methane to produce 119.10 MJ thermal energy/kg biodiesel. The total energy output (including thermal energy from combustion, electricity from PV, and biodiesel energy) is 299.22 MJ/kg. The NER for the process with the PV-biological power generation is 1.11 (Table 3-6), thus making the lignocellulosic biodiesel production an energy positive process, and completely sustainable (Figure 3-6). In the case of the CSP-biological power generation, solar heat from CSP, lignin, glycerol, and methane are used to generate steam and power a steam co-generator to produce

electricity and heat for the process uses (Figure 3-3) Since the thermal efficiency of the steam co-generator for electricity generation is 25% and boiler efficiency is 95%, the total thermal energy needed from solar heat, lignin, glycerol, and methane to produce 147.62 MJ electricity/kg biodiesel is 590.48 MJ/kg biodiesel. Subtracting the thermal energy (119.10 MJ/kg biodiesel) from combustion of lignin, glycerol and methane, the thermal energy needed from solar heat is 471.38 MJ/kg biodiesel (Figure 3-7) which requires an equivalent parabolic trough solar panel area of 0.10 m^2/kg biodiesel (6,722,000 m^2 parabolic trough solar panel for a 20 million gallon lignocellulosic biodiesel process). Using the CSP-biological power generation, the NER of the studied lignocellulosic biodiesel production is increased to 2.32 (Table 3-6)

The energy balance analysis demonstrates that an energy positive lignocellulosic biodiesel production can be achieved by integrating biodiesel fermentation technology with solar and biological power generation. Considering the total energy output, the CSP-biological power generation uses a smaller solar panel area (0.10 m^2/kg biodiesel) to generate more energy (627.98 MJ/kg biodiesel) to power the process than the PV-biological power generation with corresponding panel area and energy output of 0.19 m^2/kg biodiesel and 299.22 MJ/kg biodiesel (Table 3-6). However, the largest energy demand of the studied lignocellulosic biodiesel production is electricity (Figure 3-5), the CSP-biological power generation with steam co-generation system has 25% thermal efficiency for electricity generation, which leads to a huge amount of extra heat. The lignocellulosic biodiesel production only uses 25% of this thermal energy, and the remaining 75% of the thermal energy is not needed by the biodiesel production process. This means that the CSP-biological power generation is not exactly matched with energy demand of the lignocellulosic biodiesel production. In addition, as an on-site power plant unit, the simple configuration is an important criterion. CSP-biological power generation consists of solar collector, thermal storage tank, boiler, turbine, generator, and condenser, which is a fairly complicated system (Zhang, Baeyens, Degreve, & Caceres, 2013).

As for the PV-biological power generation, since it directly converts sunlight into electricity, and it avoids the low thermal efficiency issue of heat-to-electricity conversion. Even though the NER for the process combined with the PV-biological power unit (1.11) is lower than the one with the CSP-biological power unit (2.32) (Table 3-6), the PV-biological power unit has much better energy distribution between electricity and heat than the latter. 100% and 92% of the electricity and heat from the combined PV-biological power unit are used for the biodiesel production process (Table 3-6). In addition, the PV-biological power unit has a relatively simple configuration that consists of PV panel, electricity storage, and boiler for steam generation (Figure 3-2). Therefore, in order to achieve a simple and efficient self-sustaining biodiesel production system, the PV-biological power system is preferred as the power unit to be integrated with the fungal biodiesel production process.

3.5 Conclusion

This study has characterized a rudimentary mass and energy balance for a solar-bio-powered lignocellulosic biodiesel refinery. Aerobic fungal fermentation and pretreatment are the largest energy consumers in the refinery, which makes the energy balance unfavorable for lignocellulosic biodiesel production. Co-hydrolysis serves to save both significant amounts of water and energy. Incorporating solar and biological energy production into the process provides a sustainable approach to achieving an energy neutral (or positive) lignocellulosic biodiesel refinery system. The new concept of solar-bio-powered process can be further extended to other energy-intensive aerobic fermentation processes, so that aerobic biofuel and chemical production can be sustainably realized

4 Conclusion

While petroleum-based fossil fuels play an essential role in the world's economy, their increased consumption and environmental impact have raised concerns. This research, which utilizes filamentous fungal fermentation on lignocellulosic biomass as an alternative scheme for biodiesel production, represents one possible production substitute for petroleum-based diesel fuel.

Chapter 2 focused on the conversion of several different lignocellulosic sources to monomeric sugars, and using a co-hydrolysis process to grow the filamentous fungus *Mortierella isabellina* for bio-oil production. The conclusions from this study were:

- Co-hydrolysis of lignocellulosic biomass with filamentous fungal fermentation can successfully produce bio-oil for use as a feedstock for biodiesel
- Corn stover exhibited the highest sugar yield of all feedstocks examined in the study at
- The optimal condtions for corn stover pretreatment were
- Co-hydrolysis of lignocellulosic

Chapter 3 focused on a life cycle energy balance of a proposed lignocellulosic biodiesel biorefinery utilizing filamentousfungal fermentation for lipid production. The conclusions from this study were as follows:

- Several co-products are produced as a result of the lignocellulosic biodiesel refinery including: lignin remaing from the pretreatment and enzymatic hydroslysis of corn stover, fermentation effluent which can be incorporated into an anaerobic digestion scheme, and glycerol produced as a by-product of biodiesel refining. All of these co-proucts can be combusted to help off-set the energy inputs to the refinery sytem with lignin combustion providing the greatest total energy output of the co-products.

- Even with supplemental power produced from co-products, the overall net energy balance is significantly negative (even when ignoring electricity usage downstream of fermentation). The largest single energy input for the entire system is electricity usage for the aerobic fermentation stage, accounting for approximately 50% of all energy input to the system. This number may be even significantly higher when you consider the fact that no efficiency losses were factored in when converting the kWh of electricity usage to the common energy units of MJ, or the fact that the energy for air compressor usage may be underreported.
- Sensitivity analysis shows that lipid yield and productivity as well as the associated fermentation utilities are the most critical factors influencing the overall energy balance for the system and efforts to create a more energy neutral system should focus on these unit operations foremost.
- Incorporating solar PV and CSP technologies can create a positive overall energy balance with CSP requiring a smaller solar panel area to generate more energy compared to the PV. However, the extra heat energy produced from CSP is difficult to convert to electricity (the largest single input to the biorefinery system). Couple that with the complexity of CSP systems, and it is clear that a more careful analysis between the two technologies is warranted before deciding which to be the better supplement for such a biorefinery.

APPENDICES

Appendix A: Chapter 2 Tables and Figures

Table 2-1 Lignocellulosic feedstock composition

	Cellulose	Xylan	Lignin
Biomass	**(% w/w)**	**(% w/w)**	**(% w/w)**
Corn stover	36.3	22	18.6
Switchgrass	37.4	22.1	20.5
Miscanthus	34.2	19	22.9
Giant reed	29.7	19.2	22.1

Table 2-2 Corn stover pretreatment conditions and results

Dilute acid pretreatment parameters				**Sugar yields**		**Inhibitory compounds**		
Acid (% w/w)	**Temp (°C)**	**Time (h)**	**Log CS**	**Glu + Xyl (g/g dry biomass)**	**Glu + Xyl (% of theoretical yield)**	**Acetate (g/L)**	**HMF (mg/L)**	**Furfural (g/L)**
1	120	1	1.29	0.455	69.5	3.49	53.05	0.07
2	120	1	1.65	0.503	76.8	3.83	77.57	0.17
3	120	1	1.85	0.51	78	3.88	82.39	0.3
1	120	2	1.57	0.475	72.6	3.53	65.14	0.14
2	120	2	1.9	0.516	78.8	3.94	92.53	0.43
3	120	2	2.1	0.505	77.1	4.01	72.36	0.64
1	120	3	1.97	0.507	77.4	3.66	86.14	0.3
2	120	3	2.22	0.527	80.5	4.21	96.76	0.8
3	120	3	2.35	0.491	75	4.18	93.32	0.91
1	130	1	1.62	0.513	78.4	3.64	89.98	0.25
2	130	1	1.94	0.545	83.3	4.08	109.7	0.63
3	130	1	2.18	0.513	78.5	4.02	98.69	0.59
1	130	2	1.92	0.519	79.4	3.5	114.5	0.3
2	130	2	2.18	0.512	78.3	3.97	110.8	0.7
3	130	2	2.37	0.474	72.4	4.17	107.2	0.97
1	130	3	2.04	0.512	78.2	3.78	99.73	0.33
2	130	3	2.5	0.521	79.6	4.04	102	0.72
3	130	3	1.98	0.507	77.5	4.43	95.29	0.99
Mean	/	/	/	0.506 ± 0.021	77.3 ± 3.2	3.92 ± 0.29	91.8 ± 17.8	0.54 ± 0.31

Table 2-3 Switchgrass pretreatment conditions and results

Dilute acid pretreatment parameters				Sugar yields		Inhibitory compounds		
Acid (% w/w)	**Temp (°C)**	**Time (h)**	**Log CS**	**Glu + Xyl (g/g dry biomass)**	**Glu + Xyl (% of theoretical yield)**	**Acetate (g/L)**	**HMF (mg/L)**	**Furfural (g/L)**
1	120	1	1.33	0.388	57.9	3.47	98.54	0.22
2	120	1	1.65	0.421	62.9	3.8	165.8	0.28
3	120	1	1.84	0.428	64	4.02	164.1	0.39
1	120	2	1.57	0.42	62.7	3.78	165.9	0.31
2	120	2	1.93	0.436	65.2	3.81	170.6	0.43
3	120	2	2.09	0.422	63.1	4.08	140.8	0.87
1	120	3	1.89	0.432	64.6	3.91	183.2	0.52
2	120	3	2.22	0.426	63.7	4.13	135.6	1.03
3	120	3	2.32	0.422	63.1	4.5	100.8	1.32
1	130	1	1.85	0.434	64.9	3.67	187.4	0.48
2	130	1	2.04	0.438	65.5	4.05	168.6	0.88
3	130	1	2.18	0.421	62.9	4.11	140.4	0.81
1	130	2	2	0.433	64.7	3.79	191.4	0.57
2	130	2	2.14	0.44	65.8	4.2	146.9	0.96
3	130	2	2.37	0.415	62.1	4.4	136.3	1.17
1	130	3	2.13	0.437	65.3	3.88	198.7	0.56
2	130	3	2.5	0.433	64.7	4.41	155	1.29
3	130	3	2.65	0.399	59.6	4.61	132.8	1.56
Mean	/	/	/	0.425 ± 0.015	63.5 ± 2.05	4.04 ± 0.32	155.4 ± 28.3	0.77 ± 0.41

