Version of Record: https://www.sciencedirect.com/science/article/pii/S0960852422001092
Manuscript_018ad861558c58cf74d531960294c9a5

Jan 17, 2022

Plant Biomass Fermentation by the Extreme Thermophile *Caldicellulosiruptor bescii* for Co-Production of Green Hydrogen and Acetone: Technoeconomic Analysis

Ryan G. Bing[#1], Christopher T. Straub[#1], Daniel B. Sulis[2], Jack P. Wang[2], Michael W.W. Adams[3], and Robert M. Kelly[1,*]

[#] *Contributed equally to this work*

[1] *Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC 27695*

[2] *Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC 27695*

[3] *Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602*

Submitted to: *Bioresource Technology* (December 2021)

Revision submitted: January, 2022 (BITE-D-21-09115)

Running Title: Thermophilic Biorefinery for Acetone and H_2 Production

***Address Correspondence to:** Robert M. Kelly
Department of Chemical and Biomolecular Engineering
North Carolina State University
Raleigh, NC 27695
Phone: 919-515-6396
Email: rmkelly@ncsu.edu

ABSTRACT: A variety of chemical and biological processes have been proposed for conversion of sustainable low-cost feedstocks into industrial products. Here, a biorefinery concept is formulated, modeled, and analyzed in which a naturally (hemi)cellulolytic and extremely thermophilic bacterium, *Caldicellulosiruptor bescii*, is metabolically engineered to convert the carbohydrate content of lignocellulosic biomasses (i.e., soybean hulls, transgenic poplar) into green hydrogen and acetone. Experimental validation of *C. bescii* fermentative performance demonstrated 82% carbohydrate solubilization of soybean hulls and 55% for transgenic poplar. A detailed technical design, including equipment specifications, provides the basis for an economic analysis that establishes metabolic engineering targets. This robust industrial process leveraging metabolically engineered *C. bescii* yields 206 kg acetone and 25 kg H_2 per metric ton of soybean hull, or 174 kg acetone and 21 kg H_2 per metric ton transgenic poplar. Beyond this specific case, the model demonstrates industrial feasibility and economic advantages of thermophilic fermentation.

KEYWORDS: Acetone, Green hydrogen, *Caldicellulosiruptor bescii*, Extreme thermophiles, Technoeconomic analysis

1. INTRODUCTION

Many process concepts have been proposed for conversion of biomass into biofuels and bio-based chemicals that involve a range of feedstocks, chemical treatments, and mesophilic microbial hosts (Bing et al., 2021). While short-term interest in establishment of these processes on an industrial-scale ebbs and flows with fluctuation in the price of oil, longer-term perspectives that incorporate climate change and diminishing supplies of fossil fuels necessitate the establishment of biorefineries based on lignocellulose (Usmani et al., 2021).

The path to a robust low carbon bioeconomy is not yet clear. Challenges with lignocellulosic ethanol scale-up to an industrial process (Raj et al., 2021) have precipitated a reevaluation of the economics and state of this technology. This has induced a pivot toward 'Gen 1.5' biofuels, which are generated from underutilized portions of agricultural products. Example feedstocks include soybean hulls (seed coat), corn kernel fiber, and sweet sorghum bagasse, which are generated after removal of high starch and soluble sugar components (Beri et al., 2020; Li et al., 2013; Liu & Li, 2017). As with Gen 2 feedstocks, Gen 1.5 feedstocks are typically high in cellulose and hemicellulose. . Unlike Gen 2 feedstocks, Gen 1.5 feedstocks tend to be significantly lower in lignin and therefore less recalcitrant to biotransformation. Gen 1.5 feedstocks offer an industrial entry point for lignocellulosic biofuel technology by utilizing low value streams from existing processes. This eliminates the risk associated with development of infrastructure to grow and process Gen 2 feedstocks before lignocellulosic biofuel technology is demonstrated at industrial scale. Here, soybean hulls are considered as an example case for Gen 1.5 feedstocks. Soybean hull (seed coat) represents 7-8% of total soybean seed dry weight (Liu & Li, 2017). Hulls are removed during the production of soybean oil (~18% of seed) and meal (~74% of seed); hull by-product is often sold as low value animal feed (Johnson, 2008). Soybean hulls are a lignocellulosic portion of the seed, yet its composition is quite different from the more commonly proposed lignocellulosic feedstocks, such as corn stover, switchgrass, and poplar. Wood and grass feedstocks typically contain 40-50% cellulose (glucose), 25-30% hemicellulose (primarily xylose with smaller amounts of other carbohydrates), and 20-30% lignin (Sharma & Saini, 2020). In comparison, soybean hulls typically contain 29-51% cellulose, 10-20% hemicellulose, 1-4% lignin, 6-15% pectin, and 6-14% protein (Liu & Li, 2017).

Soybean hulls have been processed with various types of pretreatments (acid, enzymatic, thermo-mechanical) to release fermentable sugars and simultaneous saccharification and fermentation to produce ethanol (Liu & Li, 2017). These processes are limited by the energy and cost of treatments required to produce fermentable sugars. An ideal microorganism for processing lignocellulosic substrates is able to both degrade and extensively convert the carbohydrate fraction into bioproducts. Correct selection of microbe-feedstock pairs is central to the industrial potential of these processes (Bing et al., 2021). Identification and engineering of feedstocks to match microbial degradation and conversion capabilities is possible, as demonstrated with *Caldicellulosiruptor bescii* for transgenic poplar (Straub et al., 2020b; Straub et al., 2019b) and soybean hulls (this work). Degradation of corn fiber (another Gen 1.5 substrate) has been demonstrated by *Clostridium thermocellum* co-cultures with hemicellulolytic microbes (Beri et al., 2020). *C. thermocellum* is limited by the inability to degrade and convert C_5 saccharides into desired products (Beri et al., 2020), which prompted work to develop a co-culture with hemicellulolytic microbes to utilize C_5 sugars. However, the most effective hemicellulolytic co-culture partner (newly isolated *Herbinix* spp. Strain LL1355) is not genetically tractable at present, and does not produce significant amounts of desirable products (Beri et al., 2020). Carbohydrate conversion must ultimately result in a product of value at high yield and volumetric productivity, aligning with an economic purification process.

Considered here is the technoeconomic analysis of the extremely thermophilic conversion of soybean hulls as well as transgenic poplar to industrial chemicals (acetone and green H_2), based on the naturally lignocellulolytic bacterium, *C. bescii* (T_{opt} 78°C) that natively ferments both C_6 and C_5 sugars to acetate, lactate, H_2 and CO_2. *Caldicellulosiruptor* species are able to produce H_2 near the theoretical Thauer limit (4 mol H_2/glucose) which is favorable for coproduction with acetone (Straub et al., 2020a). Alternative products, such as ethanol, require additional reduced cofactors (such as NADH) that result in the reduction or elimination of H_2 to achieve high selectivity to target molecules. Additionally, high fermentation temperatures enable *in situ* vapor phase separation of acetone at atmospheric pressure (Straub et al., 2020a). From biological and industrial viewpoints, *C. bescii* is an ideal host for co-production.

Over the last decade, a robust genetic system has been established for *C. bescii* (Chung et al., 2012; Lipscomb et al., 2016), allowing significant genetic manipulations with demonstrated genomic stability. This has permitted work toward ethanol production in C. bescii (Williams-Rhaesa et al., 2018). Further, C. bescii was recently engineered to produce acetone - rather than its native acetate - as its primary carbon fermentation product at high selectivity (Straub et al, 2020a) while maintaining H2 production as its means of anaerobic electron disposal. The requirements and strategies to reach the yield, titer, and productivity metrics utilized in this analysis are detailed further in that work and efforts are ongoing toward this end. The question arises as to whether a biomass to bio-products process, operating at extremely thermophilic temperatures (≥ 70°C), can be technically and economically viable. This prospect is addressed through a detailed technoeconomic analysis and experimental substrate evaluation with *C. bescii*, for conversion of soybean hulls (Gen 1.5) or transgenic poplar (Gen 2). Evaluation of both Gen 1.5 and Gen 2 substrates tests feasibility for a currently available biomass as the main case, and provides insight into a possible Gen 2 substrate future.

2. MATERIALS AND METHODS

2.1 Process Modeling in Aspen, Equipment Sizing, & Capital Costs

Mass and energy balances were determined utilizing basic chemical engineering analysis. Storage tanks were sized based upon the estimated volumetric holding tank requirements for a proposed plant. Once energy and mass balances were determined, distillation towers and a selected set of key heat exchangers and compressors were modeled in Aspen Plus V10 (Aspen Technology Inc., https://www.aspentech.com) to determine the physical parameters of the equipment. For heat exchangers, the mass flow rates were fixed as were the inlet and outlet temperatures of the process. The software then determined physical parameters, such as length, diameter, number of tubes, and tube diameter. Process stream parameters, such as ΔP across the heat exchanger, were also generated in the software. UNIQUAC Equation of State was used for all processes, except for three product gas streams (compressor 2, heat exchanger 4, and heat exchanger 5) where LK-PLOCK was used due to the high pressure/H_2 content of those streams.

