A Sustainable Lignocellulosic Biodiesel Production Integrating Solar- and Bio-power Generation

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Supplemental Material

The major unit operations in a lignocellulosic biodiesel refinery capable of producing 10 million gallons of biodiesel a year include (1) corn stover collection & 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) wastewater treatment, and (8) solar-bio-power generation. The boundary for the process is shown in Figure 1. The details of individual unit operations are described as follow.

1 Corn stover collection and transportation

The agricultural residue - corn stover is the selected 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 other dedicated energy crops will be developed. In our scenario corn stover is treated as an agricultural residue from corn production, and as such, only energies related to 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 at the biorefinery gate [1, 2]. The following values were used in this study: (1) collection/transport to local storage of 196.90 MJ/t, (2) local storage inputs of 30.50 MJ/t, (3) corn stover compaction for transport of 233.70 MJ/t, and (4) transport of compacted corn stover to end users 62.4 MJ/t. We also assume that harvesting corn stover likewise removes nutrients that 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 assumed for this study are 8.80, 0.60, and 7.20 kg/t dry matter, respectively [1, 3]. Energy inputs for N, P, and K fertilizer production were 47.70, 13.35, and 8.09 MJ/kg, respectively, which are calculated from GREET 1.8c.

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 (cellulose, hemicellulose, and lignin). This allows hydrolytic enzymes to penetrate and hydrolyze the carbohydrate polymers to their monomeric sugar constituents, which can readily be metabolized for fungal lipid production. The composition of corn stover is 36.3% (w/w) cellulose, 22.0% (w/w) xylan, and 18.6% (w/w) lignin [4].

Physical treatment is the initial size reduction of the biomass. This process is accomplished using a hammer mill, which has energy inputs of 180 MJ/t of herbaceous biomass [5]. A combined hydrolysis process is then used to carry out corn stover pretreatment and enzymatic saccharification, while process co-products such as lignin are utilized for additional energy production. Combined hydrolysis refers to two separate steps using dilute sulfuric acid and dilute sodium hydroxide solutions to first pretreat corn stover, and then directly followed by enzymatic saccharification on the mixture of acid and alkali treated slurries without detoxification and liquid–solid separation [4]. Our previous study found optimal corn stover pretreatment conditions to be 10% solid loading (w/w) and 2% acid (w/w) and 2% alkali (w/w) at 130°C for 1 hr [4]. Energy consumption to heat the pretreatment slurry to its desired temperature is calculated using the following equation.

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

where 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 (3.96 kJ/kg°C), and ΔT is the temperature difference between the initial slurry temperature (15°C) and final desired temperature (130°C) [6]. Latent heat of vaporization is not factored into the equation due to the fact that the reaction occurs in a pressurized reactor.

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. Thus, total heat recovered is calculated using equation 1, with the exception of ΔT being 80°C. The recovered heat is used to maintain the enzymatic reaction temperature of 50°C. Hydrolytic enzymes are then added to the cooled slurry at 50°C to cleave the hydrolytic bonds in the remaining cellulose and hemicellulose chains. An enzyme loading of 47 kg-protein/t-biomass was used in our previous study resulting in a total sugar yield of 74.2% [4]. The lignin solubility of 13% at our pretreatment conditions is consistent with a previous study [7].

Both pretreatment and hydrolysis reactors are assumed to be 200 m^3 in effective size (5.0 meter in diameter and 10.0 meter in height) with continuous agitation through the reaction solution. The agitation times for pretreatment and hydrolysis are 1 hours and 72 hours, respectively. The parameters for agitating both reactors are listed in Table S1. The electricity use for the agitation is calculated as follows:

$$P = \frac{P_g}{\eta_g} \quad (2)$$

where P_g and P are agitation power (W) and total electrical power (W), respectively. η_g is the global efficiency for agitation. P_g is described by equation 3 [8].

$$P_g = N_P \rho N^3 D^5 \quad (3)$$

Here N_p is the power number for propeller, ρ is the fluid density (kg/m^3), N is the agitation speed (revolutions/s), and D is the agitator diameter (m). Values for each parameter are listed in Table 2.