Table 2-4 Giant reed pretreatment conditions and results

Dilute acid pretreatment parameters				Sugar yields		Inhibitory compounds		
Acid (% w/w)	**Temp (°C)**	**Time (h)**	**Log CS**	**Glu + Xyl (g/g dry biomass)**	**Glu + Xyl (% of theoretical yield)**	**Acetate (g/L)**	**HMF (mg/L)**	**Furfural (g/L)**
1	120	1	1.36	0.262	43.8	4.22	102.8	0.06
2	120	1	1.67	0.305	50.9	4.79	125.8	0.17
3	120	1	1.87	0.311	51.9	4.96	116.8	0.26
1	120	2	1.58	0.303	50.6	4.55	134.6	0.18
2	120	2	1.89	0.319	53.3	4.86	121	0.37
3	120	2	2.08	0.314	52.4	5	86.8	0.62
1	120	3	1.95	0.319	53.2	4.69	109.2	0.32
2	120	3	2.23	0.332	55.3	4.98	73.8	0.76
3	120	3	2.42	0.315	52.6	5.21	66.47	1.06
1	130	1	1.74	0.341	56.9	4.81	155.6	0.37
2	130	1	1.99	0.344	57.3	5.21	85.13	0.96
3	130	1	2.17	0.327	54.5	5.02	68.68	0.91
1	130	2	1.94	0.332	55.5	5.12	201.8	0.37
2	130	2	2.25	0.349	58.1	6.43	174.2	0.89
3	130	2	2.46	0.342	56.9	6.93	114.7	0.88
1	130	3	2.24	0.328	54.8	4.67	126.3	0.41
2	130	3	2.47	0.319	53.3	5.14	74.69	1.11
3	130	3	2.63	0.304	50.6	5.52	72.83	1.49
Mean	/	/	/	0.32 ± 0.02	53.4 ± 3.35	5.12 ± 0.56	111.7 ± 37.7	0.62 ± 0.40

Table 2-5 Miscanthus pretreatment conditions and results

Dilute acid pretreatment parameters				Sugar yield		Inhibitory compounds		
Acid (% w/w)	**Temp (°C)**	**Time (h)**	**Log CS**	**Glu + Xyl (g/g dry biomass)**	**Glu + Xyl (% of theoretical yield)**	**Acetate (g/L)**	**HMF (mg/L)**	**Furfural (g/L)**
1	120	1	1.22	0.24	43.8	3.87	270.6	0.04
2	120	1	1.62	0.301	54.8	5.27	352.2	0.18
3	120	1	1.84	0.327	59.6	5.84	307.6	0.31
1	120	2	2	0.32	58.3	6.34	199.5	0.75
2	120	2	1.5	0.267	48.6	4.52	351.1	0.15
3	120	2	1.77	0.314	57.2	5.45	331.4	0.3
1	120	3	1.84	0.313	57.1	5.46	347.2	0.4
2	120	3	2.1	0.334	60.8	6.27	209.9	0.65
3	120	3	2.37	0.328	59.8	6.74	111.1	1.02
1	130	1	1.66	0.327	59.5	5.5	424.3	0.44
2	130	1	1.98	0.355	64.7	6.31	263.1	0.71
3	130	1	2.18	0.355	64.7	6.79	123.5	0.83
1	130	2	2.16	0.339	61.7	4.61	124.2	0.5
2	130	2	2.33	0.336	61.2	4.97	78	0.92
3	130	2	2.52	0.339	61.8	5.19	74.65	0.81
1	130	3	2.04	0.315	57.4	5.47	420.6	0.56
2	130	3	2.39	0.325	59.2	6.25	200	1
3	130	3	2.61	0.302	55	6.98	105	1.01
Mean	/	/	/	0.319 ± 0.028	58.1 ± 5.18	5.66 ± 0.86	238.6 ± 117.2	0.59 ± 0.33

Table 2-6 *Mortierella isabellina* lipid production on synthetic and lignocellulosic media

Carbon source	**carbon (mol/mol)**	**Glucose (g/L)**	**Xylose (g/L)**	**Cell mass (X, g/L)**	**Lipid (L, g/L)**	**%Yield L/X (g/g)**	**Lipid productivity (g/L/d)**
Synthetic hydrolysate	70.30 ± 4.8	13.66 ± 0.37	14.42 ± 0.78	10.40 ± 0.23	3.15 ± 1.13	30.66 ± 11.77	1.11 ± 0.40
Corn stover hydrolysate	68.90 ± 2.20	15.00 ± 0.04	12.60 ± 0.04	12.84 ± 0.23	3.18 ± 0.02	24.82 ± 0.75	0.87 ± 0.01
Switchgrass hydrolysate	71.50 ± 0.30	13.30 ± 0.10	15.30 ± 0.50	12.55 ± 0.37	4.40 ± 0.44	35.62 ± 3.30	0.90 ± 0.09
Miscanthus hydrolysate	71.00 ± 0.30	13.20 ± 0.10	15.20 ± 0.10	12.28 ± 0.02	3.71 ± 0.45	32.21 ± 3.18	0.76 ± 0.09
Giant reed hydrolysate	68.40 ± 2.6	12.10 ± 0.10	15.10 ± 0.10	13.75 ± 0.87	3.02 ± 0.31	21.18 ± 0.96	0.53 ± 0.05

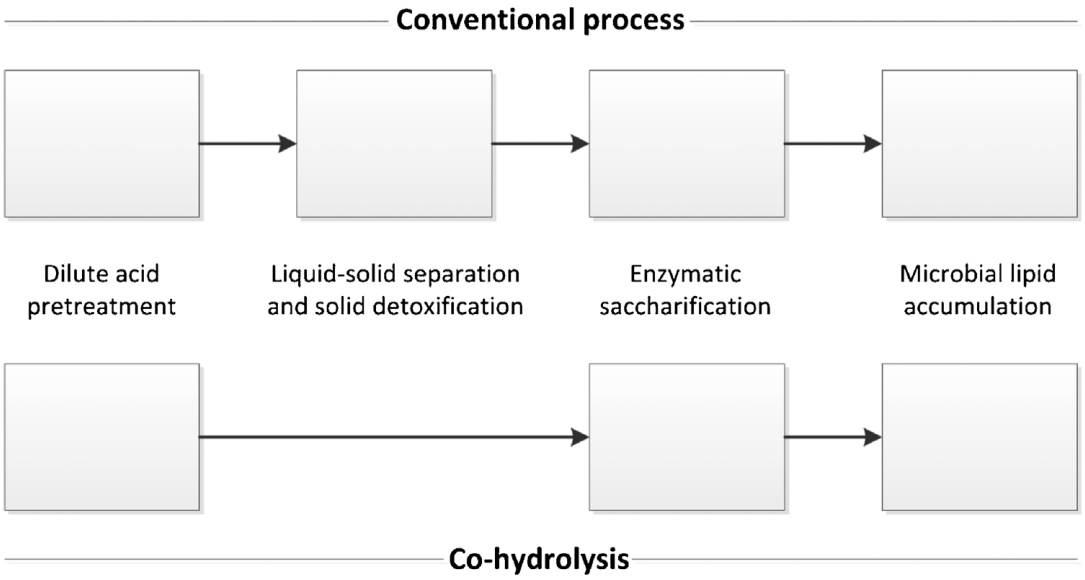

Figure 2-1 Conventional and co-hydrolysis process of dilute acid pretreatment and enzymatic saccharification operation for microbial lipid production (modified from Struder)

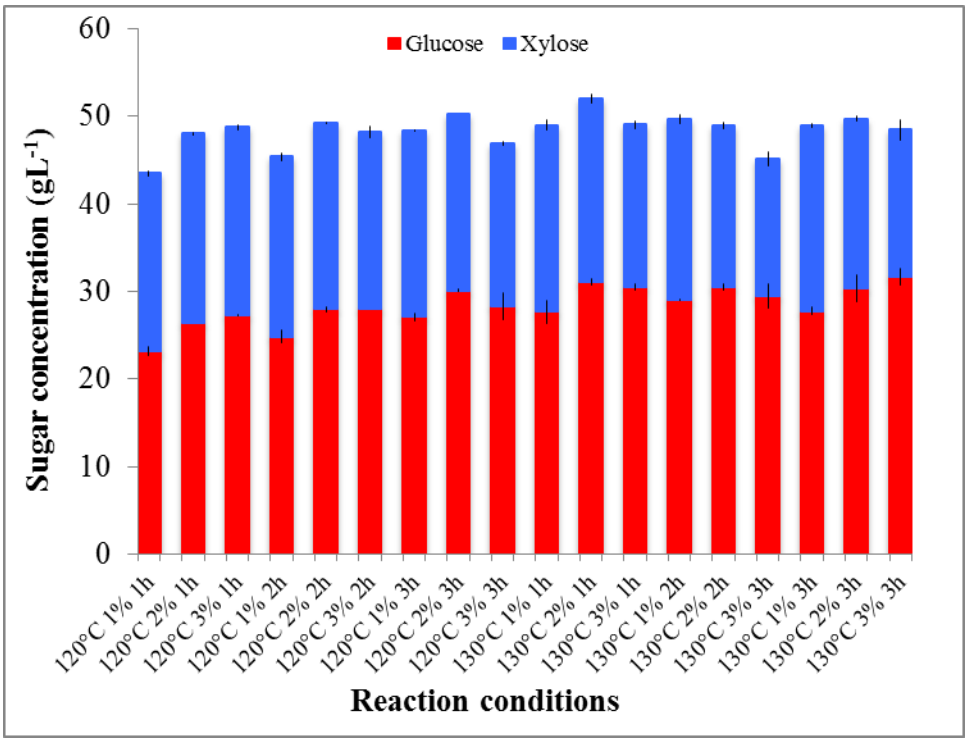

Figure 2-2 Glucose and xylose production from corn stover under different pretreatment conditions