Equipment costs were estimated utilizing available design guidelines (Couper et al., 2012; Green & Perry, 2008), accounting for size, materials of construction and pressure rating. A 'module factor' was utilized to account for accessory equipment, such as piping, instrumentation, insulation, foundations, and electrical wiring. In addition, an 'installation factor' for each piece of equipment was multiplied by the base cost to account for installation costs. Finally, the Chemical Engineering Plant Cost Index (CEPCI) was utilized to update the costs to be current as of May 2021. A 2011 NREL technoeconomic study that designed a plant to produce ethanol from corn stover (Humbird et al., 2011) was used as a reference for costing certain equipment, as indicated (adjusted for size accordingly).

Total Capital Investment was calculated by estimating other direct cost (warehouse, site development, additional piping), indirect costs (prorated costs, field expenses, offices, project contingency, miscellaneous other costs), and land price. Values for these items were estimated using methods described in the 2011 NREL study and other sources (Humbird et al., 2011; Towler & Sinnott, 2013). Other direct costs are taken as percentages of the installed equipment costs, indirect costs are percentages of the total direct costs.

2.2 Economic Analysis of Process

Capital costs for equipment were determined, as described in the 'Equipment Sizing' section. Soybean hull price was obtained from literature (Huang et al., 2016). Acetone price was based upon market data obtained from Intratec (Intratec.us). H_2 price range was obtained from a European Commission's hydrogen strategy report. (European Commission, 2020).

Utility costs were determined from various sources based on independent assumptions. Cost of electricity was obtained from the U.S. Energy Information Administration (EIA) for industrial electricity prices in Iowa (average of entire year) and thus $0.07/ kWh was used (U.S. Energy Information Administration, 2021). Since this facility is co-located with a soybean or transgenic poplar processing facility, process water (30°C, $0.08/m^3) and low pressure steam (150°C, 400kPa, $0.015/kg) were priced (Ulrich & Vasudevan, 2006) as if purchased from the adjoining facility, resulting in some offset of capital and operating costs. As a result, no capital costs are included for a steam generation facility, nor for process water cooling towers. A chiller system is included to provide glycol chilling at -15°C ($3.35/GJ).

Plant labor, maintenance, engineering, supervision, and administrative services were obtained from the 2011 NREL lignocellulosic ethanol report (Humbird et al., 2011). The data contained salaries for multiple positions (in 2007 dollars) along with a 90% premium for labor overburden (benefits, safety, general facilities, and similar expenses). The number of employees was reduced from the NREL report, since there is no primary feedstock storage area, no enzyme production unit, and no pre-treatment area.

2.3 Quantitative Saccharification of Substrates

Compositional analysis of lignocellulosic substrates (soybean hull and poplar), before and after fermentation, was done via the Klason procedure (Wang et al., 2018). The acid soluble fraction was neutralized with $CaCO_3$ and filtered through a 0.22 µm PES syringe filter

(Genesee Scientific 25-243). Acid-solubilized sugars were determined with a Waters Arc HPLC (RI detector) and a Shodex SP0810 sugar column with 0.6 mL/min water mobile phase at 85°C.

2.4 Transgenic Poplar Production

Transgenic poplar (*Populus trichocarpa* genotype Nisqually-1) was produced in this study using *Agrobacterium tumefaciens* (strain C58) following the established method (Song et al., 2006). An RNA-interference construct (i20; (Wang et al., 2018)) was used to target the transcriptional suppression of a monolignol biosynthetic gene, *PtrC3H3* (Gene ID: Potri.006G033300.1). Putative transgenic plantlets were produced and genomic DNA was extracted from immature leaves using the Qiagen DNeasy Plant Mini Kit (Invitrogen/Life Technologies, Grand Island, NY). Genome integration of the transgene was then confirmed by PCR using gene-specific primers (Wang et al., 2018). Following PCR confirmation, the transgenic line (i20-3-1) was vegetatively propagated by *in vitro* tissue culture, and 3 clonal copies were transferred to a greenhouse along with 3 wildtype poplar controls. The wildtype and transgenic *poplar* were planted in ½ Miracle-Gro Soil (Scotts Miracle-Gro products, Maysville, OH, USA) and ½ Metro-Mix 200 (Sun Gro, Bellevue, WA, USA) at 16 h light/8 h dark cycles, with supplement lighting of ~300 µE m^{-2} s^{-1} (Song et al., 2006). All wildtype and transgenic poplar used for analysis were 6 months old. De-barked stem wood samples were harvested and air-dried at room temperature for solubilization experiments.

2.5 Bacterial Strains, Growth Conditions, and Substrate Solubilization Experiments

Caldicellulosiruptor bescii (DSM 6725) was grown on in modified defined DSM671 media, as previously described, with the addition of 20 mM MOPS (3-(N-morpholino)propanesulfonic acid) (Zurawski et al., 2015). Cultures were grown at 75°C, 150 RPM, and 50mL working volume in 125mL total volume seal serum bottles with N_2/CO_2 (80/20 v/v) headspace using an New Brunswick Innova 42 incubator shaker.

Soybean hulls and poplar were prepared by milling, sieving, and washing, as previously described (Straub et al., 2019b), except washing and drying was performed at 65°C. Soybean hulls were generously provided by Dr. Marko Hakovirta (Department of Forest Biomaterials, NC State University), who acquired them from a commercial vendor (previously used in another study (Faradilla et al., 2021)). The vendor indicated that the hulls contained 48.1% cellulose, 19.7% hemicellulose, 4.3% lignin, 0.8% fat, 11.8% protein, and 3.7% ash (dry weight percentages), as received (not milled or washed).

For substrate solubilization experiments, all mass loadings were based on carbohydrate content. Freezer stocks of *C. bescii* were recovered in media with 2 g/L Avicel, 2 g/L beechwood xylan (Biosynth Carbosynth YX45751), and 1 g/L cellobiose. After 48 hours, a 2% inoculum was transferred to media with 4.5 g/L desired substrate (soybean hulls or poplar), 0.2 g/L Avicel, 0.2 g/L beechwood xylan, and 0.1 g/L cellobiose. After 3 days, a 2% inoculum was passaged to 5 g/L substrate. After 3 days, cells were passaged for a starting density of 10^7 cells/ml on 5 g/L substrate (previously weighed and exact amount recorded) in triplicate for mass solubilization experiments. After 7 days, cultures were harvested and analyzed as previously described (Straub et al., 2019b), except drying steps were carried out at 75°C. Dried post-fermentation material was saved and quantitative saccharification was done, as described above.

3. RESULTS AND DISCUSSION

C. bescii has been examined for its ability to solubilize and metabolize lignocellulosic substrates, such as switchgrass, poplar, and genetically engineered lines of these substrates (Straub et al., 2020b; Straub et al., 2019b; Zurawski et al., 2017). While it performed exceptionally well on a few lines of genetically engineered low lignin poplar trees, such feedstocks are not available in industrial quantities at present. The prospects for using a lignocellulosic agricultural by-product feedstock is closer at hand. Here, both types of opportunities are considered.

Soybean hulls are a commercially available feedstock that align with the natural capabilities of *C. bescii*. As previously discussed, soybean hulls are rich in carbohydrate content and low in lignin. Additionally, soybean hulls are of uniform composition and of small particle size. These factors have significant impacts on carbohydrate accessibility and conversion (Li et al., 2016; Vidal et al., 2011). Looking towards Gen 2 feedstocks, transgenic poplar was also considered, especially lines that can be extensively solubilized by *C. bescii* without significant growth defects in the trees (Straub et al., 2020b).

3.1 Substrate Validation

C. bescii fermentation of selected feedstocks was assessed to support process design assumptions, including carbohydrate consumption and mass loadings. These variables are key to sizing bioreactors and completing material and energy balances. Carbohydrate and lignin composition of soybean hulls, wildtype poplar, and transgenic poplar (line i20-3-1) were determined before and after 5 g/L carbohydrate loading *C. bescii* fermentations (**Figure 1**). Pre-fermentation composition showed similar total carbohydrate content but differing distributions among carbohydrate types and lignin content (**Figure 1A**). As expected, wildtype poplar had the highest lignin, and soybean hulls the lowest. Substrates were subjected to minimal pretreatment (milled and washed with 65°C water to remove soluble sugars). Wildtype *C. bescii* fermented the substrates predominantly into acetate (**Figure 1B**). *C. bescii* solubilized 82% of the soybean hull total carbohydrate (cellulose, hemicelluloses, galactomannan, pectin) and 55% of the transgenic poplar line carbohydrate (**Figure 1C**). Both of these substrates were significantly less recalcitrant in comparison to wildtype poplar, which solubilized at 31%. *C. bescii* solubilized glucose (86%), xylose (92%), galactose (80%), and mannose (100%) to a greater extent than arabinose (17%) from soybean hulls (**Figure 1D**). The arabinose is likely contained mostly in pectin, and represents only 6% of the total mass of soybean hulls. The process model assumed that 65% of soybean hulls are fermentable by *C. bescii*. The experimentally determined carbohydrate consumption (55%) was used for the i20-3-1 transgenic poplar line case.

