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Biochemical production of ethanol and fatty acid ethyl esters from switchgrass: A comparative analysis of environmental and economic performance

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ABSTRACT

As advances in biotechnology have continued at a rapid pace, interest in the biochemical production of so-called “drop-in” fuels has increased as a way to avoid the well-known shortcomings of ethanol as a fuel molecule and to potentially exploit the processing advantages of a water-immiscible fuel to reduce product recovery costs and energy requirements. In the current study, processes to produce either ethanol or a representative fatty acid ethyl ester (FAEE) via the fermentation of sugars liberated from lignocellulosic materials pretreated in acid or alkaline environments are analyzed in terms of economic and environmental metrics. Simplified process models are introduced and employed to estimate fuel production, greenhouse gas emissions, net energy consumption, minimum fuel selling price, and water consumption for both processes. Monte Carlo analyses were carried out to identify key sources of uncertainty and variability, and an analysis of the impact of potential improvements to the FAEE process was performed. We find that the near-term performance of processes to produce FAEE is significantly worse than that of ethanol production processes for all metrics considered, primarily due to poor fermentation yields and higher electricity demands for aerobic fermentation. Even if these issues are addressed in the longer term, the reduced cost and energy requirements of FAEE separation processes will be at least partially offset by inherent limitations in the relevant metabolic pathways that constrain the maximum yield potential of FAEE from biomass-derived sugars.

Abbreviations: AFEX, ammonia fiber expansion; CAFI, Consortium for Applied Fundamentals and Innovation; DA, dilute acid; FAEE, fatty acid ethyl ester; GHG, greenhouse gas; HHV, higher heating value; LAP, laboratory analytical procedure; MFSP, minimum fuel selling price; SHF, separate (or sequential) hydrolysis and fermentation.

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1. Introduction

The utilization of lignocellulosic materials for biofuels production will be necessary if biomass-derived transportation fuels are to be produced on a large enough scale to effect meaningful reductions in fossil fuel use in the transportation sector [1], and the liberation and microbial conversion of sugars from these feedstocks has been pursued as one potential route to industrial-scale biofuels production [2–4]. Ethanol is currently the only commercially relevant fuel derived from plant sugars, and its long history of industrial fermentative production made it an obvious choice for initial efforts to produce fuels from cellulosic materials. After decades of technology development, the past several years have seen the emergence of a nascent cellulosic ethanol industry, with commercial- or demonstration-scale plants now operating in North America, Europe, and China [5]. A range of proposed biochemical processes to convert lignocellulosic biomass to ethanol have been analyzed by numerous researchers, in the form of case studies based on detailed techno-economic models [6–8], life-cycle analyses [9], and comparisons between competing process configurations and technologies [10–12].

Despite ethanol's process technology maturity and efficient production in microbes [13], it suffers from a relatively low energy density (23.5 MJ/L versus 34.7 MJ/L for gasoline), and its corrosivity and miscibility with water presents challenges for the use of existing gasoline distribution infrastructure for ethanol-rich fuel blends. These well-known shortcomings have driven interest in the production of alternative high energy density, infrastructure-compatible fuel molecules from plant-derived sugars, commonly referred to as "drop-in" or "fungible" fuels, which has become possible with recent advances in metabolic engineering [14]. Prominent examples of candidate molecules include short-chain (C4 and C5) alcohols [15], isoprenoid derivatives such as farnesene (a precursor to farnesane) [16], and fatty acid derivatives such as alkanes [17] and fatty acid ethyl esters (FAEE) [18]. The water-immiscibility of these so-called "advanced biofuels" may significantly reduce the need for distillation operations for product recovery, potentially reducing the energy requirements of the fuel production process. However, claims that advanced biofuels will offer superior performance to cellulosic ethanol must be supported by comparative analyses of the respective production processes in terms of relevant economic and environmental metrics. To date such studies are lacking in the literature, with the work of Huang and Zhang [19] comparing the energetics of the microbial production of ethanol, butanol, fatty acid ethyl ester, and hydrogen serving as one of the few exceptions. The current paper attempts to address this gap by pursuing the following objectives: (1) conduct an assessment of the relative economic and environmental performance of representative processes to produce ethanol and a water-immiscible biofuel from lignocellulosic material under the current state of technology development, (2) investigate the effect of uncertainty and variability in process parameters and the relative importance of these parameters in determining the process performance by conducting a Monte Carlo analysis of the relevant processes, and (3) explore the long-term prospects for improving the performance of the fermentation and product recovery sections of the water-immiscible biofuel production processes, and the implications for their relative competitiveness versus the pathways for producing cellulosic ethanol.