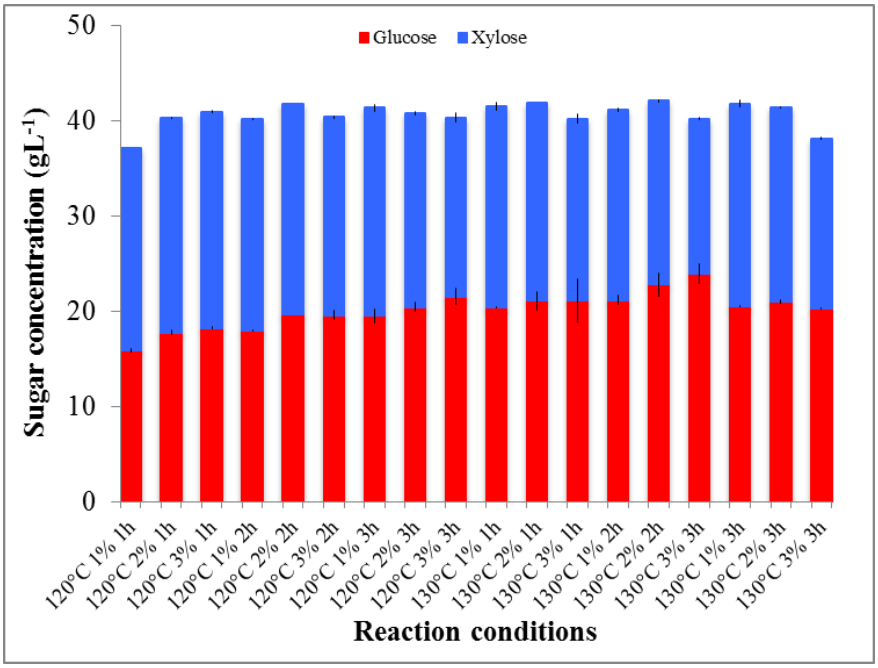

Figure 2-3 Glucose and xylose production from switchgrass under different pretreatment conditions

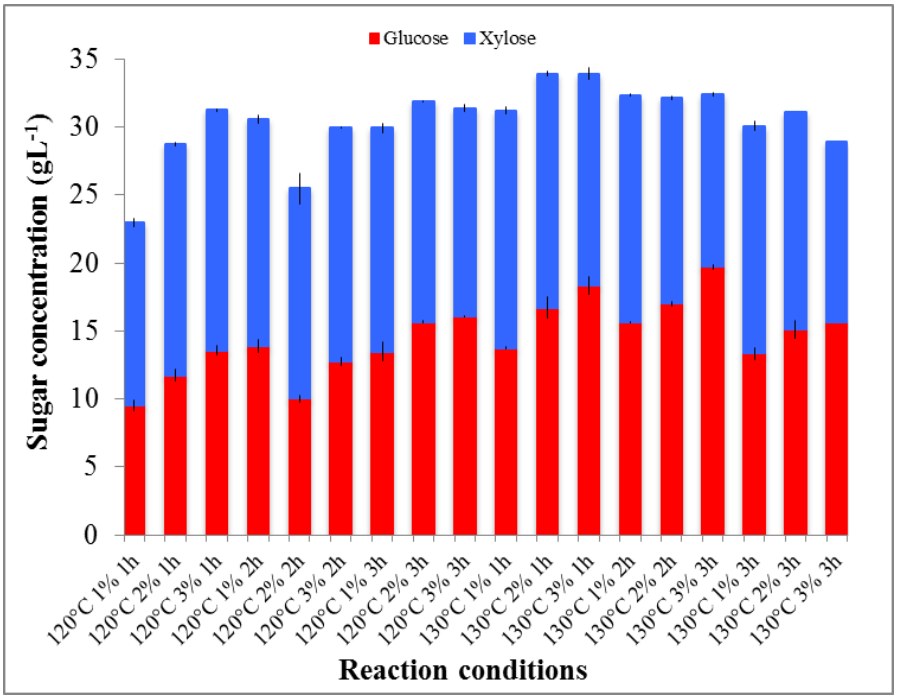

Figure 2-4 Glucose and xylose production from giant reed under different pretreatment conditions

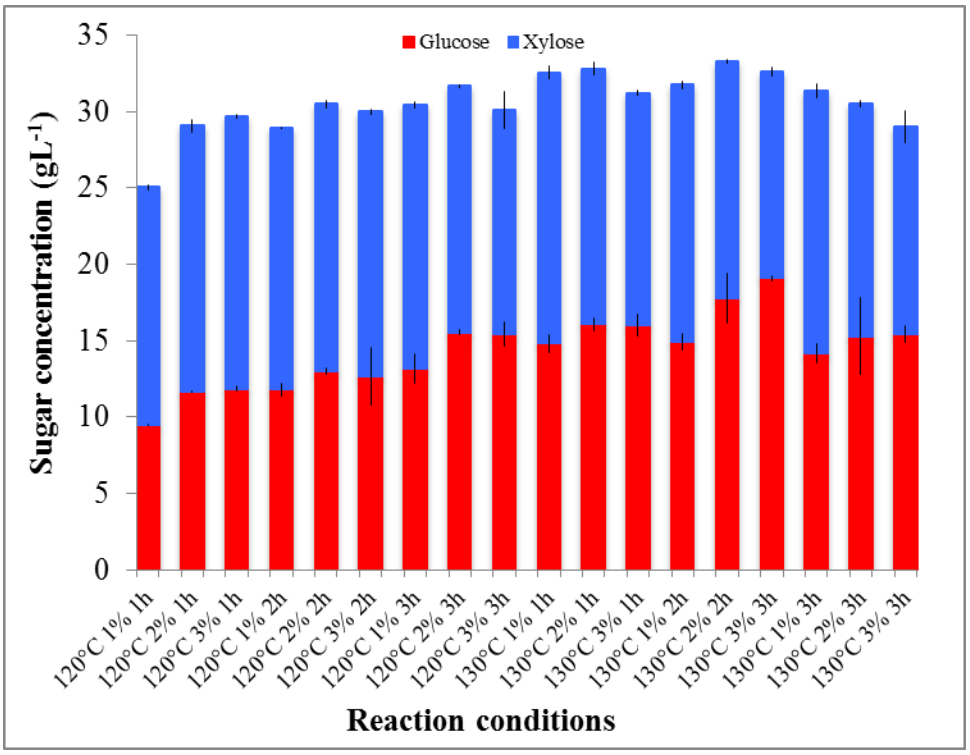

Figure 2-5 Glucose and xylose production from miscanthus under different pretreatment conditions

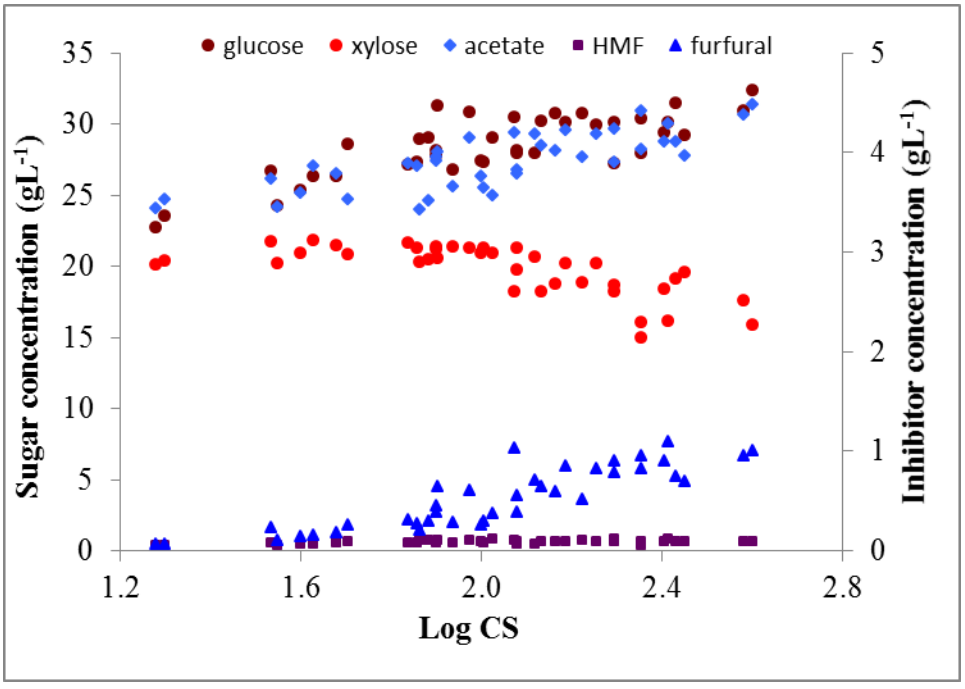

Figure 2-6 Combined severity factor effect on the conversion of glucose, xylose, acetic acid, HMF, and furfural from corn stover

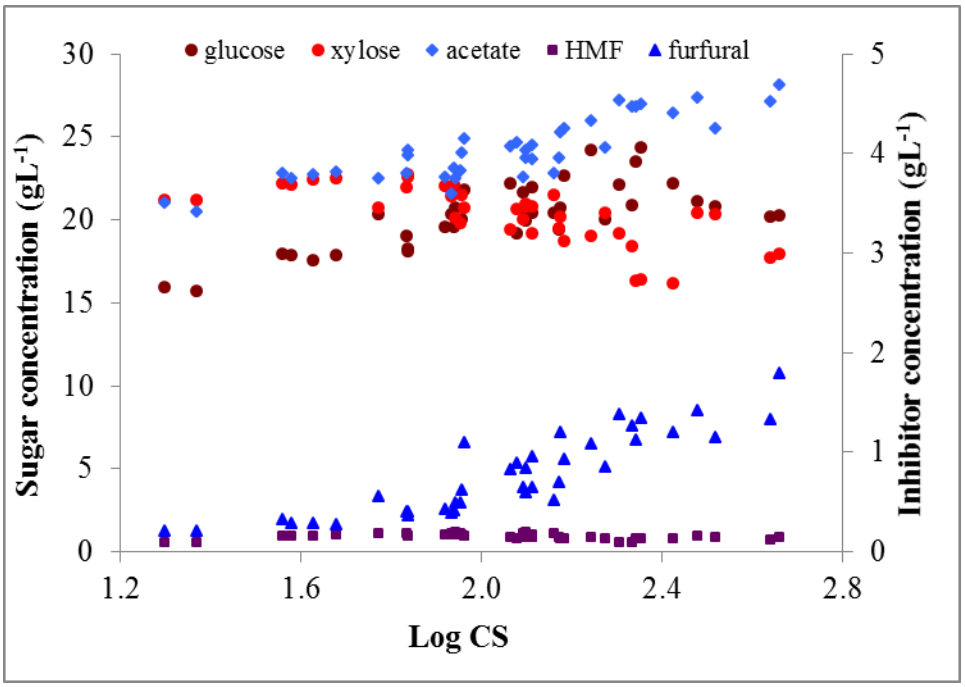

Figure 2-7 Combined severity factor effect on the conversion of glucose, xylose, acetic acid, HMF, and furfural from switchgrass