The hydrolysis slurry has a relatively high viscosity due to the alkali pretreatment. Our experiments indicate that vacuum filtration does not work for such a high viscosity slurry. A pressure filter is thus selected as the liquid-solid separation unit to obtain the enzymatic hydrolysate in this study. The filtration operates under 50°C, and is able to recover 99% of the sugars in the hydrolysis slurry [9]. The energy consumption of the pressure filter is 309.64 kJ/kg dry biomass residue.

Since the fermentation temperature is 25°C, the enzymatic hydrolysate must be cooled down before inoculating the fungal seed. Considering the location of the studied biorefinery, the average groundwater temperature is 15°C. Therefore, the incoming water for the process is used to cool down the enzymatic hydrolysate as well to recover the heat and prepare the hydrolysate for the fermentation. The heat recovery is calculated by equation 1 again, with the ΔT being 25°C and $C_{p\text{-solution}}$ being 4.18 kJ/kg°C,

3 Lignin processing

The biomass residues remaining after enzymatic hydrolysis consist primarily of the insoluble lignin fraction at 80% (w/w) of the dry residue. Considering the fact that lignin is the second most abundant terrestrial polymer on our planet after cellulose, the amount of lignin produced will be significantly increased along with the implementation of lignocellulosic biorefineries to generate biofuels. Lignin valorization then becomes vital to improving the economic performance of the second-generation biofuel product and enhancing the sustainability of lignocellulosic biorefining [10, 11]. Potential paths to valorize the residual lignin include carbon fiber composites for structural materials, solid fuels for energy generation, and low-molecular-weight aromatic compounds for value-added chemical production [11-13]. Compared to lignin solid fuel for power generation, carbon fiber composites and value-added aromatic compound production are still in the research and development stage. Therefore, our study utilizes lignin as solid fuels to generate energy for the process uses.

The wet biomass residue from the pressure filter are dried to further reduce its moisture content for 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.

$$E_{lignin} = M_{wl} \times W \times [(C_{p-water} \times \Delta T) + \Delta H_v] + [M_{wl} \times (1 - W)] \times (C_{p-lignin} \times \Delta T) \quad (4)$$

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 percentage 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 (4.19 kJ/kg-°C), $C_{p\text{-lignin}}$ is the specific heat capacity of solid lignin (1.10 kJ/kg-°C) [14], and ΔT is the temperature difference between the initial biomass residue temperature (50°C) and the drying temperature (75°C).

4 Fungal lipid fermentation

Our previous experimental data of the *M. isabellina* from the flask and fermentor cultures on the combined hydrolysate were used to carry out the mass and energy balance for the fungal lipid fermentation [4]. A fungal cell mass concentration of 17.2 g/L, comprised of 29.7% lipid was reported under a carbon:nitrogen ratio (mol:mol) of 65.3 from a 96-hour batch culture. This equates to a lipid yield of 0.069 g-lipid/g-corn stover.

Energy consumption is a major consideration in aerobic fermentation systems. Many components can affect the final power consumption such as agitation, air compression, and refrigeration. Identifying the operating conditions and unit operations is a key step towards accurately accounting for all energy inputs. Due to the fact that the *M. isabellina* fermentation is exothermic, the reaction heat needs to be removed to maintain the fermentation temperature (25°C). The oxygen uptake rate for the fungus is 1.0 mol oxygen/kg dry fungal biomass/hr. Since the amount of energy released from the consumption of one mole oxygen for aerobic cultures is 460 kJ/mol oxygen consumed [8], the reaction heat generated during the fermentation can be calculated by multiplying oxygen uptake and energy release per mol oxygen consumption. Centrifugal water-cooled chillers are selected to cool the fermentation broth.

Considering the fact that industrial fermenters for aerobic cultivation are typically in the range of 100 to 250 m^3 [15], 200 m^3 in effective volume (5.0 meter in diameter and 10.0 meter in height) is selected as the fermenter size for fungal lipid accumulation. The fermenters are

continuously stirred with air sparged through the fermentation broth. The aeration rate is 1 m^3 air/m^3 broth/minute. The culture time is 96 hours. Energy input for the fermentation, largely electricity for agitation, air compression, and cooling, is calculated as follows [16].