3.2 Capacity Basis for Commercial Scale Process

Prior economic analyses for lignocellulosic ethanol have identified the feedstock collection radius as a critical factor, placing an upper limit on plant size and some benefits for larger economies of scale (Humbird et al., 2011; Wang et al., 2017). In contrast to a seasonal feedstock, such as corn stover, soybean hulls are continuously produced by processing facilities throughout the year. By-product utilization creates significant benefits by eliminating collection and storage logistical issues.

The relatively high density of soybean processing plants in the U.S. Midwest creates an opportunity for multiple sites to deliver hulls to a hypothetical facility. Ideally, the facility would be co-located with a large soybean processing facility (>50 million bushels per year) and receive the remainder of its feedstock requirements from other facilities located within 100 km to minimize transportation costs. Here, a plant was designed and sized to utilize 550 mt/day of hulls, with a 90% plant utilization factor, which is equivalent to 180,000 mt hulls/year. At 8% of soybean total mass, this would require ~83 million bushels (2.25 million mt) of processed soybeans per year. Annual U.S. production of soybeans (~115 million mt soybeans in 2020/21 (Foreign Agricultural Service, 2021)) would support up to ~50 identical facilities of the type modeled in this study. While all calculations are performed on a dry basis, the hulls would be delivered ‘wet’ to prevent the biomass source from expending resources to dry the product.

3.3 Metabolically Engineered Microorganism Development Targets

The proposed process is dependent upon the development of a biocatalyst to perform the conversion of soybean hulls to acetone and H_2, which requires plausible assumptions for technical targets (**Table 1**). This study addresses the rationale and evidence that metabolic engineering targets for *C. bescii* are achievable.

Maximal acetone titer was set to 20 g/L. This acetone titer is low enough to be below the toxicity of level of acetone to *C. bescii* (23.3 g/L), yet high enough to allow for acetone enrichment of the fermenter vapor, facilitating *in situ* acetone separation (Straub et al., 2020a). Lower titers of acetone would require additional vacuum or higher temperatures during fermentation to enable the same process flow. The acetone titer assumed here sets targets for future metabolic engineering work for *C. bescii*. Less volatile alternative products, like ethanol, would require significantly higher titers, higher temperatures, or lower pressures to match the performance of acetone in a similar process. Clearly, volatile products significantly leverage the advantage of thermophilic fermentation.

A critical hurdle to achieving the envisioned bioprocess is the yield and productivity of the metabolically engineered strain. Carbohydrate utilization rate, essentially the catalytic rate of reaction for the process, determines fermenter residence time. While *C. bescii* has been grown on high loadings of cellulose and switchgrass, its metabolic activity ceased after only a portion of the substrate was consumed (Basen et al., 2014). Further studies utilizing a fixed substrate continuous flow reactor confirmed that *C. bescii* ceases consuming substrate due to entering stationary phase (Straub et al., 2019a). *C. bescii* is estimated to utilize carbohydrate at >0.7 g/L/h based on available data for related organisms (**Table 1**).

Carbohydrate consumption at 0.7 g/L/h was assumed for the models in this process design. After accounting for 12% carbon loss to biomass and non-target products, acetone is produced at 0.20 g/L/h along with H_2 at 0.025 g/L/h. To achieve this, metabolic engineering efforts will require *C. bescii* to undergo adaptive laboratory evolution that has been widely applied to achieve productivity and titer improvements in various biocatalyst strains (Sandberg et al., 2019). Adaptive laboratory evolution has been used successfully to achieve industrially relevant ethanol titers in two thermophilic bacteria, *Thermoanaerobacterium saccharolyticum* and *Clostridium thermocellum*, and is the basis for the modest parameter estimates used here (**Table 1**). Further details of key elements required to obtain a strain that will produce at high titer and yield are described in the initial demonstration of acetone production by *C. bescii* (Straub et al., 2020a). Considering these ongoing efforts to metabolically engineer *C. bescii*, examination of the proposed process and economics supports development targets.

3.4 Process Description

Key points of the process are described in the following sub-sections. The fermentation system is designed to be the rate-limiting step of the process. All other unit operations are designed with a capacity up to 25% above the fermentation output. This design decision assumes that fermentation is the most capital-intensive portion of the plant and bioreactor capacity should not go un-utilized. It also allows for operational improvement efficiency programs in fermentation to increase output without additional capital.

3.4.1 Fermentation

Fermentation (**Figure 2**) is conducted with an initial 100 g/L loading of soybean hulls. Over the first four days, acetone titer increases to 20 g/L and initiates the steady state production phase, where acetone is produced at the same rate as it evaporates and exits the fermentation vessel with H_2, CO_2, and some water vapor (**Figure 3**). High fermentation temperature (70°C) and slight vacuum (89 kPa) enable the 'bioreactive distillation' process concept (Straub et al., 2020a) to capture acetone product from the fermentor vapor phase. Two soybean hull additions (50 g/L per addition) are made to compensate for fermentation mass loss and thus further extending the fermentation. Upon cessation of fermentation at approximately 400 h, the remaining acetone is stripped by lowering the fermenter pressure. Remaining water and solids are discharged for water recycle and solids processing operations.

3.4.2 Fermentation Off-Gas Processing

Due to its high volatility at 70°C and 89 kPa, nearly the entirety of acetone produced is removed as vapor before liquid leaves the fermentation vessel. Thus, products of interest are recovered from the vapor stream. Fermentor vapor is cooled and compressed to condense water and acetone (stored, ready for distillation), leaving a high-pressure gas mixture of H_2/CO_2 with <1% acetone (**Figure 2**). Residual acetone is removed from the H_2/CO_2 stream via mesoporous silica adsorbers (20 g acetone/g-particle capacity) (Hung et al., 2009). Two adsorbers are used, one online, while the other is recharged with minimal heated waste CO_2.

H_2 is then purified via pressure swing adsorption (PSA) and waste CO_2 is utilized to backfill fermenters while the remainder is vented to the atmosphere. (Note that the high-pressure low temperature PSA CO_2 stream is well suited for CO_2 liquefaction and geological sequestration, but this option is not explored further here.) The H_2/CO_2 stream is nearly 60 mol% H_2 and is well suited to PSA which is standard for purifying H_2 from steam methane reforming which contains 80-90 mol% H_2 with the balance being primarily CO_2 (LeValley et al., 2014). In general, selective binding of CO_2 to a zeolite or activated carbon at high pressure and low temperature is preferred to separate CO_2 from H_2 (Wiessner, 1988). This analysis assumes H_2 is purified to 99.9 mol% with 85% recovery (Wiessner, 1988). The system uses eight adsorber tanks with a centralized valving system and a high-pressure buffering tank to minimize pressure disturbances from the product gas recovery process.

Storing H_2 is an unresolved issue within the envisioned H_2 energy economy and presents a similar issue here. The only H_2 pipelines in the U.S. are located along the Gulf Coast (Hydrogen and Fuel Cell Technologies Office, 2021), far from most soybean processing facilities. It could alternatively be liquified and shipped, but this presents further issues and expenses. However, this issue is not considered here.

3.4.3 Acetone Distillation

Two continuous columns are used to distill the condensed water and acetone mixture (**Figure 2**), the first providing a pure water stream (99.99 wt% water) to the water recycle system, while the distillate contains 93.25 wt% acetone. The second column improves acetone purity to 98.77 wt% (primary contaminant is dissolved CO_2). Acetone distillate from the second column is at 30°C and is stored for shipment. While acetone/water systems do not form an azeotrope, as seen in ethanol/water systems, molecular sieves are an alternative. Further design iterations may explore the use of a molecular sieve in place of the second column.