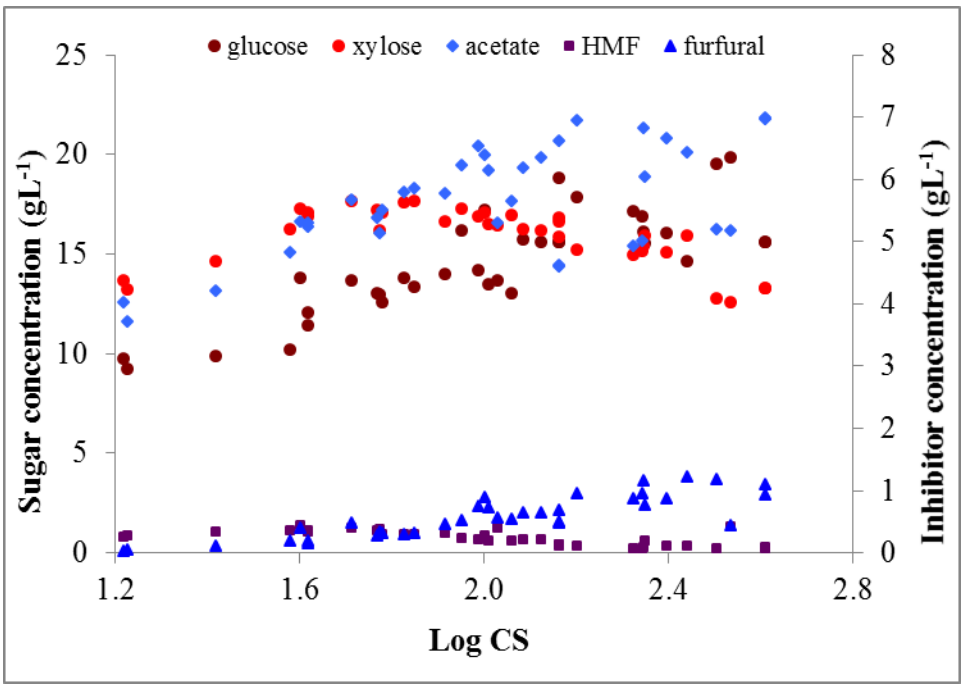

Figure 2-8 Combined severity factor effect on the conversion of glucose, xylose, acetic acid, HMF, and furfural from giant reed

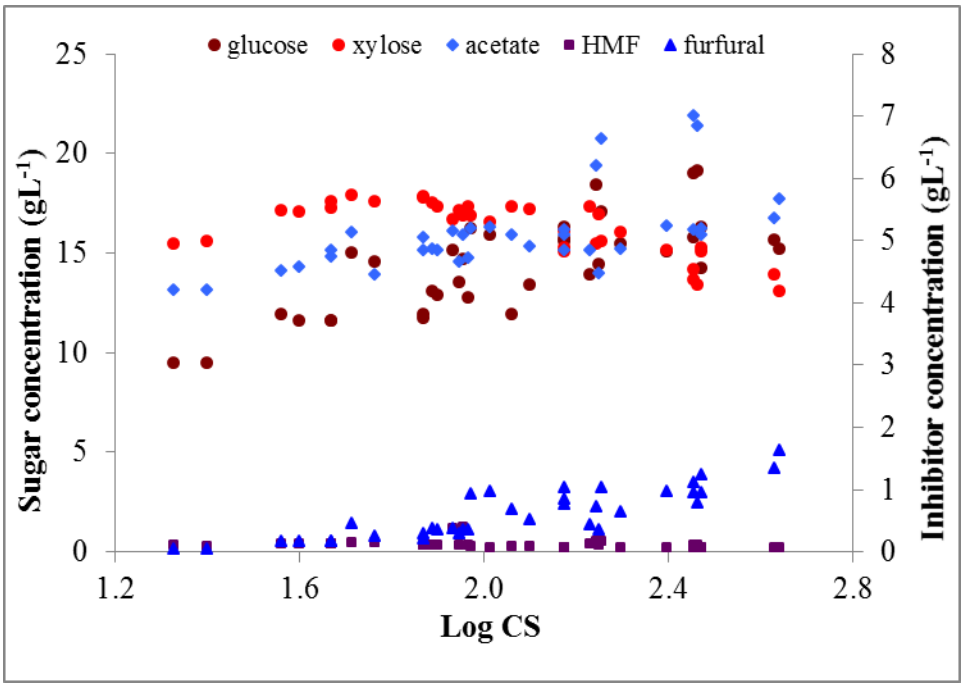

Figure 2-9 Combined severity factor effect on the conversion of glucose, xylose, acetic acid, HMF, and furfural from miscanthus

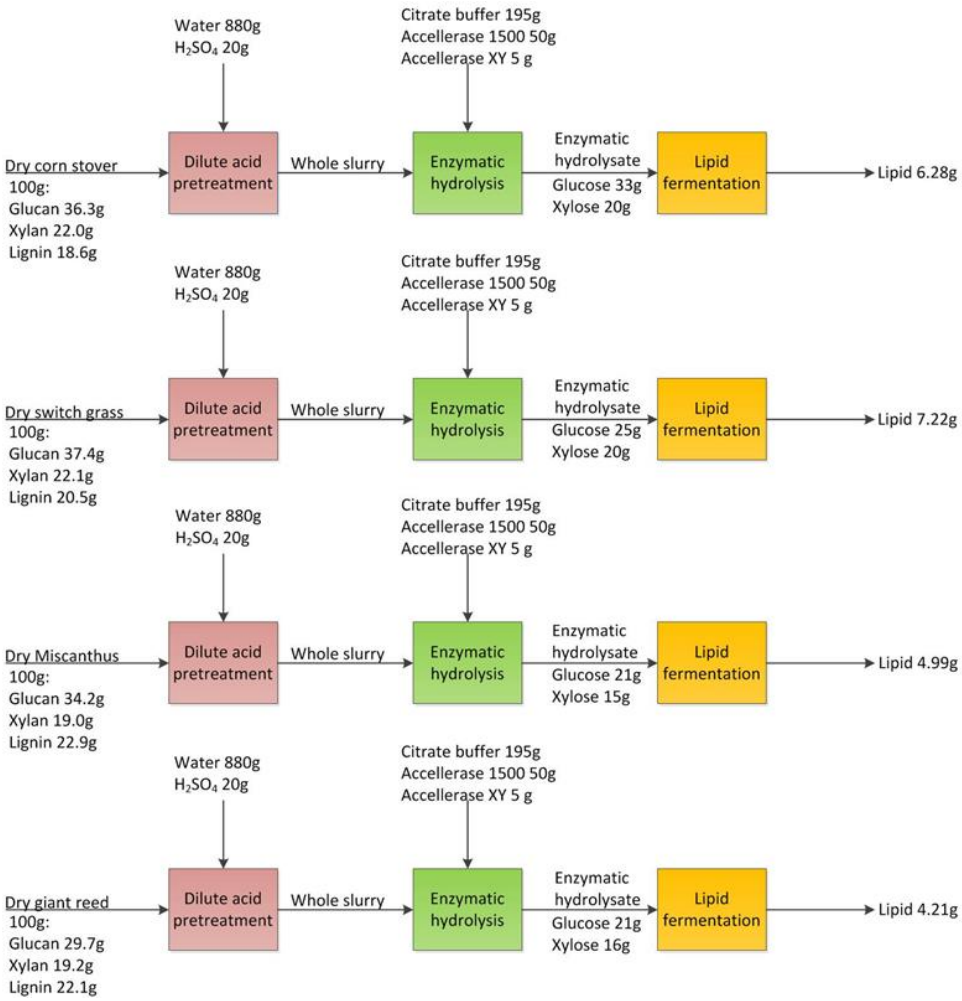

Figure 2-10 Mass balance of microbial lipid accumulation from corn stover, switchgrass, miscanthus, and giant reed co-hydrolysis

Appendix B: Chapter 3 Tables and Figures

Table 3-1 Reactor parameters for pretreatment, enzymatic hydrolysis, and aerobic fungal fermentation

Parameter	Value	Unit
Pretreatment reactor		
N_p (power number for marine propeller)	0.35	-
D (agitator diameter)	1.25	m
ρ (liquid density)	1000	kg/m^3
N (rotation speed)	1.5	rotation/s
η_g (global efficiency for agitation)	0.7	-
Hydrolysis reactor		
N_p (power number for marine propeller)	0.35	-
D (agitator diameter)	1.25	m
ρ (liquid density)	1000	kg/m^3
N (rotation speed)	1.5	rotation/s
η_g (Global efficiency for agitation)	0.7	-
Fermentor		
P_0 (atmospheric pressure)	1.0×10^5	N/m^2
P_1 (compressor exit pressure)	3.0×10^5	N/m^2
P_2 (pressure at the bottom of the fermentor)	2.5×10^5	N/m^2
N_p (power number for marine propeller)	0.35	-
D (agitator diameter)	1.25	m
ρ (liquid density)	1000	kg/m^3
N (rotation speed)	1.5	rotation/s
Q (air flow)b	0.83	m^3/s
η_g (global efficiency for agitation)	0.7	-
η_c (global efficiency for compressor)	0.5	-
Chiller efficiency	0.6	kW/ton
Heat released per mol oxygen consumed during the fungal fermentation	460	kJ/mol O_2 consumed

Table 3-2 Mass balance data for proposed biorefinery

Inventory	Input (per kg biodiesel)	Output (per kg biodiesel)
Stover collection and transport		
Nitrogen fertilizer	0.146 kg	-
Phosphorus fertilizer	0.00995 kg	-
Potassium fertilizer	0.119 kg	-
Diesel (harvest)	0.0749 kg	-
Diesel (transport)	0.0200 kg	-
Corn stover	-	16.592 kg
Pretreatment and enzymatic hydrolysis		
Corn stover	16.592 kg	-
Sulfuric acid	3.320 kg	-
Alkali	3.320 kg	-
Water	146.008 kg	-
Enzyme	0.652 kg	-
Hydrolysate	-	146. 630 kg
Hydrolysis residue (wet)	-	19. 288 kg
Lignin processing		
Wet hydrolysis residue	19.288 kg	-
Dry lignin rich residue	-	3.858 kg
Aerobic fermentation		
Hydrolysate	146.630 kg	-
Compressed air (3 bar)	422.294 m^3	-
Fungal biomass (wet)	-	13. 665 kg
Fermentation liquid effluent	-	132. 964 kg
Fungal drying		
Wet fungal biomass	13.665 kg	-
Dry fungal biomass	-	2.733 kg
Lipid extraction		
Dry fungal biomass	2.733 kg	-
Hexane	0.00308 kg	-
Water	2.652 kg	-
Fungal lipid	-	1.041 kg
Fungal biomass residue	-	1.692 kg
Wastewater	-	2.652 kg

Table 3-2 (cont'd)