$$P = \frac{P_g}{\eta_g} + \frac{P_c}{\eta_C} + P_r \quad (5)$$

Where P_g, P_c, P_r and P are required agitation energy (W), compressed energy (W), cooling energy (W) and total electrical power (W), respectively. η_g and η_C are the global efficiencies for agitation and compression, respectively. Compressed power is described by the following equations.

$$P_c = \alpha_1 Q \quad (6)$$

$$\alpha_1 = \frac{\gamma}{\gamma - 1} P_0 \left[\left(\frac{P_1}{P_0} \right)^{\frac{\gamma - 1}{\gamma}} - 1 \right] \quad (7)$$

Where Q is the air volumetric flow (m^3/s), α_1 is the coefficient described by equation 7, 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 8 below.

$$P_g = \left(0.90 + 2.1 \, e^{-\frac{7.32 Q P_0}{P_2}} \right) N_P \rho N^3 D^5 \quad (8)$$

Where N_p is the un-aerated power number, ρ is the fluid density (kg/m^3), N is the agitation speed (revolutions/s), P_2 is the pressure at the bottom of the vessel (N/m^2), and D is the agitator diameter (m) [16].

The chiller efficiency is assumed at 0.6 kW/ton (defined as kW electricity needed to remove a ton of cooling water that is equivalent to 12,661 kJ per hour). The electricity demand for cooling the fermenters can then be calculated using heat of the fermentation reaction and chiller efficiency (Supplemental material). Values for each parameter are listed in Table S1.

After the fermentation, the fungal biomass is harvested and dewatered by a pressure filter again. The unit energy consumption of the pressure filter is also set at 309.64 kJ/kg dry fungal biomass residue.

5 Fungal biomass drying

The moisture content of the wet fungal biomass after the dewatering is 75%. Fungal biomass needs to be dried for the following process steps. The drying temperature is 100°C, corresponding to similar conditions used in yeast spray drying, while the incoming biomass temperature is 25°C. Energy to dry the wet fungal biomass can similarly be calculated using equation 4 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 2,244 kJ/kg at 100°C, and ΔT is 75°C. The specific heat capacity for fungal biomass ($C_{p\text{-}f}$) was calculated based on the composition of the dry cell mass shown in equation 9 [17].

$$C_{p-f} = 1.424 \times X_h + 1.549 \times X_p + 1.675 \times X_f + 0.837 \times X_a \quad (9)$$

Here X is the mass fraction, and the subscripts h, p, f, and a, represent carbohydrate (0.43), protein (0.22), fat (0.30), and ash (0.05), respectively. The coefficients represent the specific heat capacity for each mass fraction and are expressed in units of kJ/kg-°C [17]. Therefore, the calculated $C_{p\text{-}f}$ is 1.50 kJ/kg-°C.

6 Lipid extraction and transesterification

Different from other oleaginous microbes (i.e., yeasts), our experiments demonstrate that the lipids in *M. isabellina* can be extracted without mechanical disruption of its cell membrane. Hexane has shown to be an effective method to extract fungal lipids directly from dried fungal cells. Therefore, lipid extraction is modeled using a soybean oil extraction processes without additional or specialized equipment [18]. Mass and energy data regarding fungal lipid extraction and transesterification were based on the U.S. Soybean Board's 2010 life cycle analysis study [19]. Consistent with industry-wide practices for soybean biodiesel production, extracted oil is converted to biodiesel through an alkali catalyzed transesterification process. 0.12 kg of glycerol co-product is produced for every 1 kg of biodiesel processed. Glycerol possesses a relatively large LHV of 16 MJ/kg, and therefore it is assumed to be combusted to produce heat in this study.

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, and recycle the water back to the process. Anaerobic digestion (AD) first utilizes the organic matter in the effluent to produce methane biogas as an energy by-product and prepare the effluent with less nutrient content for the following aerobic treatment. A continuously stirred 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 fermentation effluent to the desired temperature. The electricity energy is assumed at 2% of the thermal energy produced by methane combustion, and the heat demand for heating the reactor will follow equation 1, with Cp of 4.187 kJ/kg-°C and ΔT of 10°C. Total methane production is based on a measured yield of 0.25 kg-CH_4/kg-COD destroyed. The effluent after being treated anaerobically with a COD of 7 g/L then undergoes an aerobic treatment. The required 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 [20]. The effluent from aerobic wastewater treatment process still has 1.7% Na_2SO_4. A RO and hydrated lime treatment are then implemented to simultaneously produce reclaimed water and concentrated NaOH solution for the pretreatment uses, and improve the process efficiency. The RO 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. The hydrated lime is then applied on the brine solution to generate $CaSO_4$ and concentrated NaOH solution. $CaSO_4$ is settled and removed from the

solution. The concentrated 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 [21].