3.4.4 Solid By-Products and Water Recycle

Even though soybean hulls are readily solubilized by *C. bescii*, both soluble and insoluble solids remain in the fermenter effluent (‘whole stillage’). Insoluble solids are separated by centrifuge and further dewatered by a vacuum belt filter (**Figure 2**). Approximately two-thirds of clarified liquid is recycled to fermentation (‘backset’); the remainder is sent through evaporators to create a dissolved solids syrup and evaporated water is recycled. This is common practice in corn ethanol plants, where the syrup is typically 25-35% dry matter and is mixed in with the distiller’s grains (Adom et al., 2014). Wetcake is produced at a rate of 110 mt/day dry basis (actual 70% moisture), while syrup is produced at 19.3 mt/day dry basis at 25% solids. For this economic evaluation, it is assumed that neither wetcake nor syrup provide revenue to the plant, but also no expense for disposal is taken. All costs for producing wetcake and syrup are accounted for in capital and operating expenses. Ideally, these streams would have value as animal feed, biomethane digester feedstock, or as soil amendments.

3.5 Energy and Carbon Balance Evaluation

3.5.1 Thermophilic Biocatalyst Impacts

Feasibility of a process is not solely a function of fermentation temperature, but rather determined by analysis of energy balances and economics that consider all unit operations. Heat loss to the environment is proportional to surface area, with volume to surface area ratio decreasing with increasing volume. A 2” spray-on polyurethane coating to the fermenters provides sufficient insulation ($R = 2.1\ m^2 \cdot K/W = 12\ ft^2 \cdot °F \cdot h/BTU$) to result in energy loss of only 1 kJ/m^3/day, assuming outside temperatures averaging 10°C and operational temperature of

70°C. For this process, heat loss from fermentation to the environment accounts for less than 2% of the overall utility expenses of the design. It is far exceeded by the energy required to evaporate and condense both acetone and water. Heating and cooling utility considerations are far more impacted by gas/liquid phase transitions, such as those that occur during distillation, water recovery, or drying solid by-products, rather than the heating and cooling of aqueous streams. Energy lost to the environment from the thermophilic fermenters is a very small drawback, considering the benefits gained by separating product from the vapor stream. Additionally, microbial carbohydrate fermentation will release heat, possibly offsetting heat losses to the environment. This is not evaluated here, but should be considered in the future to determine proper insulation or heating/cooling requirements.

Direct use of steam injection for heating tanks, as well as steam condensate recovery, provides additional benefits. In this model, approximately 300 m^3 water is lost via wetcake and syrup by-products each day. However, this is made up for by approximately 350 m^3 water, either directly from steam injection or by routing steam condensate to the recycle water tank. This is a productive way to extend benefits of purchased steam.

In theory, extreme thermophiles growing at elevated temperatures (≥ 70°C) should be highly resistant to contaminating microorganisms from the surrounding environment. In this assumption, no clean-in-place (CIP) equipment was included in the design. Seed fermenters can be directly steamed to sterilize between seed batches. However, there is always the possibility that a contaminating strain or even an undesired genetic mutant will colonize the plant. This remains an issue that can only be fully understood in practice at scale.

3.5.3 CO_2 Emissions

For a renewable energy facility to be truly sustainable, it must significantly lower the overall carbon footprint when compared to the alternatives. Accounting for the carbon footprint of the soybean hull feedstock is complicated. There is large variability in estimating carbon footprint for soybean cultivation (Castanheira & Freire, 2013), even prior to the processing facility, thus developing a rationale for assigning a given fraction of that footprint to the hulls presents further ambiguity. Assignment of carbon emissions due to production of soybean hulls are not resolved here and only readily quantifiable components are evaluated (**Table 2**).

Carbon content of dry hulls is approximately 50% by mass. At the plant design feed rate of 550 mt/day, full combustion of hulls would generate ~1,000 mt CO_2. Approximately, one quarter of that carbon will be biologically generated during fermentation (259 mt CO_2/day). Remaining carbon originating from the hulls is contained in the products - acetone, wetcake, and syrup. End use of these products determines their life-cycle CO_2 contributions.

Emissions from operation of the facility also require consideration. Generation of steam via combustion of natural gas results in another 70.2 mt CO_2/day. Assuming electricity generation from current grid sources in the U.S. Midwest, CO_2 emissions related to electricity consumption account for 23.5 mt CO_2/day. Use of external energy sources thus results in a total of 93.7 mt CO_2/day. However, there is enough energy contained within produced H_2 (1912 GJ/day) that it could be utilized for both electricity and steam production (both via high temperature fuel cell – e.g. molten salt) to replace natural gas (1395 GJ/day) and electricity (210 GJ/day) requirements at the cost of reducing revenue from selling the hydrogen. Current market price for renewable hydrogen would make this an economically unattractive use of the H_2. H_2 prices of ~$1.00/kg or lower are needed before the H_2 value would be lower than the offset utility costs. Yet this remains an option if H_2 transportation to market remains as a hurdle.

3.5.4 Opportunities for Energy Efficiency Improvements

This analysis seeks to establish a commercial process paradigm that addresses engineering details. However, there are significant energy recovery opportunities that could be considered in future iterations. Many such recovery options were not included in this analysis to prevent obscuring the key process flows. These potential improvements are not included in energy balances nor economic evaluation, but are mentioned for completeness. Additionally, there are likely many more energy recovery synergies, other than those mentioned below, that could be implemented after operation of a commercial scale facility and years of experience.

There are many streams at >70°C that are good candidates for heat recovery, such as those in the off-gas processing heat exchangers (**Figure 2**). These streams could be used to offset the steam heat required for the thin stillage evaporators (especially, if evaporators are operated at slight vacuum). Another opportunity is to utilize energy of expansion of CO_2 exiting pressure swing adsorption for cooling to reduce the duty of chilled glycol refrigeration system. Furthermore, there will be significant heat generation from microbial activity, especially at high carbohydrate solubilization levels. Heat generation in cultures of *C. bescii* has not been examined, but could partially offset steam requirements and heat losses.

3.6 Economic Evaluation

As with most industrial chemicals and fuels, process economics are significantly influenced by feedstock cost, yield, and product selling price. To determine those factors that are most influential, variation in prices for soybean hulls, acetone, and H_2 were considered. However, the potential value of by-products (wetcake and syrup) were not considered in any scenario, although they may have significant impact.

3.6.1 Feedstock & Product Prices

The proposed process is based upon a soybean hull feed, assumed to cost \$90-\$150/mt (Huang et al., 2016); although soybean prices fluctuate, they have not changed significantly since 2016 (Foreign Agricultural Service, 2021). Revenue generated from the sale of acetone and H_2 markedly influence process economics. While the mass of acetone produced is eight times that of H_2, the relative value (\$/kg) of H_2 is much higher. Currently, acetone is primarily produced as a by-product of phenol production (Zakoshansky, 2007) and prices are subject to high variability, fluctuating between \$0.57-\$1.72/kg since 2007 in the USA (IntraTec Solutions, LLC). Acetone prices were based on average market data with a baseline acetone price of

\$0.956/kg. The low price of natural gas has created an extremely competitive market for H_2. The European Commission's July 2020 report on H_2 strategy listed H_2 prices: grey H_2 (fossil-based) at 1.50 € /kg (\$1.77/kg, July 2020), blue H_2 (fossil-based with carbon capture) at 2 €/kg (\$2.36/kg), and green H_2 (renewable) at 2.5-5.5 €/kg (\$2.94-\$6.48/kg) (European Commission, 2020). A broad price range (\$1.00 - \$5.00/kg) for the green H_2 produced in this process was examined with a baseline of \$3.00/kg. Baseline prices were used with the material balance to calculate their daily revenue or expense (**Table 3**).

3.6.2 Capital Costs

After sizing equipment and determining base equipment cost, scaling factors were applied to determine the cost of installed equipment, which accounts for the piping, instruments, insulation, and installation costs for with the equipment. Total Capital Investment accounts for other direct and indirect costs as percentages based on installed equipment costs (**Table 4**).

A 2011 National Renewable Energy Laboratory (NREL) design for a 2000 mt/day corn stover to ethanol (61 MMGPY or 231 million L/yr) facility was used as a comparison (Humbird et al., 2011). A gallon of ethanol equivalent (GPYeq) is utilized to compare the total combustion energy contained within the products, with a total of ~20 MMGPYeq (12.9 MMGPYeq for acetone and 7.1 MMGPYeq for H_2) for the facility.

While the modeled process and facility are roughly a third of the total MMGPYeq capacity of the 2011 NREL study, comparison of unit operations required for total installed costs provides a clear picture of benefits from a consolidated bioprocess in which neither enzyme production nor pre-treatment are required (**Table 4**). Lack of pre-treatment chemicals also avoids wastewater treatment costs. Further, energy requirements of this process did not suggest utilizing byproduct wetcake for powering a combined heat and power turbogenerator.