In support of these objectives, a simplified process model representing the biochemical production of ethanol and an FAEE from acid- and alkaline-pretreated switchgrass was constructed in Microsoft® Excel, based largely on detailed models of biofuels production constructed in chemical process simulation software packages [6,8,20]. Construction of a simplified model in Excel enabled the incorporation of multiple pathways from biomass to biofuel within a unified framework, and facilitated comprehensive Monte Carlo analysis in a fraction of the time that would be required using detailed models in process simulation software. The Monte Carlo analyses in turn provided valuable insight into the relative impacts of process parameters based on both their intrinsic influence on the model as well as the variability and uncertainty in their values. The ability to conduct this type of sensitivity analysis is crucial for identifying key parameters and process steps in early-stage technologies such as those currently under consideration, for which performance at scale is uncertain. The analytical approach taken here is similar to that presented by Spatari and coworkers [9], who employed a stochastic model of ethanol production based on acid and alkaline pretreatment methods in an analysis yielding life-cycle environmental metrics. This approach was extended to include both environmental and economic metrics, as well as an evaluation of technologies to produce FAEE from lignocellulosic biomass.

2. Scope of analysis

The current analysis encompasses biofuel production pathways that have been recognized in the literature as leading candidates for commercialization, and that have been the subject of intensive research efforts within government, industry, and academia. The choices of feedstock, process technologies, and fuels are not intended to represent the full scope of biochemical pathways from biomass to biofuels under development, and do not reflect the authors' endorsement of the chosen pathways.

2.1. *Feedstock*

Switchgrass (*Panicum virgatum*) was selected as the model feedstock for the current analysis. This highly productive perennial warm-season grass is native to the eastern U.S. and is one of the most widely studied examples of a potential dedicated energy crop [21,22]. Composition ranges in terms of key chemical components are listed in Table 1, along with estimated costs, higher heating values (HHV), and moisture content values representative of a single late-fall (October) harvest with field drying. The greenhouse gas (GHG) emissions associated with the production, harvest, and transportation of switchgrass are addressed in Section 3, and feedstock costs are discussed in Section 3.1 below.

The composition of switchgrass is influenced by a variety of factors, including cultivar, soil type, harvest date, and

Table 1 – Selected properties of switchgrass. Composition values are moisture-free weight percent.

Feedstock property	Low[a]	Baseline[b]	High[a]
HHV, MJ/kg[c]	17.9	18.1	18.4
Typical moisture content[d]	10%	15%	20%
Cellulose content	31%	34%	45%
Xylan content	20%	23%	26%
Other sugar polymer content	2%	4%	6%
Lignin content	17%	19%	22%
Ash content range	4.5%	7%	8.5%
Extractives & protein content	5%	13%	17%
Feedstock cost, $/dry metric ton[e]	$60	$130	$180

a Composition ranges compiled from Refs. [26–30].
b Baseline values taken from Refs. [8,26].
c Estimated from holocellulose and lignin content based on the relationship proposed by Demirbas [31].
d Compiled from Refs. [32,33].
e Compiled from Refs. [71–73].

agricultural inputs [23–25]. The wide ranges of composition values listed in Table 1 underscore the importance of accounting for such variability in analyses of biofuels processes employing cellulosic materials as feedstock.