Transesterification		
Fungal lipid	1.0413 kg	-
Methanol	0.0922 kg	-
Water	0.000 kg	-
Biodiesel	-	1.000 kg
Glycerol	-	0.120 kg
wastewater	-	0.156 kg
Anaerobic digestion		
Wastewater (from fermentation and liquid extraction)	135.772 kg	-
Anaerobic digestion effluent	-	134.816 kg
Methane	-	0.956 kg
Aerobic waste treatment		
Anaerobic digestion effluent	134.816 kg	-
Reclaimed water	-	132.120 kg
Reverse osmosis		
Anaerobic digestion effluent	132.120 kg	-
Reclaimed water	-	105.696 kg
Brine water (18.25% Na_2SO_4)	-	26.424 kg
Lime treatment		
Brine water (18.25% Na_2SO_4)	26.424 kg	-
Lime	2.444 kg	-
NaOH solution (10% w/w)	-	6.408 kg
Wet $CaSO_4$	-	22.460 kg

Table 3-3 Energy flow of 1 kg biodiesel production without solar energy input

Unit operations	Electricity (MJ/kg biodiesel)	Heat (MJ/kg biodiesel)	Fossil fuel (MJ/kg biodiesel)	Total energy (MJ/kg biodiesel)
Energy demand				
Corn stover collection & transportation	-	-	-13.23	-13.23
Pretreatment & enzymatic hydrolysis	-5.23	-37.45	-	-42.68
Lignin drying	-0.76	-38.22	-	-38.98
Fungal lipid fermentation	-132.42		-	-132.42
Fungal biomass drying	-0.54	-27.02	-	-27.56
Lipid extraction & transesterification	-6.61	-1.17	-	-7.78
Wastewater treatment	-2.06	-5.68	-	-7.74
Energy product				
Biodiesel	-	-	37.5	37.50
Lignin processing (lignin)	-	75.3	-	75.30
Glycerol		2.27		2.27
Water treatment (Methane)	-	47.79	-	47.79

Table 3-4 Sensitivity analysis on energy balance for the lignocellulosic biodiesel production without combined solar bio-power generation

Key unit operations	Parameter	Values		Corresponding base value (MJ/kg biodiesel)	Change on net energy output (%)
		Base value	Sensitivity range		
Corn stover collection & transportation	N fertilizer	0.15 kg/kg biodiesel	0.12-0.18 g/kg biodiesel	6.96	± 1.22
	Fossil fuel	0.10 kg/kg biodiesel	0.08-0.12 kg/kg biodiesel	5.15	± 0.91
Pretreatment & enzymatic hydrolysis	Solid loading	10%	8-12 %	42.68	± 7.50
	Pretreatment temperature	130°C	104-156°C	37.45	± 6.58
	Pretreatment time	2 h	1.6-2.4 h	37.45	± 6.58
	Hydrolysis time	72 h	57.6-86.4 h	2.22	± 0.39
Lignin process	Drying temperature	75°C	60-90°C	38.22	± 6.70
Fungal lipid fermentation	Agitation and aeration	90 rpm and 0.5 vvm	72-108 rpm and 0.4-0.6 vvm	111.83	± 19.66
Fungal biomass drying	Culture time	96 h	76.8-115.2 h	132.42	± 23.27
	Drying temperature	105°C	84-126°C	27.56	± 4.84
Entire process	Biodiesel yield	0.063 (kg biodiesel/kg corn stover)	0.050-0.076 kg biodiesel/kg corn stover	270.39	± 47.52

Table 3-5 Solar and biological power generation

Energy generation sources	**Location: Meade, KS**
PV-biological power	
Electricity generated by the PV ($MJ/m^2/day$)	1.8
Thermal energy from lignin combustion (MJ/kg biodiesel)	71.54
Thermal energy from glycerol combustion (MJ/kg biodiesel)	2.16
Thermal energy from methane combustion (MJ/kg biodiesel)	45.4
CSP-biological power	
Thermal energy from the parabolic trough collector ($MJ/m^2/day$)	14.04
Thermal energy from lignin combustion (MJ/biodiesel)	71.54
Thermal energy from glycerol combustion (MJ/biodiesel)	2.16
Thermal energy from methane combustion (MJ/biodiesel)	45.4

Table 3-6 Energy balance of a 20 million gallon lignocellulosic biodiesel refinery using solar and biological power generation

		Without combined solar and biological power generation	**With combined solar and biological power generation**	
			PV	**CSP**
Energy demand				
Process thermal energy demand (MJ/kg biodiesel)		-109.54	-109.54	-109.54
Process electricity demand (MJ/kg biodiesel)		-147.62	-147.62	-147.62
Process fossil energy demand (MJ/kg biodiesel)		-13.23	-13.23	-13.23
Total energy demand (MJ/kg biodiesel)		-270.39	-270.39	-270.39
Energy output				
Biodiesel (MJ/kg biodiesel)		37.5	37.5	37.5
Biological power	Thermal energy (MJ/kg biodiesel)	119.1	-	-
	Electricity (MJ/kg biodiesel)	-	-	-
Combined solar and biological power generation	Thermal energy (MJ/kg biodiesel)	-	119.1	442.86
	Electricity (MJ/kg biodiesel)	-	147.62	147.62
	Solar panel area (m2/kg biodiesel)	-	0.19	0.1
Total energy output (MJ/kg biodiesel)		156.6	299.22	627.98
Overall energy balance				
Net electricity output (MJ/kg biodiesel)		-147.62	-	0
Net thermal energy output (MJ/kg biodiesel)		9.56	9.56	333.32
Net energy output (MJ/kg biodiesel)		-113.79	28.83	357.59
Net energy ratio (NER)		0.58	1.11	2.32

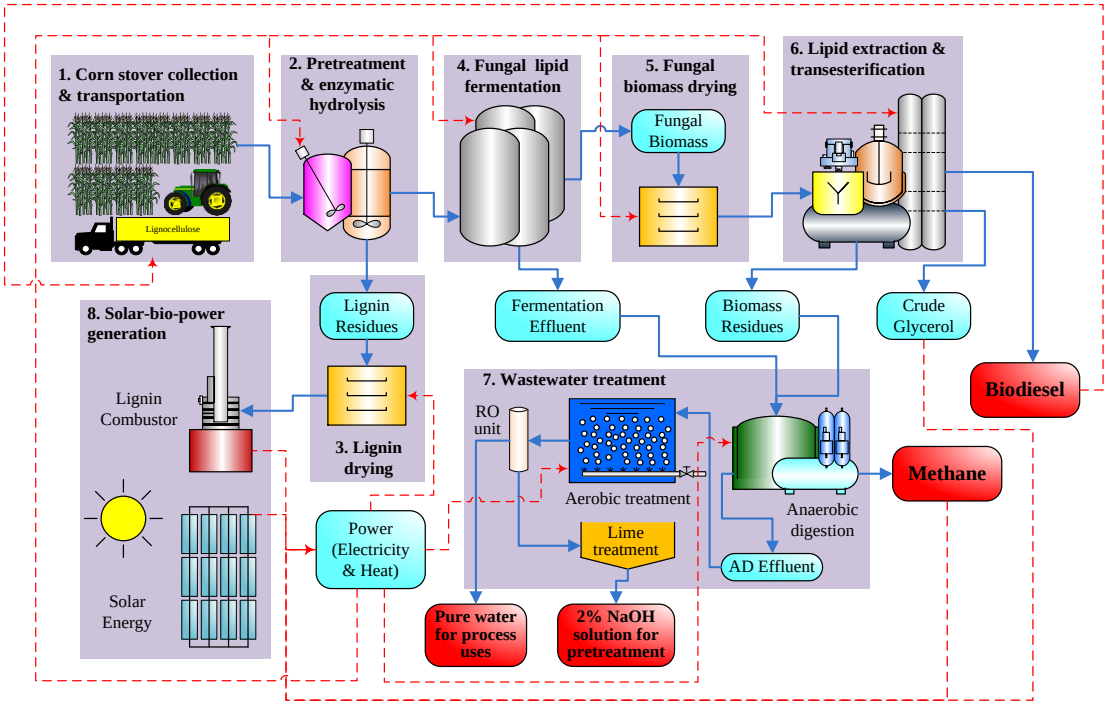

Figure 3-1 Solar-bio-powered lignocellulosic biodiesel refinery mass (blue lines) and energy (red lines) flows

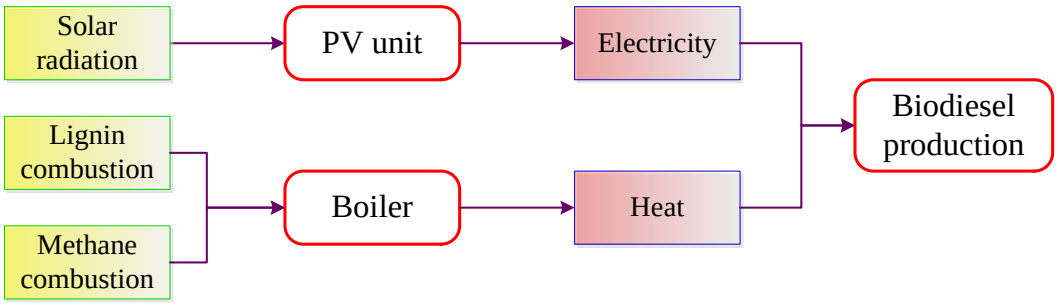

Figure 3-2 Photovoltaic-biological power generation flow diagram

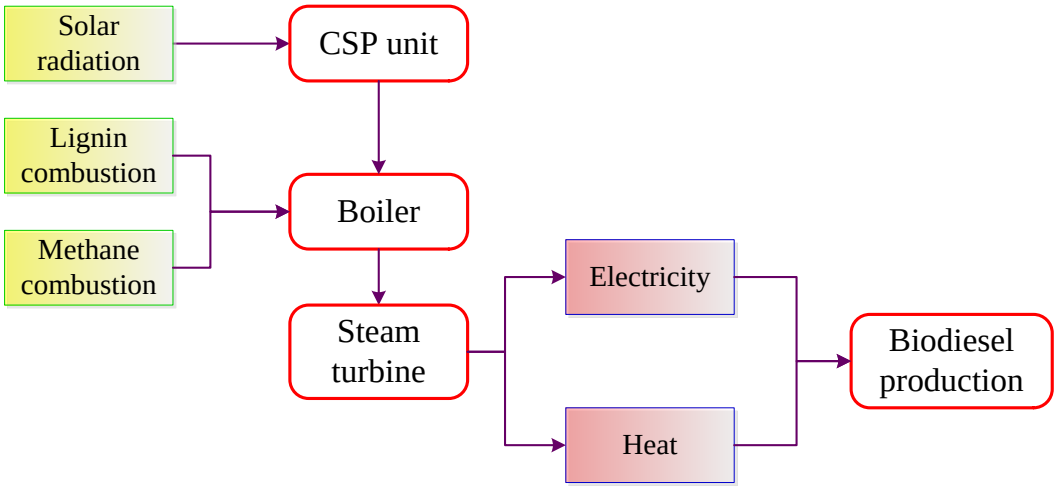

Figure 3-3 Concentrated solar power (CSP) and biological power generation flow diagram