8 Combined solar and biological power generation

Two solar technologies, photovoltaics (PV) and concentrated solar power (CSP), were investigated to be combined with lignin, glycerol, 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 delineate 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/glycerol combustion unit (Fig 2a). The PV module is for the electricity generation only. 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 [22]. The electricity generated from the PV module is used to mainly power the unit operations in the lignocellulosic biodiesel production. The lignin/methane/glycerol combustion unit (boilers) 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 [23, 24]. The electricity conversion efficiency (from solar radiation to alternative current) of the thin film PV is 12%. The thermal efficiencies of boilers for lignin/methane combustion are set at 95%.

The parasitic load for the PV-biological power generation was set at 5% of the energy output of the power generation, which refers to the energy used to power pumps and fans for the lignin/methane/glycerol combustion of thermal energy production [25]. The parasitic load is assumed 100% from the PV.

Parabolic trough technology is currently a proven commercial CSP technology on the market [26]. The parabolic trough solar system is capable of concentrating solar energy to generate steam up to 400°C [26]. The CSP-biological power generation uses a parabolic solar trough power technology to integrate with lignin/glycerol/methane combustion to generate power (electricity and heat) for the process uses (Fig. 2b). The CSP-biological power generation includes parabolic solar trough receiver, boilers, and steam turbine cogeneration. Combining solar thermal energy with lignin/glycerol/methane combustion has advantages of solving unsteady energy flow issues of solar radiation (using lignin/methane/glycerol 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 [26]. The average solar radiation available to be extracted by the parabolic trough receiver is 18 MJ/m^2/day at Meade County, Kansas [23]. Solar-radiation-to-steam thermal efficiency (considering radiation and convection receiver losses, piping and storage thermal losses, and heat-medium to steam thermal losses) was assumed to be 70%. The thermal efficiency of the boilers was set at 95%. The electricity and thermal efficiency of the steam turbine cogeneration were 25% and 60%, respectively. The parasitic load for the CSP-biological power generation was set at 10% of the energy output of the power generation, which refers to the energy used by pumps, fans, and the positioning of the

parabolic troughs for both CSP and biological power generation [27]. The parasitic load is also 100% from the CSP.

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Table S1. Parameters of reactors for pretreatment, enzymatic hydrolysis, and aerobic fungal fermentation at an industrial scale of 200 m^3

Parameters	Value	Unit
Pretreatment reactor (200 m^3)[a]		
N_p (power number for propeller)	0.35	-
D (Agitator diameter)	1.65	m
ρ (liquid density)	1000	kg/m^3
N (agitation speed)[b]	1.50	rotation/s
η_g (Global efficiency for agitation)	0.70	-
Hydrolysis reactor (200 m^3)[a]		
N_p (power number for propeller)	0.35	-
D (Agitator diameter)	1.65	m
ρ (liquid density)	1000	kg/m^3
N (agitation speed)[b]	1.50	rotation/s
η_g (Global efficiency for agitation)	0.70	-
Fermentor (200 m^3)[a]		
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 propeller)	0.35	-
D (Agitator diameter)	1.65	m
ρ (liquid density)	1000	kg/m^3
N (agitation speed)[c]	3	rotation/s
Q (air flow)[d]	3.34	m^3/s
η_g (Global efficiency for agitation)	0.70	-
η_c (Global efficiency for compressor)	0.50	-
Chiller efficiency	0.60	kW/ton
Heat released per mole oxygen consumed during the fungal fermentation	460	kJ/mol O_2 consumed

a. The geometry of the reactors is 5 meter in diameter and 12 meter in height. The height for 200 m^3 effective volume is at 10 meter. The impeller is propeller.
b. The agitation speed for pretreatment and hydrolysis reactors is 90 rpm.
c. The agitation speed for fermenters is 180 rpm.
d. The air flow is 1 vvm.