2.2. *Fuels*

Ethyl hexadecanoate (ethyl palmitate) – chemically similar to 1st generation biodiesel molecules derived from plant oils and animal fats – was selected as a representative water-immiscible fuel molecule for comparison with ethanol. Steen and coworkers have demonstrated the production of C_{12}–C_{18} fatty acid ethyl esters (FAEE) including ethyl hexadecanoate from glucose and xylose in recombinant *Escherichia coli* (*E. coli*) strains [18]. The relevant properties of ethanol and ethyl hexadecanoate are listed in Table 2. Many of the conclusions derived from the current analysis will be more broadly applicable to other water-immiscible biofuels; however, the fact that FAEEs are included in the broader definition of biodiesel is an important advantage, as it obviates the need to undergo potentially lengthy fuel certification processes.

2.3. *Process description*

The biochemical processes under consideration to produce liquid fuels from lignocellulosic biomass include four major operations: biomass pretreatment to disrupt the structure of the lignocellulosic material; hydrolysis of cellulose, xylan, and other sugar polymers via the action of enzymes to yield their respective monomeric sugars (saccharification); microbial fuel production; and fuel recovery and purification [3,4,37]. In addition, industrial processes will require operations to recover valuable residual streams, treat and dispose of waste, and generate process steam and electricity. Four biofuel production pathways were examined in the current analysis, based on acid and alkaline pretreatment technologies to produce ethanol and FAEE. The key process steps and their primary mass and energy inputs and outputs are depicted schematically in Fig. 1.

The current analysis focuses on processes employing either the dilute acid pretreatment process developed by NREL [6] or the ammonia fiber expansion (AFEX) process developed at Michigan State University and described in a recent techno-economic analysis by Laser and coworkers [8]. The separate (or sequential) hydrolysis and fermentation (SHF) process configuration is assumed in all cases, with hydrolyzing enzymes purchased from an off-site vendor and co-fermentation of glucose and xylose occurring in a single microbe. Fermentation to ethanol proceeds anaerobically, whereas fermentation to FAEE is currently aerobic. Ethanol is recovered and purified via distillation followed by a vapor-phase molecular sieve adsorption operation, while the water-immiscible FAEE product is separated using a pair of centrifuges in series and a final vacuum drying step to remove residual moisture. The feedstock handling equipment, wastewater treatment facilities, steam and electricity generation plant, and auxiliary systems are identical for all pathways under consideration, with the process described by NREL [6] serving as the basis for analysis. A more detailed description of the process pathways under consideration is presented in the accompanying Supplementary Material.

3. Modeling & analysis approach

The primary objective of the current analysis is to compare the relative economic and environmental performance of biochemical processes to produce ethanol and FAEE from switchgrass. To this end, a deterministic model incorporating the four biomass-to-biofuels pathways of interest was constructed in Microsoft® Excel, consisting of equipment-level mass and energy balances as well as estimates of project investment costs, operating expenses, and revenues. A plant size capable of processing 2000 dry metric tons of switchgrass per day was selected for all pathways, as proposed by Aden and coworkers [38] for initial biorefineries utilizing cellulosic materials. The process model is primarily based on the techno-economic analysis by Humbird and coworkers at NREL [6], which examines the production of ethanol using dilute acid pretreatment. The process described by Laser and coworkers [8] served as the basis for an AFEX pretreatment module, and additional modules were constructed to represent aerobic fermentation and recovery of FAEE. The model yields an estimate of the minimum fuel selling price based on a 20-year discounted cash flow analysis, as well as overall mass and thermal conversion efficiency, electricity production

Table 2 – Selected properties of ethanol and ethyl hexadecanoate (FAEE).

Property	Ethanol	Ethyl hexadecanoate
Chemical formula	C_2H_6O	$C_{18}H_{36}O_2$
Molecular weight, g/mol	46.07	284.48
HHV, MJ/kg	29.7[a]	39.8[b]
Density @ 20 °C, kg/L	0.79[a]	0.86[a]
Water solubility in fuel @ 20 °C, g/L	Miscible	1.3[c]

a Value taken from Ref. [34].
b Value estimated based on data from Ref. [35].
c Value estimated based on data from Ref. [36].