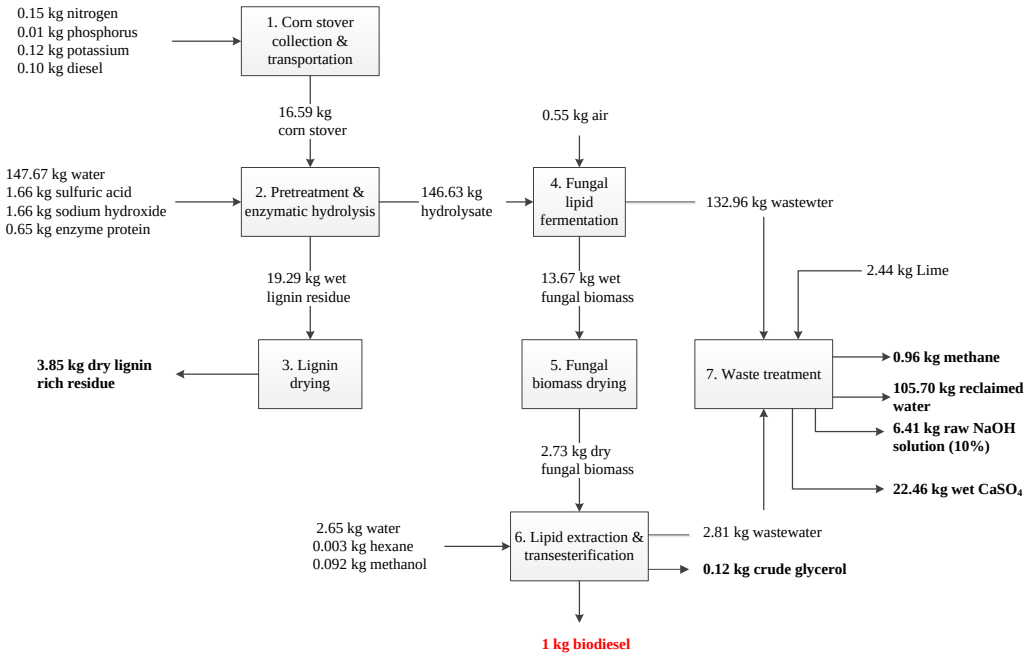

Figure 3-4 Mass flow for the production of 1 kg of fungal biodiesel

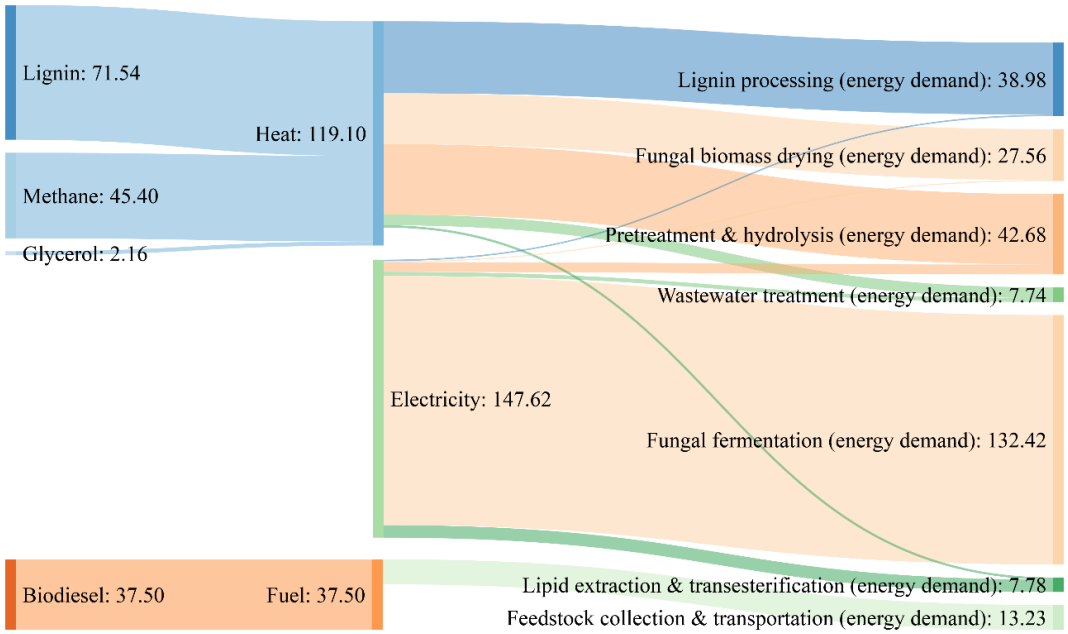

Figure 3-5 Energy balance without solar-bio-power generation

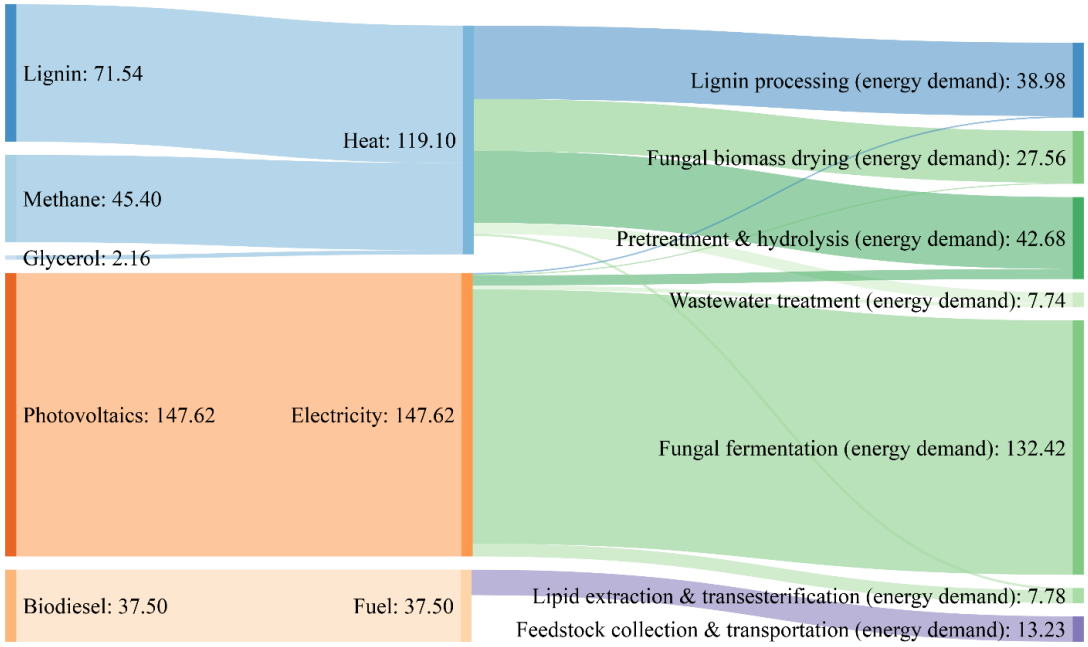

Figure 3-6 Energy balance with Photovoltaic-biological power generation

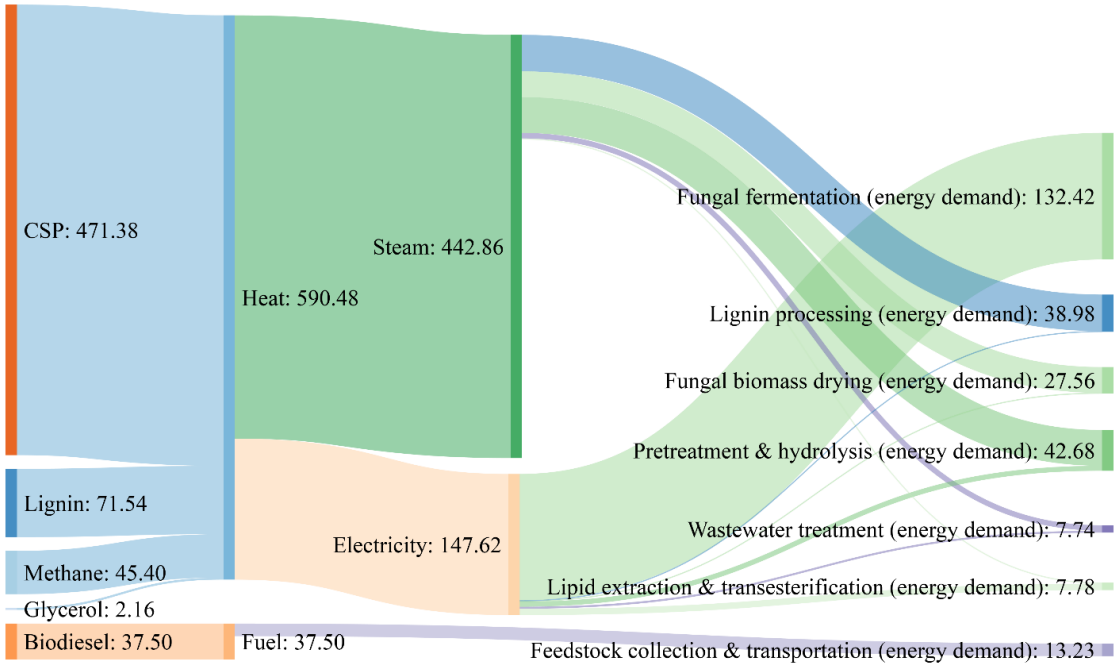

Figure 3-7 Energy balance with concentrated solar power-biological power generation

BIBLIOGRAPHY

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Alves, S., & Vasconcelos, J. (1996). Optimisation of Agitation and Aeration in Fermenters. *Bioprocess Engineering*, 119-123.

Anderson, W. F., Dien, B. S., Brandon, S. K., & Peterson, J. D. (2008). Assessment of Bermudagrass and Bunch Grasses as Feedstock for Conversion to Ethanol. *Applied Biochemistry and Biotechnology*, 13-21.

Benko, Z., Andersson, A., Szengyel, Z., Gaspar, M., Reczey, K., & Stalbrand, H. (2007). Heat Extraction of Corn Fiber Hemicellulose. *Applied Biochemistry and Biotechnology*, 136-140.

Brechbill, S., & Tyner, W. E. (2009). *The Economics of Renewable Energy: Corn Stover and Switchgrass.* Purdue University Extension.