This comparison highlights one drawback of utilizing *C. bescii* under the current assumptions. Relatively low volumetric productivity compared to other biocatalysts, such as yeast, necessitate significant capital allocated to fermentation volume. While total installed equipment capital requirements are less than half of the total capital investment, this does not add significant operating costs nor negate the attractive economics. Yet, it does identify an important technical target for metabolic engineering and adaptive laboratory evolution.

3.6.3 Operating Costs and Bottom Line

Beyond initial capital investment, daily material costs, and utility costs, there are other expenses, including operations, maintenance, and administration (**Table 5**). A bottom line as earnings before interest, tax, depreciation, and amortization (EBITDA) was calculated. EBITDA is not profit nor free cash flow and does not account for fair return on investment nor the type of financing utilized to obtain capital and start-up funding. The annual EBITDA summary for baseline prices can be seen in **Table 5**. With the modeled capital costs, estimates for start-up costs and non-capital expenses, a blanket 15-year depreciation timeline for capital, 30% overall tax rate, and average inflation of 2.3%, an EBITDA of \$13.4M/year is required to earn a 15% internal rate of return (IRR) or \$8.5M/year for 10% IRR over a 30-year period.

The three factors that most heavily influence the EBITDA margins are soybean hull feedstock cost, acetone selling price, and H_2 selling price. Holding all other factors constant, these key materials were evaluated for IRR of 10% and 15%, with an IRR of 10% leading to more favorable conditions (**Figure 4**). Looking at an IRR of 15% a selling price of H_2 \$1.77/kg, competitive with H_2 production from fossil sources, is viable for average acetone sale price (\$956/mt), if soybean hulls are purchased at \$118/mt or cheaper. Higher hull prices are feasible if acetone sale price is higher. Higher price for soybean hulls (\$150/mt) can make sense for a renewable hydrogen price (\$3/kg) and slightly above average acetone price (\$964/mt). At fossil hydrogen prices and high hull prices, acetone would need to sell for higher than average prices (\$980/mt at IRR 10%, \$1111/mt at IRR 15%). It should be noted that whole soybean price for 2020/21 ~\$450-500/mt with soy protein meal ~\$440/mt and soybean oil ~\$1,200/mt. Since soybean hull is the least valuable and smallest portion of the bean, it stands to reason that unless soybean prices rise significantly in the future, the \$150/mt scenario is unlikely.

It is worth noting that there is currently a $1.01 credit per ethanol equivalent gallon available in the U.S. to fuels meeting the qualifications to be considered cellulosic fuels. There are a variety of requirements, including CO_2 reductions compared to petroleum-based alternatives. No subsidies or tax credits were considered, but these would substantially improve the attractiveness of such an investment.

3.7 Feedstock Flexibility

This study utilized soybean hulls as a ‘bridge’ concept, with the long-term goal of utilizing either pre-treated or engineered lignocellulose substrates. The biocatalyst under consideration here (*C. bescii*) consumes a variety of carbohydrates without catabolite repression (VanFossen et al., 2009), and can be considered insensitive to variation in carbohydrate composition. In practice, this means that *C. bescii* can utilize grain starches (Gen. 1), soybean hulls (Gen. 1.5), or more traditional lignocellulosic feedstocks (Gen. 2), despite their respective carbohydrate compositional differences. While utilizing a Gen. 1 feedstock is not ideal from an environmental perspective, it does provide a contingency option if soybean hulls are unavailable, or if there are solids handling technical challenges encountered in the first iteration at commercial scale.

Looking forward to Gen. 2 feedstocks, *C. bescii* would likely require pre-treatment for many current feedstocks (e.g., corn stover or wildtype poplar). However, other low lignin substrates, such as corn cobs (Liu et al., 2010) and other Gen 1.5 substrates (corn fiber, sweet sorghum bagasse), could potentially be utilized by the same facility. Maintaining the ability to utilize a Gen. 1, Gen. 1.5, or Gen. 2 feedstock provides risk reduction for capital investment, with flexibility to adapt to more complex feedstocks over time, and versatility to operate with the most economic and environmentally suitable feedstock combinations. Recent work to produce transgenic poplar lines with reduced recalcitrance has shown to pair well with *C. bescii* (Straub et al., 2020b). Line i20-3-1 is a recently generated poplar line with no apparent growth defects compared to wildtype poplar (WT3-1) in 6-month old trees. *C. bescii* was able to solubilize carbohydrate from i20-3-1 at 55% (**Figure 1C**). This is not too far from the 65% utilization assumed in the modeled process. Further work to improve poplar trees could result in a Gen 2 feedstock that could be a drop-in replacement for the modeled soybean hulls. Examples of possible Gen 1, 1.5, and 2 feedstock price per accessible carbohydrate are shown in **Table 6**. Usage of transgenic poplar (55% mass utilization), in the modeled process would result in ~15% decrease in products, unless additional fermentors were added to the process. In order to maintain production rate, an additional 1 to 2 bioreactors would be needed (additional installed equipment cost ~$1.1M-$2.2M). Poplar feedstock costs would likely be significantly less than soybean hulls (~$50/mt (Wood Resources International, 2017)), which could justify this additional investment. Overestimating extra installed equipment costs at $10M plus other increased operating and indirect costs, with fossil hydrogen prices ($1.77/kg), required acetone sell prices are below average for IRR 10% ($699/mt, EBITDA $11.0/year) and for IRR 15% ($898/mt, EBIDTA $17.2M/year), indicating feasibility of the process for transgenic poplar.

4. CONCLUSIONS

Significant advancements are still needed to develop *C. bescii* to produce acetone at 0.2 g/L/h with >20 g/L effective titer. But if metabolic engineering targets can be achieved, the reasonable economics of the TEA model demonstrates the benefits and feasibility of industrial use of extremely thermophilic fermentation (≥ 70°C). High temperature fermentation enables the use of a unique separation technology (‘bioreactive distillation’) and reduction of sterilization costs, with minimal increase to facility energy requirements. Opportunity exists to consider thermophilic fermentation for production of a variety of biofuels and bioproducts.

ACKNOWLEDGMENTS

This work was supported in part by the US Department of Agriculture (2018-67021-27716), the US National Science Foundation (CBET-1802939) and the US Department of Energy BER Awards DE-SC0019391 and DE-SC0022192. CT Straub acknowledges support from a US DoEd GAANN Fellowship (P200A160061). RG Bing acknowledges support from an NIH Biotechnology Traineeship (NIH T32 GM008776-16).

REFERENCES

1. Adom, F., Fan, J., Davis, J., Dunn, P., Shonnard, D. 2014. Compositional analysis of defatted syrup from a corn ethanol dry-Grind process as a feedstock for biobased products. *ACS Sustainable Chem. Eng.*, **2**(5), 1139-1146.
2. Basen, M., Rhaesa, A.M., Kataeva, I., Prybol, C.J., Scott, I.M., Poole, F.L., Adams, M.W. 2014. Degradation of high loads of crystalline cellulose and of unpretreated plant biomass by the thermophilic bacterium *Caldicellulosiruptor bescii*. *Bioresour. Technol.*, **152**, 384-392.
3. Beri, D., York, W.S., Lynd, L.R., Peña, M.J., Herring, C.D. 2020. Development of a thermophilic coculture for corn fiber conversion to ethanol. *Nat. Commun.*, **11**(1937).
4. Bing, R.G., Sulis, D.B., Wang, J.P., Adams, M.W., Kelly, R.M. 2021. Thermophilic microbial deconstruction and conversion of natural and transgenic lignocellulose. *Environ. Microbiol. Rep.*, **13**(3), 272-293.
5. Castanheira, É.G., Freire, F. 2013. Greenhouse gas assessment of soybean production: implications of land use change and different cultivation systems. *J. Cleaner Prod.*, **54**, 49-60.
6. Chung, D., Farkas, J., Huddleston, J.R., Olivar, E., Westpheling, J. 2012. Methylation by a unique α-class N4-cytosine methyltransferase is required for DNA transformation of *Caldicellulosiruptor bescii* DSM6725. *PloS one*, **7**(8), e43844.