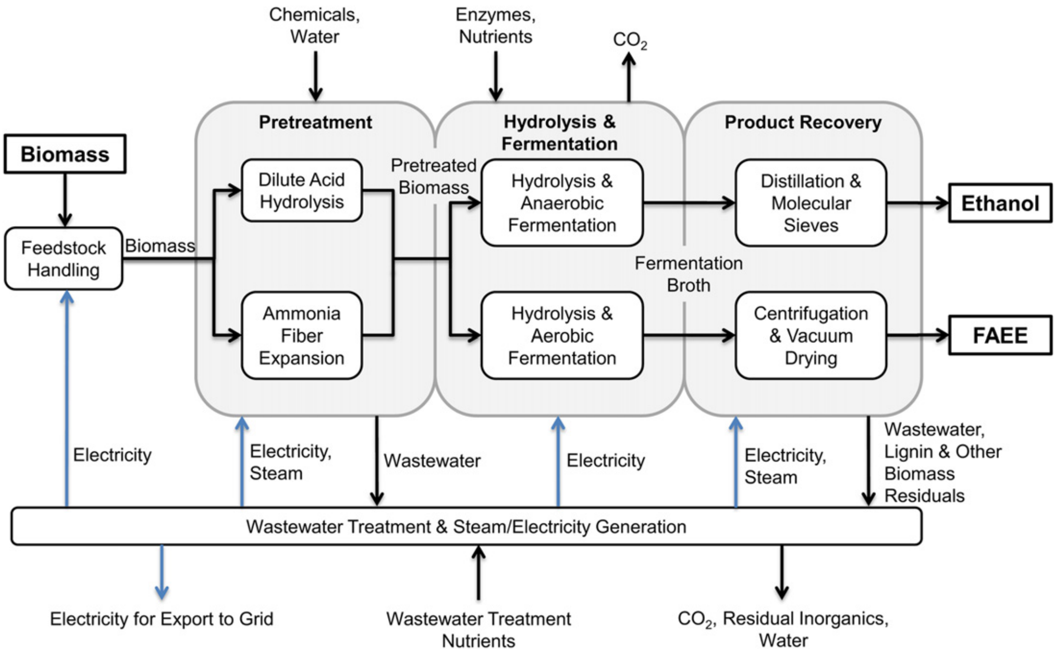

Fig. 1 – Process pathways evaluated in this study for the conversion of cellulosic feedstocks to ethanol and biodiesel (FAEE).

or consumption, process water consumption, and net GHG emissions. Further details regarding the process model can be found in the Supplementary Material.

Greenhouse gas emissions attributed to the conversion process were estimated by tabulating the emissions associated with the main process inputs as well as the avoided emissions associated with the co-generation of electricity. Estimates of GHG emissions associated with switchgrass production and chemical inputs are from Ref. [40]. We adopt the estimate of Hsu and coworkers [41] for GHG emissions due to cellulase production and assume that it is also valid for xylanase production; however, as these researchers note, this value is uncertain due to the early-stage and proprietary nature of cellulase production technology. As no location is specified for the biofuels production facility, the emissions associated with electricity production are based on the average generation mix for the U.S. national grid [40]. Emissions associated with the biorefinery infrastructure and land-use changes associated with switchgrass production are neglected.

3.1. *Monte Carlo analysis*

Many of the technologies envisioned for biochemical conversion of biomass to biofuels are still under development, and their performance in commercial-scale facilities is unproven. Monte Carlo analysis techniques were employed in order to explicitly incorporate this uncertainty and variability in estimates of overall process performance. Probability distributions based on literature data were constructed for a subset of input parameters to the process model; where data were sparse or unavailable (e.g., FAEE fermentation performance), the authors employed engineering judgment based on prior research experience to construct input parameter distributions. The Crystal Ball add-in for Excel was utilized to perform 10,000 Monte Carlo trials for each combination of pretreatment technology and fuel, yielding stochastic distributions for each metric of interest. Input parameters were represented using the beta-PERT probability distribution, requiring only estimates of the minimum, maximum and most likely values [42].