Brosse, N., El Hage, R., Sannigrahi, P., & Ragauskas, A. (2010). Dilute Sulphuric Acid and Ethanol Organosolv Pretreatment of Miscanthus x Giganteus. *Cellulose Chemistry and Technology*, 71-78.

Chandel, A. K., & Singh, O. V. (2011). Weedy Lignocellulosic Feedstock and Microbial Metabolic Engineering: Advancing the Generation of 'Biofuel'. *Applied Microbiology and Biotechnology*, 1289-1303.

Chatzifragkou, A., Fakas, S., Galiotou-Panayotou, M., Komaitis, M., Aggelis, G., & Papanikolaou, S. (2010). Commercial Sugars as Substrates for Lipid Accumulation in Cunninghamella echinulata and Mortierella isabellina Fungi. *Lipid Science and Technology*, 1048-1057.

Chen, M., Zhao, J., & Xia, L. (2009). Comparison of Four Different Chemical Pretreatments of Corn Stover for Enhancing Enzymatic Digestibility. *Biomass and Bioenergy*, 1381-1385.

Chen, X., Li, Z., Zhang, X., Hu, F., Ryu, D. D., & Bao, J. (2009). Screening of Oleaginous Yeast Strains Tolerant to Lignocellulose Degradation Compounds. *Applied Biochemistry and Biotechnology*, 591-604.

Cheng, K.-K., Cai, B.-Y., Zhang, J.-A., Ling, H.-Z., Zhou, Y.-J., Ge, J.-P., & Xu, J.-M. (2008). Sugarcane Bagasse Hemicellulose Hydrolysate for Ethanol Production by Acid Recovery Process. *Biochemical Engineering Journal*, 105-109.

Chowdhury, R., Viamajala, S., & Gerlach, R. (2012). Reduction of Environmental and Energy Footprint of Microalgal Biodiesel Production Through Material and Energy Integration. *Bioresource Technology*, 102-111.

Cohen, Z., & Ratledge, C. (2010). *Single Cell Oils - Microbial and Algal Oils (2nd Edition).* AOCS Press.

Coyle, W. T. (2010). *Next-Generation Biofuels: Near-Term Challenges and Implications for Agriculture.* United States Department of Agriculture.

de Vrije, T., de Haas, G. G., Tan, G. B., Keijsers, E., & Claassen, P. (2002). Pretreatment of Miscanthus for Hydrogen Production by Thermotoga Elfii. *International Journal of Hydrogen Energy*, 1382-1390.

Decker, S. R., Brunecky, R., Tucker, M. P., Himmel, M. E., & Selig, M. J. (2009). High-Throughput Screening Techniques for Biomass Conversion. *Bioenergy Research*, 179-192.

Delgenes, J., Moletta, R., & Navarro, J. (1996). Effects of Lignocellulose Degradation Products on Ethanol Fermentations of Glucose and Xylose by Saccharomyces Cerevisiae, Zymomonas Mobilis, Pichia Stipitis, and Candida Shehatae. *Enzyme and Microbial Technology*, 220-225.

Demirbas, A. (2009). Progress and Recent Trends in Biodiesel Fuels. *Energy Conversion and Management*, 14-34.

Dey, P., Banerjee, J., & Maiti, M. K. (2011). Comparative Lipid Profiling of Two Endophytic Fungal Isolates – Colletotrichum Sp. and Alternaria Sp. Having Potential Utilities as Biodiesel Feedstock. *Bioresource Technology*, 5815-5823.

Dien, B. S., Jung, H.-J. G., Vogel, K. P., Caslter, M. D., Lamb, J. F., Iten, L., . . . Sarath, G. (2006). Chemical Composition and Response to Dilute-Acid Pretreatment and Enzymatic Saccharification of Alfalfa, Reed Canarygrass, and Switchgrass. *Biomass and Bioenergy*, 880-891.

Doran, P. M. (2013). *Bioprocess Engineering Principles.* Academic Press.

Economou, C. N., Aggelis, G., Pavlour, S., & Vayenas, D. V. (2011). Single Cell Oil Production From Rice Hulls Hydrolysate. *Bioresource Technology*, 9737-9742.

Economou, C. N., Makri, A., Aggelis, G., Pavlou, S., & Vayenas, D. V. (2010). Semi-Solid State Fermentation of Sweet Sorghum for the Biotechnological Production of Single Cell Oil. *Bioresource Technology*, 1385-1388.

Emami, S., Tabil, L. G., & Adapa, P. (2015). Effect of Glycerol on Densification of Agricultural Biomass. *International Journal of Agricultural and Biological Engineering*, 64-73.

Fakas, S., Papanikolaou, S., Batsos, A., Galiotou-Panayotou, M., Mallouchos, A., & Aggelis, G. (2009). Evaluating Renewable Carbon Sources as Substrates for Single Cell Oil Production by Cunninghamella Echinulata and Mortierella Isabellina. *Biomass and Bioenergy*, 573-580.

Fei, Q., Chang, H., Shang, L., Choi, J.-d.-r., Kim, N., & Kang, J. (2011). The Effect of Volatile Fatty Acids as a Sole Carbon Source on Lipid Accumulation by Cryptococcus Albidus for Biodiesel Production. *Bioresource Technology*, 2695-2701.

Felby, C., Klinke, H., Olsen, H., & Thomsen, A. (2003). Ethanol from Wheat Straw Cellulose by Wet Oxidation Pretreatment and Simultaneous Saccharification and Fermentation. *Applications of Enzymes to Lignocellulosics*, 157-174.

Georgieva, T. I., Hou, X., Hilstrom, T., & Ahring, B. K. (2008). Enzymatic Hydrolysis and Ethanol Fermentation of High Dry Matter Wet-Exploded Wheat Straw at Low Enzyme Loading. *Applied Biochemistry and Biotechnology*, 35-44.

Gong, Z., Wang, Q., Shen, H., Hu, C., Jin, G., & Zhao, Z. K. (2012). Co-Fermentation of Cellobiose and Xylose by Lipomyces Starkeyi for Lipid Production. *Bioresource Technology*, 20-24.

Green, M. A. (2007). Thin-film Solar Cells: Review of Materials, Technologies and Commercial Status. *Journal of Materials Science: Materials in Electronics*, 15-19.

Hu, C., Wu, S., Wang, Q., Jin, G., Shen, H., & Zhao, Z. K. (2011). Simultaneous Utilization of Glucose and Xylose for Lipid Production by Trichosporon Cutaneum. *Biotechnology for Biofuels*, 383-387.

Hu, C., Zhao, X., Zhao, J., Wu, S., & Zhao, Z. K. (2009). Effects of Biomass Hydrolysis By-Products on Oleaginous Yeast Rhodosporidium Toruloides. *Bioresource Technology*, 4843-4847.

Huang, C., Wu, H., Liu, Q.-p., Li, Y.-y., & Zong, M.-h. (2012). Effects of Aldehydes on the Growth and Lipid Accumulation of Oleaginous Yeast Trichosporon Fermentans. *Biotechnology for Biofuels*, 4606-4613.

Huang, C., Zong, M.-h., Wu, H., & Liu, Q.-p. (2009). Microbial Oil Production from Rice Straw Hydrolysate by Trichosporon Fermentans. *Bioresource Technology*, 4536-4538.

Hui, L., Wan, C., Hai-tao, D., Xue-jiao, C., & Yu-hua, Z. (2010). Direct Microbial Conversion Of Wheat Straw into Lipid by a Cellulolytic Fungus of Aspergillus Oryzae A-4 in Solid-State Fermentation. *Bioresource Technology*, 7556-7562.

Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Aden, A., . . . Dudgeon, D. (2011). *Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol.* National Renewable Energy Laboratory.

Ingersoll Rand. (2015). Retrieved from http://www.ingersollrandproducts.com/am-en/products/air/centrifugal-air-compressor/standard-pressure-centrifugal-air-compressor-/45-70-m3min-1600-2350-cfm/modelspec/12996

Jorgensen, H., Vibe-Pedersen, J., Larsen, J., & Felby, C. (2007). Liquefaction of Lignocellulose at High-Solids Concentrations. *Biotechnology and Bioengineering*, 862-870.

Khanal, S. K. (2009). *Anaerobic Biotechnology for Bioenergy Production: Principles and Applications.* John Wiley and Sons, Inc.

Kim, Y., & Parker, W. (2008). A Technical and Economic Evaluation of the Pyrolysis of Sewage Sludge for the Production of Bio-Oil. *Bioresource Technology*, 1409-1416.

Klinke, H. B., Thomsen, A. B., & Ahring, B. K. (2004). Inhibition of Ethanol-Producing Yeast and Bacteria by Degradation Products Produced During Pre-Treatment of Biomass. *Applied Microbiology and Biotechnology*, 10-26.

Krohn, B. J., & Fripp, M. (2012). A Life Cycle Assessment of Biodiesel Derived from the "Niche Filling" Energy Crop Camelina in the USA. *Applied Energy*, 92-98.

Lee, Y. Y., Iyer, P., & Torget, R. W. (2001). *Dilute-Acid Hydrolysis of Lignocellulosic Biomass. Advances in Biochemical Engineering*, 93-115.

Lewandowski, I., Scurlock, J. M., Lindvall, E., & Christou, M. (2003). The Development and Current Status of Perennial Rhizomatous Grasses as Energy Crops in the US and Europe. *Biomass and Bioenergy*, 335-361.

Liao, W., Wen, Z., Hurley, S., Liu, Y., Liu, C., & Chen, S. (2005). Effects of Hemicellulose and Lignin on Enzymatic Hydrolysis of Cellulose from Dairy Manure. *Applied Biochemistry and Biotechnology*, 121-124.

Lloyd, T. A., & Wyman, C. E. (2005). Combined Sugar Yields for Dilute Sulfuric Acid Pretreatment of Corn Stover Followed by Enzymatic Hydrolysis of the Remaining Solids. *Bioresource Technology*, 1967-1977.

Lopez, J. A., Santos, M. M., Perez, A. F., & Martin, A. M. (2009). Anaerobic Digestion of Glycerol Derived from Biodiesel Manufacturing. *Bioresource Technology*, 5609-5615.

Lu, Y., Wang, Y., Xu, G., Chu, J., Zhuang, Y., & Zhang, S. (2010). Influence Of High Solid Concentration on Enzymatic Hydrolysis and Fermentation of Steam-Exploded Corn Stover Biomass. *Applied Biochemistry and Biotechnology*, 360-369.

Lynd, L. R., Weimer, P. J., van Zyl, W. H., & Pretorius, I. S. (2002). Microbial Cellulose Utilization: Fundamentals and Biotechnology. *Microbiology and Molecular Biology Reviews*, 506-577.