7. Couper, J.R., Penney, W.R., Fair, J.R. 2012. *Chemical Process Equipment : Selection and Design. 3rd ed*. Butterworth-Heinemann Elsevier, Oxford.
8. Erickson, M.J., Dobbins, C., Tyner, W.E. 2011. The economics of harvesting corn cobs for energy. *Crop Manag.*, **10**(1), 1-8.
9. European Commission. 2020. Hydrogen strategy for a climate-neutral Europe. https://ec.europa.eu/energy/sites/ener/files/hydrogen_strategy.pdf
10. Faradilla, R.F., Lucia, L., Hakovirta, M. 2021. Hydrothermal carbonization of soybean hulls for the generation of hydrochar: A promising valorization pathway for low value biomass. *Environ. Nanotechnol. Monit. Manag.*, **16**, 100571.
11. Foreign Agricultural Service. 2021. Oilseeds: World Markets and Trade, October 2021, (Ed.) U.S.D.o. Agriculture, USDA. https://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf
12. Green, D.W., Perry, R.H. 2008. *Perry's Chemical Engineers' Handbook. 8th ed*. McGraw-Hill, New York.
13. Herring, C.D., Kenealy, W.R., Shaw, A.J., Covalla, S.F., Olson, D.G., Zhang, J., Sillers, W.R., Tsakraklides, V., Bardsley, J.S., Rogers, S.R. 2016. Strain and bioprocess improvement of a thermophilic anaerobe for the production of ethanol from wood. *Biotechnol. Biofuels*, **9**(1), 125.
14. Holwerda, E.K., Thorne, P.G., Olson, D.G., Amador-Noguez, D., Engle, N.L., Tschaplinski, T.J., Van Dijken, J.P., Lynd, L.R. 2014. The exometabolome of *Clostridium thermocellum* reveals overflow metabolism at high cellulose loading. *Biotechnol. Biofuels*, **7**(1), 155.
15. Huang, H., Long, S., Singh, V. 2016. Techno‐economic analysis of biodiesel and ethanol co‐production from lipid‐producing sugarcane. *Biofuel. Bioprod. Biorefin.*, **10**(3), 299-315.
16. Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Aden, A., Schoen, P., Lukas, J., Olthof, B., Worley, M., Sexton, D., Dudgeon, D. 2011. Process design and economics for conversion of lignocellulosic biomass to ethanol. in: *Dilute-Acid Pretreatment and Enzymatic*

Hydrolysis of Corn Stover, (Ed.) U.S.D.o. Energy, National Renewable Energy Laboratory. Golden, Co. https://www.nrel.gov/docs/fy11osti/47764.pdf

17. Hung, C., Bai, H., Karthik, M. 2009. Ordered mesoporous silica particles and Si-MCM-41 for the adsorption of acetone: A comparative study. *Sep. Purif. Technol.*, **64**(3), 265-272.
18. Hydrogen and Fuel Cell Technologies Office. 2021. Hydrogen Pipelines, (Ed.) U.S.D.o. Energy. Washington, D.C. https://www.energy.gov/eere/fuelcells/hydrogen-pipelines
19. Johnson, L.A. 2008. Oil Recovery from Soybeans. in: *Soybeans*, (Eds.) L.A. Johnson, P.J. White, R. Galloway, AOCS Press, pp. 331-375.
20. Kenner, B., Jiang, H., Russel, D. 2021. Economic Research Serive and Foreign Agricultural Service Situtaion and Outlook Report: Outlook for U.S. Agricultural Trade: November 2021, (Ed.) U.S.D.o. Agriculture, USDA. https://www.ers.usda.gov/webdocs/outlooks/102735/aes-118.pdf
21. LeValley, T.L., Richard, A.R., Fan, M. 2014. The progress in water gas shift and steam reforming hydrogen production technologies–A review. *Int. J. Hydrogen Energ.*, **39**(30), 16983-17000.
22. Li, C., Aston, J.E., Lacey, J.A., Thompson, V.S., Thompson, D.N. 2016. Impact of feedstock quality and variation on biochemical and thermochemical conversion. *Renew. Sust. Energ. Rev.*, **65**, 525-536.
23. Li, S., Li, G., Zhang, L., Zhou, Z., Han, B., Hou, W., Wang, J., Li, T. 2013. A demonstration study of ethanol production from sweet sorghum stems with advanced solid state fermentation technology. *Appl. Energy*, **102**, 260-265.
24. Lipscomb, G.L., Conway, J.M., Blumer-Schuette, S.E., Kelly, R.M., Adams, M.W. 2016. A highly thermostable kanamycin resistance marker expands the tool kit for genetic manipulation of *Caldicellulosiruptor bescii. Appl. Environ. Microbiol.*, **82**(14), 4421-4428.
25. Liu, H.-M., Li, H.-Y. 2017. Application and conversion of soybean hulls. in: *Soybean-the basis of yield, biomass and productivity*, (Ed.) M. Kasai, IntechOpen. Rijeka, Croatia.

26. Liu, K., Lin, X., Yue, J., Li, X., Fang, X., Zhu, M., Lin, J., Qu, Y., Xiao, L. 2010. High concentration ethanol production from corncob residues by fed-batch strategy. *Bioresour. Technol.*, **101**(13), 4952-4958.
27. Raj, T., Chandrasekhar, K., Naresh Kumar, A., Banu, J.R., Yoon, J.-J., Bhatia, S.K., Yang, Y.-H., Varjani, S., Kim, S.-H. 2021. Recent advances in commercial biorefineries for lignocellulosic ethanol production: Current status, challenges and future perspectives. *Bioresour. Technol.*, 126292.
28. Sandberg, T.E., Salazar, M.J., Weng, L.L., Palsson, B.O., Feist, A.M. 2019. The emergence of adaptive laboratory evolution as an efficient tool for biological discovery and industrial biotechnology. *Metab. Eng.*, **56**, 1-16.
29. Sharma, D., Saini, A. 2020. Cellulosic Ethanol Feedstock: Diversity and Potential. in: *Lignocellulosic Ethanol Production from a Biorefinery Perspective*, Springer Singapore, pp. 23-63.
30. Shaw, A.J., Podkaminer, K.K., Desai, S.G., Bardsley, J.S., Rogers, S.R., Thorne, P.G., Hogsett, D.A., Lynd, L.R. 2008. Metabolic engineering of a thermophilic bacterium to produce ethanol at high yield. *Proc. Natl. Acad. Sci.*, **105**(37), 13769-13774.
31. Song, J., Lu, S., Chen, Z.-Z., Lourenco, R., Chiang, V.L. 2006. Genetic transformation of *Populus trichocarpa* genotype Nisqually-1: a functional genomic tool for woody plants. *Plant Cell Physiol.*, **47**(11), 1582-1589.
32. Straub, C.T., Bing, R.G., Otten, J.K., Keller, L.M., Zeldes, B.M., Adams, M.W., Kelly, R.M. 2020a. Metabolically engineered *Caldicellulosiruptor bescii* as a platform for producing acetone and hydrogen from lignocellulose. *Biotechnol. Bioeng.*, **117**(12), 3799-3808
33. Straub, C.T., Bing, R.G., Wang, J.P., Chiang, V.L., Adams, M.W.W., Kelly, R.M. 2020b. Use of the lignocellulose-degrading bacterium *Caldicellulosiruptor bescii* to assess recalcitrance and conversion of wild-type and transgenic poplar. *Biotechnol. Biofuels*, **13**, 43.

34. Straub, C.T., Khatibi, P.A., Otten, J.K., Adams, M.W.W., Kelly, R.M. 2019a. Lignocellulose solubilization and conversion by extremely thermophilic *Caldicellulosiruptor bescii* improves by maintaining metabolic activity. *Biotechnol. Bioeng.*, **116**(8), 1901-1908.
35. Straub, C.T., Khatibi, P.A., Wang, J.P., Conway, J.M., Williams-Rhaesa, A.M., Peszlen, I.M., Chiang, V.L., Adams, M.W.W., Kelly, R.M. 2019b. Quantitative fermentation of unpretreated transgenic poplar by *Caldicellulosiruptor bescii*. *Nat. Commun.*, **10**(1), 3548.
36. Towler, G., Sinnott, R. 2013. *Chemical engineering design: principles, practice and economics of plant and process design*. *2nd ed*. Butterworth-Heinemann Elsevier, Oxford.
37. U.S. Energy Information Administration. 2021. Electric Power Monthly, Vol. April 2021. Washington, D.C., pp. 1-284. https://www.eia.gov/electricity/monthly/
38. Ulrich, G.D., Vasudevan, P.T. 2006. How to estimate utility costs. in: *Chemical Engineering*, Vol. 113, pp. 66-69. https://terpconnect.umd.edu/~NSW/chbe446/HowToEstimateUtilityCosts-UlrichVasudevan2006.pdf
39. Usmani, Z., Sharma, M., Awasthi, A.K., Lukk, T., Tuohy, M.G., Gong, L., Nguyen-Tri, P., Goddard, A.D., Bill, R.M., Nayak, S.C., Gupta, V.K. 2021. Lignocellulosic biorefineries: The current state of challenges and strategies for efficient commercialization. *Renew. Sust. Energ. Rev.*, **148**, 111258.
40. VanFossen, A.L., Verhaart, M.R., Kengen, S.M., Kelly, R.M. 2009. Carbohydrate utilization patterns for the extremely thermophilic bacterium *Caldicellulosiruptor saccharolyticus* reveal broad growth substrate preferences. *Appl. Environ. Microbiol.*, **75**(24), 7718-7724.
41. Vidal, B.C., Dien, B.S., Ting, K., Singh, V. 2011. Influence of feedstock particle size on lignocellulose conversion—a review. *Appl. Biochem. Biotechnol.*, **164**(8), 1405-1421.
42. Wang, J.P., Matthews, M.L., Williams, C.M., Shi, R., Yang, C., Tunlaya-Anukit, S., Chen, H.-C., Li, Q., Liu, J., Lin, C.-Y., Naik, P., Sun, Y.-H., Loziuk, P.L., Yeh, T.-F., Kim, H., Gjersing, E.S., Todd, Shuford, C.M., Song, J., Miller, Z., Huang, Y.-Y., Edmunds, C.W., Liu, B., Sun,

Y., Lin, Y.-C.J., Li, W., Chen, H., Peszlen, I., Ducoste, J.J., Ralph, J., Chang, H.-M., Muddiman, D.C., Davis, M.F., Smith, C., Isik, F., Sederoff, R., Chiang, V.L. 2018. Improving wood properties for wood utilization through multi-omics integration in lignin biosynthesis. *Nat. Commun.*, **9**(1579).