The technical parameter distributions related to the pretreatment, enzymatic hydrolysis, and fermentation processing steps are given in Table 3. As hydrolysis performance is known to depend on the pretreatment method, independent hydrolysis parameter distributions were developed for pathways incorporating dilute acid and AFEX technologies. Pretreatment process condition ranges were compiled from previous studies utilizing switchgrass as feedstock [43–50], with the ‘most likely’ parameter values representing the optimized conditions reported by the most recent Biomass Refining Consortium for Applied Fundamentals and Innovation project (CAFI 3) [44]; the only exception was the ‘most likely’ value of the solids loading for dilute acid hydrolysis, which was selected to be midway between the CAFI 3 optimum of 10% and the value of 30% employed by NREL in their latest techno-economic model [6]. Laser and coworkers [8] assume 99.5% recovery of ammonia following AFEX pretreatment; however, because this technology is unproven at commercial scale we also investigate the effect of higher ammonia losses.

Most investigations of biomass pretreatment techniques employ variations of NREL’s Laboratory Analytical Procedure (LAP) for enzymatic saccharification [51] to determine their effectiveness, and the parameter ranges selected to represent hydrolysis conditions and performance for the current analysis are based on results from similar trials utilizing dilute acid- and AFEX-pretreated switchgrass [43–45,47–50,52], with

Table 3 – Pretreatment, hydrolysis, and fermentation parameter ranges and 'most likely' values used in Monte Carlo analysis (see text for references).

	Pretreatment and hydrolysis parameter	Dilute acid			AFEX		
		Low	High	Most likely	Low	High	Most likely
Pretreatment	Solids loading (catalyst-free basis), wt%	10%	30%	20%	30%	90%	33%
	Temperature, C	130	200	140	70	180	140
	Residence time, min	2	60	40	5	40	30
	Sulfuric acid loading, mg/g dry biomass	5	200	100	N/A	N/A	N/A
	Ammonia/biomass mass ratio	N/A	N/A	N/A	0.3	2.0	1.5
	Ammonia recovery for recycle	N/A	N/A	N/A	90%	99.5%	97%
Hydrolysis	Hemicellulose conversion[a]	60%	95%	85%	50%	98%	75%
	Cellulose conversion[a]	65%	90%	75%	70%	95%	80%
	(Maximum) solids loading, wt%[b]	10%	25%	20%	10%	25%	20%
	Residence time, hr	24	168	72	24	168	72
	Cellulase loading, mg enzyme/g cellulose	5	40	20	5	40	15
	Hemicellulase loading, mg/mg cellulase	N/A	N/A	N/A	0	0.5	0.33
	Temperature, C	40	70	50	40	70	50
	Enzyme cost, \$/kg	\$7	\$15	\$10	\$7	\$15	\$10

Fermentation parameter	Ethanol			FAEE		
	Low	High	Most likely	Low	High	Most likely
Yield on glucose, % of theoretical maximum[c]	85%	95%	90%	10%	50%	30%
Yield on xylose, % of theoretical maximum[c]	70%	90%	80%	0%	40%	20%
Residence time, h	24	72	48	24	132	48

a Total conversion in pretreatment and hydrolysis stages.

b Includes both soluble and insoluble solids; value was adjusted as necessary in the model if the effluent stream from the dilute acid pretreatment step was more dilute than the specified hydrolysis solids loading.

c Theoretical maximum yield of ethanol and FAEE on glucose/xylose is 0.511 g/g sugar and 0.35 g/g sugar, respectively [50].