Mani, S., Sokhansanj, S., Tagore, S., & Turhollow, A. F. (2010). Techno-Economic Analysis of Using Corn Stover to Supply Heat and Power to a Corn Ethanol Plant – Part 2: Cost of Heat and Power Generation Systems. *Biomass and Bioenergy*, 356-364.

Marion, W., & Wilcox, S. (1994). *Solar Radiation Data Manual for Flat-Plate and Concentrating Collectors.* Golden, Colorado: National Renewable Energy Laboratory.

Meher, L. C., Sagar, D. V., & Naik, S. N. (2006). Technical Aspects of Biodiesel Production by Transesterification- a Review. *Renewable and Sustainable Energy Reviews*, 248-268.

Mills, D. (2001). Advances in Solar Thermal Electricity Technology. *Solar Energy*, 19-31.

Morey, R. V., Kaliyan, N., Tiffany, D. G., & Schmidt, D. R. (2010). A Corn Stover Supply Logistics System. *American Society of Agricultural and Biological Engineers*, 455-461.

Moser, B. R. (2009). Biodiesel Production, Properties, and Feedstocks. *In Vitro Cellular and Developmental Biology - Plant*, 229-266.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., & Ladisch, M. (2005). Features of Promising Technologies for Pretreatment of Lignocellulosic Biomass. *Bioresource Technology*, 673-686.

Murnen, H. K., Balan, V., Chundawat, S. P., Bals, B., da Costa Sousa, L., & Dale, B. E. (2007). Optimization of Ammonia Fiber Expansion (AFEX) Pretreatment and Enzymatic Hydrolysis of Miscanthus X Giganteus to Fermentable Sugars. *Biotechnology Progress*, 846-850.

Omni Tech International. (2010). *Life Cycle Impact of Soybean Production and Soy Industrial Products.* United States Soybean Board.

Papanikolaou, S., Komaitis, M., & Aggelis, G. (2005). Single Cell Oil (SCO) Production by Mortierella Isabellina Grown on High-Sugar Content Media. *Bioresource Technology*, 287-291.

Pathak, M., Pearce, J., & Harrison, S. (2012). Effects on Amorphous Silicon Photovoltaic Performance from High-Temperature Annealing Pulses in Photovoltaic Thermal Hybrid Devices. *Solar Energy Materials and Solar Cells* , 199-203.

Peng, W., Lamei, Z., Zhiming, Z., Li, W., Hui, W., Chengling, Y., & Guohong, G. (2011). Microbial Lipid Production by Co-Fermentation with Mortierella Alpina Obtained by Ion Beam Implantation. *Chemical Engineering and Technology*, 422-428.

Peng, X., & Chen, H. (2008). Single Cell Oil Production in Solid-State Fermentation by Microsphaeropsis Sp. from Steam-Exploded Wheat Straw Mixed with Wheat Bran. *Bioresource Technology*, 3885-3889.

Petrolia, D. R. (2008). The Economics of Harvesting and Transporting Corn Stover for Conversion to Fuel Ethanol: a Case Study for Minnesota. *Biomass and Bioenergy*, 603–612.

Pienkos, P. T., & Zhang, M. (2009). Role of Pretreatment and Conditioning Processes on Toxicity of Lignocellulosic Biomass Hydrolysates. *Cellulose*, 743-762.

Pradhan, A., Shrestha, D. S., McAloon, A., Yee, W., Haas, M., & Duffield, J. A. (2011). Energy Life-Cycle Assessment of Soybean Biodiesel Revisited. *Transactions of the ASABE*, 1031-1039.

Pradhan, A., Shrestha, D. S., McAloon, A., Yee, W., Haas, M., Duffield, J. A., & Shapouri, H. (2009). *Energy Life-Cycle Assessment of Soybean Biodiesel.* United States Department of Agriculture.

Price, H., Lupfert, E., Kearney, D., Zarza, E., Cohen, G., Gee, R., & Mahoney, R. (2002). Advances in Parabolic Trough Solar Power Technology. *Solar Energy Engineering*, 109-125.

Price, H., Lupfert, E., Kearney, D., Zarza, E., Cohen, G., Gee, R., & Mahoney, R. (2002). *Advances in Parabolic Trough Solar Power Technology*. Journal of Solar Energy Engineering.

Ratledge, C. (2004). Fatty Acid Biosynthesis in Microorganisms Being Used for Single Cell Oil Production. *Biochimie*, 807-815.

Ruan , Z., Zanotti, M., Zhong, Y., Ducey, C., & Liu, Y. (2013). Co-Hydrolysis of Lignocellulosic Biomass for Microbial Lipid Accumulation. *Biotechnology and Bioengineering*, 1039-1049.

Ruan, Z., & Zanotti, M. (2012). Evaluation of Lipid Accumulation from Lignocellulosic Sugars by Mortierella Isabellina for Biodiesel Production. *Bioresource Technology*, 198-205.

Scordia, D., Cosentino, S. L., Lee, J.-W., & Jeffries, T. W. (2011). Dilute Oxalic Acid Pretreatment for Biorefining Giant Reed (Arundo Donax L.). *Biomass and Bioenergy*, 3018-3024.

Scordia, D., Cosentino, S. L., Lee, J.-W., & Jeffries, T. W. (2012). Bioconversion of Giant Reed (Arundo Donax L.) Hemicellulose Hydrolysate to Ethanol by Scheffersomyces Stipitis Cbs6054. *Biomass and Bioenergy*, 296-305.

Shatalov, A. A., & Pereira, H. (2005). Kinetics of Polysaccharide Degradation During Ethanol-Alkali Delignification of Giant Reed - Part 2. Minor Carbohydrates and Uronic Acids. *Carbohydrate Polymers*, 304-313.

Shatalov, A. A., & Pereira, H. (2012). Xylose Production from Giant Reed (Arundo Donax L.): Modeling and Optimization of Dilute Acid Hydrolysis. *Carbohydrate Polymers*, 210-217.

Sheehan, J., Aden, A., Paustian, K., Killian, K., Brenner, J., Walsh, M., & Nelson, R. (2004). Energy and Environmental Aspects of Using Corn Stover for Fuel Ethanol. *Journal of Industrial Ecology*, 117-146.

Shukla, R., Sumathy, K., Erickson, P., & Gong, J. (2013). Recent Advances in the Solar Water Heating Systems: A Review. *Renewable and Sustainable Energy Reviews*, 173-190.

Singh, R. P., & Heldman, D. R. (2009). *Introduction to Food Engineering.* Academic Press.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Crocker, D. (2011). *Determination of Structural Carbohydrates and Lignin in Biomass Laboratory Analytical Procedure (LAP).* National Renewable Energy Laboratory.

Sorensen, A., Teller, P. J., Hilstrom, T., & Ahring, B. K. (2008). Hydrolysis of Miscanthus for Bioethanol Production Using Dilute Acid Presoaking Combined with Wet Explosion Pre-Treatment and Enzymatic Treatment. *Bioresource Technology*, 6602-6607.

Studer, M. H., Brethauer, S., DeMartini, J. D., McKenzie, H. L., & Wyman, C. (2011). Co-Hydrolysis of Hydrothermal and Dilute Acid Pretreated Populus Slurries to Support Development of a High-Throughput Pretreatment System. *Biotechnology for Biofuels*.

Takahashi, C. M., Takahashi, D. F., Carvalhal, M. L., & Alterthum, F. (1999). Effects of Acetate on the Growth and Fermentation Performance of Escherichia Coli KO11. *Applied Biochemistry and Biotechnology*, 193-203.

Tian, Y., & Zhao, C. Y. (2013). A Review of Solar Collectors and Thermal Energy Storage in Solar Thermal Applicatons. *Applied Energy*, 538-553.

Tomani, P. (2009). The Lignoboost Process. *Cellulose Chemistry and Technology*, 53-58.

U.S. Energy Information Administration. (2014, January 29). *Independent Statistics and Analysis U.S. Energy Information Administration*. Retrieved from U.S. Energy Information Administration: http://www.eia.gov/cfapps/ipdbproject/iedindex3.cfm?tid=79&pid=81&aid=1&cid=regions&syid=2008&eyid=2012&unit=TBPD

Van Gerpen, J. (2005). Biodiesel Processing and Production. *Fuel Processing Technology*, 1097-1107.

Vogel, H. C., & Todaro, C. L. (1997). *Fermentation and Biochemical Engineering Handbook Principles, Process Design, and Equipment.* Noyes Publications.

Voitkevich, O. V., Kabo, G. J., Blokhin, A. V., Paulechka, Y. U., & Shishonok, M. V. (2012). Thermodynamic Properties of Plant Biomass Components. Heat Capacity, Combustion Energy, and Gasification Equilibria of Lignin. *Journal of Chemical and Engineering Data*, 1903-1909.

von Gottberg, A., Pang, A., & Talavera, J. L. (2012). *Optimizing Water Recovery and Energy Consumption for Seawater RO Systems.* GE Power and Water.

Water Environment Federation. (2009). *Energy Conservation in Water and Wastewater Facilities.* McGraw-Hill Professional.

Water Environment Federation. (2009). *Energy Conservation in Water and Wastewater Facilities.* McGraw-Hill Education.

Williams, P. R., Inman, D., Aden, A., & Heath, G. A. (2009). Environmental and Sustainability Factors Associated with Next-Generation Biofuels in the U.S.: What do we Really Know? *Environmental Science Technology*, 4763-4775.

Xing, D., Wang, H., Pan, A., Wang, J., & Xue, D. (2012). Assimilation of Corn Fiber Hydrolysates and Lipid Accumulation by Mortierella Isabellina. *Biomass and Bioenergy*, 494-501.

Yang, B., & Wyman, C. E. (2006). BSA Treatment to Enhance Enzymatic Hydrolysis of Cellulose in Lignin Containing Substrates. *Biotechnology and Bioengineering*, 611-617.

Yusuf, N., Kamarudin, S. K., & Yaakub, Z. (2011). Overview on the Current Trends in Biodiesel Production. *Energy Conversion and Management*, 2741-2751.

Zhang, G., French, W. T., Hernandez, R., Alley, E., & Paraschivescu, M. (2011). Effects of Furfural and Acetic Acid on Growth and Lipid Production from Glucose and Xylose by Rhodotorula Glutinis. *Biomass and Bioenergy*, 734-740.

Zhang, H. L., Baeyens, J., Degreve, J., & Caceres, G. (2013). Concentrated Solar Power Plants: Review and Design Methodology. *Renewable and Sustainable Energy Reviews*, 466-481.

Zhu, J. Y., & Zhuang, X. S. (2012). Conceptual Net Energy Output for Biofuel Production from Lignocellulosic Biomass Through Biorefining. *Progress in Energy and Combustion Science*, 583-598.