43. Wang, Y., Ebadian, M., Sokhansanj, S., Webb, E., Lau, A. 2017. Impact of the biorefinery size on the logistics of corn stover supply–A scenario analysis. *Appl. Energy*, **198**, 360-376.
44. Wiessner, F.G. 1988. Basics and industrial applications of pressure swing adsorption (PSA), the modern way to separate gas. *Gas Sep. Purif.*, **2**(3), 115-119.
45. Williams-Rhaesa, A.M., Rubinstein, G.M., Scott, I.M., Lipscomb, G.L., Poole, F.L., Kelly, R.M., Adams, M.W. 2018. Engineering redox-balanced ethanol production in the cellulolytic and extremely thermophilic bacterium, *Caldicellulosiruptor bescii*. *Metab. Eng. Commun.*, **7**, e00073.
46. Willquist, K., Pawar, S.S., Van Niel, E.W. 2011. Reassessment of hydrogen tolerance in *Caldicellulosiruptor saccharolyticus*. *Microb. Cell Fact.*, **10**(111).
47. Wood Resources International. 2017. North American Wood Fiber Review, Fourth Quarter 2016. https://woodprices.com/wp-content/uploads/2018/10/NAWFR-4Q-2016.pdf
48. Zakoshansky, V. 2007. The cumene process for phenol-acetone production. *Pet. Chem.*, **47**(4), 273-284.
49. Zurawski, J.V., Conway, J.M., Lee, L.L., Simpson, H.J., Izquierdo, J.A., Blumer-Schuette, S., Nookaew, I., Adams, M.W., Kelly, R.M. 2015. Comparative analysis of extremely thermophilic *Caldicellulosiruptor* species reveals common and unique cellular strategies for plant biomass utilization. *Appl. Environ. Microbiol.*, **81**(20), 7159-7170.
50. Zurawski, J.V., Khatibi, P.A., Akinosho, H.O., Straub, C.T., Compton, S.H., Conway, J.M., Lee, L.L., Ragauskas, A.J., Davison, B.H., Adams, M.W.W., Kelly, R.M. 2017. Bioavailability of carbohydrate content in natural and transgenic switchgrasses for the extreme thermophile *Caldicellulosiruptor bescii*. *Appl. Environ. Microbiol.*, **83**(17).

FIGURE CAPTIONS

Figure 1: Substrate Composition and Compatibility with *Caldicellulosiruptor bescii.* [A] Quantitative saccharification for wildtype poplar (WT3-1 poplar), transgenic poplar (line i20-3-1), and soybean hulls. Glucose, xylose, galactose, arabinose, mannose and lignin (acid insoluble and soluble) were quantified. [B] Water soluble fermentation products (acetate, lactate, and pyruvate) are from *C. bescii* fermentation for 7-days at 75°C, 150 RPM. Residual sugars (glucose and xylose) are solubilized by fermentation, but not consumed. Gas products (CO_2 and H_2) were not measured. [C] Carbohydrate solubilization results from *C. bescii* fermentation calculated from insoluble mass change over the fermentation and total carbohydrate content before and after fermentation. [D] Individual sugar solubilization for *C. bescii* fermentation of soybean hulls, values represent percent solubilization of each major sugar.

Figure 2. Process Flow Diagram for Conversion of Soybean Hulls to Acetone and Hydrogen. Overview of modeled process equipment for fermentative conversion of soybean hulls. Dashed lines indicate intermittent process (post-fermentation vacuum system and solids processing). Abbreviations: HX = heat exchanger, COMP = compressor.

Figure 3. Fermentation Process Phases and Product Flows. Biomass addition indicates when additional lignocellulosic biomass is to be added to the fermentors (either soybean hull or transgenic poplar for the two modeled cases). All fermentation phases occur at 70°C, pressure changes as indicated. Steady state operation occurs at 20g/l acetone titer.

Figure 4: Feedstock and Product Price Sensitivity. Internal Rate of Return (IRR) of 10% or 15% are used as indicators of process performance. Acetone and hydrogen price combinations within the expected price ranges were solved to meet IRR values of 10% (A) or 15% (B) for a constant soybean hull price ($90, $120, or $150/mt as indicated). IRR values of 10% and 15% occur for Earnings before Interest, Taxes, Depreciation, and Amortization (EBIDTA) values of ~$8.5M and ~$13.4M respectively.

TABLES

Table 1. Process Design Assumptions

Parameter	Target Value	Explanation of Basis		Source
Substrate Fermentable content	65% Soybean hull	*C. bescii* performance soybean hull		This work
	55% Transgenic Poplar	*C. bescii* performance on i20-3-1 transgenic poplar		This work
Acetone Toxicity Limit	>20 g/L	Demonstrated growth in 23 g/L acetone		(Straub et al., 2020a)
Metabolized Carbohydrate Diverted to Biomass & Non-Target Products	<12%	Previous *C. bescii* carbon balances		(Basen et al., 2014) (Williams-Rhaesa et al., 2018)
Carbohydrate Consumption Productivity	>0.7 g/L/hr	*C. saccharolyticus* (wild-type)	0.7 g/L/h	(Willquist et al., 2011)
		T. saccharolyticum (wild-type)	1.4 g/L/h	(Shaw et al., 2008)
		T. saccharolyticum (Engineered for Ethanol)	1.7 g/L/h	(Herring et al., 2016)
		Cl. thermocellum (wild-type)	1.4 g/L/h	(Holwerda et al., 2014)

Table 2. Carbon Emission Balance						
Component	**Produced / Consumed (mt/day)**	**Carbon in Product (mt CO_2 Equiv/day)**	**CO_2 Emissions from Plant (mt/day)**		**ΔH_{comb} (kJ/kg)**	**Total (GJ/day)**
Soybean hulls	550	~1000	-		-	-
CO_2 from Fermentation	259	259	259		-	-
Natural Gas	26	70.2[a]	70.2		55,500	1395
Electricity	[b]	23.5[c]	23.5		-	210
Acetone	113.2	258	-		30,810	3487
H_2	13.5	-	-		141,580	1912
Wetcake	110	~200	-		-	-
Syrup	19.3	~35	-		-	-

[a]Natural gas is based upon generation of steam from burning natural gas without electricity co-generation at an efficiency of 85%

[b]Electricity consumption is 58,000 kWh per day

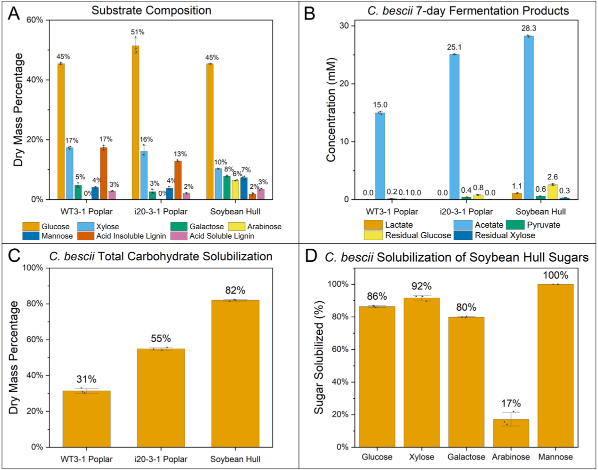
[c]CO_2 emissions from electricity are based solely on input fuels and do not account for the carbon footprint of construction, operation, or other factors.

Table 3. Baseline Process Feedstock and Product Prices and Revenue			
Component	**mt/day**	**Price per mt**	**Revenue (Expense) per Day**
Soybean Hulls (dry basis)	550	$120	($66,000)
Acetone	113.2	$956	$108,200
Hydrogen	13.5	$3000	$40,500
Wetcake (dry basis)	110	$0	-
Syrup (dry basis)	19.3	$0	-

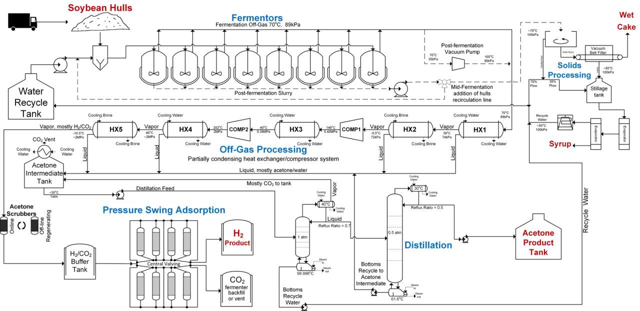
Table 4. Total Capital Investment Summary		
	2011 NREL Study (2007 10^6 USD)	**This Study (May 2021 10^6 USD)**
Plant Section Equipment Costs		
Feedstock Handling	-	$0.7
Pretreatment	$29.9	-
Neutralization/Conditioning	$3.0	-
Saccharification & Fermentation	$31.2	$19.1
On-Site Enzyme Production	$18.3	-
Distillation & Solids Recovery	$22.3	$11.2
Wastewater Treatment	$49.4	-
Storage	$5.0	$1.3
Boiler/Turbogenerator	$66.0	-
Utilities	$6.9	$2.1
Total Installed Equipment Cost	**$232.1**	**$34.4**
Other Direct Costs (warehouse, site development, additional piping)	$18.3	$4.1
Total Direct Costs (TDC)	**$250.4**	**$38.5**
Indirect Costs		
Prorated Costs (10% TDC)	$25.0	$7.6
Field Expenses (10% TDC)	$25.0	$7.6
Office, constructed (20% TDC)	$50.1	$11.0
Project Contingency (10% TDC)	$25.0	$7.6
Misc. Other Costs (10% TDC)	$25.0	$7.6
Other Capital Costs		
Land	$1.8	$1.5
Total Capital Investment	**$422.5**	**$81.2**

Table 5. Annual EBITDA with Baseline Prices	
Acetone	$35,538,403
Hydrogen	$13,308,394
Total Revenue	**$48,846,797**
Soybean Hull Feedstock	$21,681,000
Utilities	$4,616,720
Personnel & Support Services	$2,781,743
Maintenance Parts & Supplies (3%Total Installed Equipment Cost)	$1,030,582
Miscellaneous Raw Materials	$200,000
Total Expenses	**$30,310,045**
EBITDA	**$18,536,752**

Table 6. Feedstock Cost per Accessible Carbohydrate				
	Corn Kernel (Gen 1)	**Soybean Hull (Gen 1.5)**	**Corn Cob (Gen 2)**	**i20-3-1 Transgenic Poplar**
Feedstock Price ($/ dry mt)	$197[a]	$120	$100[c]	$50
Carbohydrate Content	78%[b]	77%	75%	74%
Estimated Carbohydrate Accessible to *C. bescii* (unpretreated)	95%	82%	50%	55%
Fermented Carbohydrate Cost ($/ dry mt)	$266	$190	$267	$123
[a]Estimate from 2018 to 2021 from (Kenner et al., 2021) [b]74% starch carbohydrate with 4% carbohydrate contribution from fiber [c]Estimate from (Erickson et al., 2011)				

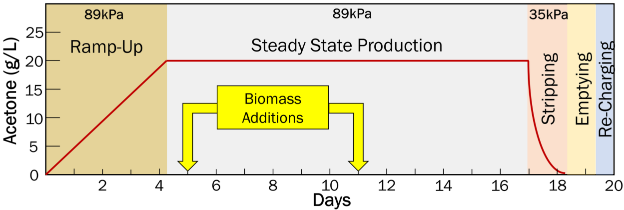


A
Substrate Composition
Dry Mass Percentage
60%
40%
20%
0%
45%
17%
5%
0%
4%
17%
3%
51%
16%
3%
0%
4%
13%
2%
45%
10%
8%
6%
7%
2%
3%
WT3-1 Poplar
i20-3-1 Poplar
Soybean Hull
Glucose
Xylose
Galactose
Arabinose
Mannose
Acid Insoluble Lignin
Acid Soluble Lignin
B
C. bescii 7-day Fermentation Products
Concentration (mM)
30
20
10
0
0.0
15.0
0.2
0.1
0.0
0.0
25.1
0.4
0.8
0.0
1.1
28.3
0.6
2.6
0.3
WT3-1 Poplar
i20-3-1 Poplar
Soybean Hull
Lactate
Acetate
Pyruvate
Residual Glucose
Residual Xylose
C
C. bescii Total Carbohydrate Solubilization
Dry Mass Percentage
100%
80%
60%
40%
20%
0%
31%
55%
82%
WT3-1 Poplar
i20-3-1 Poplar
Soybean Hull
D
C. bescii Solubilization of Soybean Hull Sugars
Sugar Solubilized (%)
100%
80%
60%
40%
20%
0%
86%
92%
80%
17%
100%
Glucose
Xylose
Galactose
Arabinose
Mannose

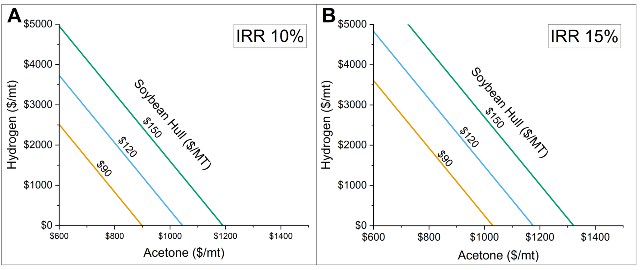


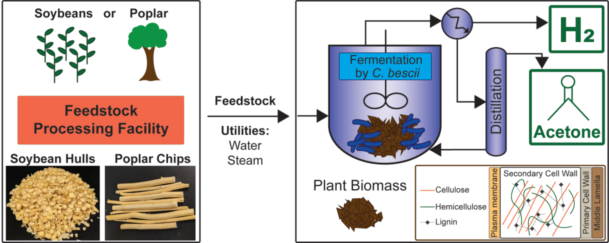
Soybean Hulls
Fermentors
Fermentation Off-Gas 70°C, 89kPa
Wet Cake
Water Recycle Tank
Post-fermentation Vacuum Pump
70°C 35kPa
100°C 90kPa
Post-fermentation Slurry
Mid-Fermentation addition of hulls recirculation line
Vacuum Belt Filter
Solids Processing
Stillage tank
Recycle Water ~50°C 100kPa
Evaporator
Syrup
Cooling Brine
Cooling Water
HX5
HX4
COMP2
HX3
COMP1
HX2
HX1
Vapor, mostly H_2/CO_2
Vapor
Liquid
CO_2 Vent
Acetone Intermediate Tank
Off-Gas Processing
Partially condensing heat exchanger/compressor system
Liquid, mostly acetone/water
Mostly CO_2 to tank
Distillation Feed
Acetone Scrubbers
Online
Off-line Regenerating
Pressure Swing Adsorption
Central Valving
H_2 Product
H_2/CO_2 Buffer Tank
CO_2 fermenter backfill or vent
Reflux Ratio = 0.7
Reflux Ratio = 0.5
1 atm
0.5 atm
99.996°C
61.6°C
Distillation
Acetone Product Tank
Bottoms Recycle to Acetone Intermediate
Bottoms Recycle Water
Recycle Water

70ºC Fermentation Phases



Mass Exiting Vessel via Vapor Stream in Each Phase (kg per 1000 L Fermenter Working Volume)				
	Ramp-Up	Steady State	Stripping	Total
Acetone	~3	30	19.5	52.4
Water	~10	40	50.5	100
CO_2	61	96	<1	157
H_2	2.7	4.4	~0	7.1


A
IRR 10%
Soybean Hull ($/MT)
$150
$120
$90
Hydrogen ($/mt)
Acetone ($/mt)
$5000
$4000
$3000
$2000
$1000
$0
$600
$800
$1000
$1200
$1400
B
IRR 15%
Soybean Hull ($/MT)
$150
$120
$90
Hydrogen ($/mt)
Acetone ($/mt)
$5000
$4000
$3000
$2000
$1000
$0
$600
$800
$1000
$1200
$1400


Soybeans or Poplar
Feedstock Processing Facility
Soybean Hulls
Poplar Chips
Feedstock
Utilities:
Water
Steam
Fermentation by C. bescii
Distillation
H_2
Acetone
Plant Biomass
Cellulose
Hemicellulose
Lignin
Plasma membrane
Secondary Cell Wall
Primary Cell Wall
Middle Lamella