a slight reduction in sugar yields for dilute acid pretreatment to account for fermentation inhibition due to the byproducts from that technology [53] (see discussion of fermentation parameters below). Despite significant efforts to optimize the conditions of dilute acid and AFEX pretreatments and the use of a consistent analytical method for hydrolysis trials, considerable variation exists in reported hydrolysis performance of pretreated switchgrass; this variation is reflected in the relatively wide hydrolysis parameter ranges listed in Table 3. One factor contributing to uncertainty regarding the performance of commercial-scale hydrolysis operations on AFEX-pretreated biomass is the fact that the enzymes typically employed for laboratory-scale saccharification trials (Genencor Spezyme® CP) are not tailored for hydrolysis of hemicellulose. Because hemicellulose is not solubilized during AFEX pretreatment as it is during dilute acid pretreatment, the addition of xylanases is necessary to achieve high yield of monomeric xylose and in turn increase the accessibility of cellulose to cellulases [54]; Gao and coworkers [55] have proposed an optimum xylanase to cellulase ratio of 1:3 for hydrolysis of AFEX pretreated switchgrass. The effectiveness of AFEX pretreatment on switchgrass has also been shown to depend on harvest date [43,56] and to a lesser extent on cultivar [44].

Another factor adding to uncertainty regarding the projected industrial hydrolysis performance of biomass pretreated via any technique is the relatively dilute (1 wt% glucan) nature of the hydrolysis slurry specified in the NREL LAP. The dilute conditions minimize sugar inhibition, which has been shown to lower enzymatic hydrolysis sugar recovery efficiencies at higher (20 wt%) solids concentration [57].

The fermentation yield parameter ranges for ethanol and FAEE production in Table 3 are given in terms of the theoretical maximum conversion based on the relevant metabolic pathways to produce each fuel molecule [58]. The parameter distributions for ethanol fermentation reflect the relatively mature state of industrial glucose fermentation; however, native strains of the two leading candidate hosts for microbial production of ethanol from switchgrass – *Saccharomyces cerevisiae* and *Zymomonas mobilis* – do not possess the ability to ferment the pentose sugars xylose and arabinose [59]. Progress has been made in developing pentose-fermenting organisms via metabolic engineering techniques [60–67], although yields and productivities still do not match those for glucose and performance at industrial scales has not been publicly demonstrated [68–70]. The performance of systems to produce FAEE is subject to even greater uncertainty; the best professional judgment of the authors based on experience with FAEE-producing *E. coli* was used to construct the parameter distributions listed in Table 3. Differences in the inhibitory effects of hydrolysates obtained using different pretreatment technologies are not explicitly accounted for in the fermentation parameter ranges, even though previous studies have indicated that AFEX pretreatment results in fewer inhibitory byproducts [53]; potential reductions in ethanol production from dilute acid-pretreated switchgrass are instead captured in lower hydrolysis conversion efficiencies.

Biomass and enzymes costs have long been acknowledged as major drivers of the cost of producing cellulosic biofuels, yet despite the attention these areas have received the costs of both are still highly uncertain. The biomass feedstock and enzyme cost distributions assumed in the current analysis are given in Tables 1,3, respectively. The delivered feedstock cost distribution spans the results of recent analyses of switchgrass costs in the U.S. Midwest [71], Great Plains [72], and Southeast [73], with transportation costs from the farm to the biorefinery assumed to be $14 per dry metric tons [74]; the 'most likely' feedstock cost corresponds to the average breakeven price in the low-cost scenario described by Jain et al. [71]. The enzyme cost distribution is based on the recent techno-economic analysis of Klein-Marchuschamer and coworkers [75].

Distributions were also assigned to the mass fractions of major chemical species in the dry switchgrass fed to the process, with the minimum and maximum values corresponding to the low and high values, respectively, listed in Table 1.

The performance of the biomass conversion processes was evaluated based on the following economic and environmental metrics: (1) fuel production per metric ton of dry biomass, (2) minimum fuel selling price, (3) net electricity production, (4) process water consumption, and (5) process GHG emissions. Process water consumption does not include water associated with the production of feedstocks, other chemical inputs, or grid electricity used in the process.

4. Results and discussion

4.1. Near-term process performance

Stochastic distributions of the chosen economic and environmental performance metrics are presented in Figs. 2,3 for each of the four modeled pathways from switchgrass to fuel. As discussed in Section 3.1 the input parameter ranges employed to generate these stochastic distributions were selected to be representative of the current state of technology for the respective processes; it is assumed that the input parameter ranges are appropriate for an "nth plant" among a hypothetical fleet of similar biorefineries as described in Ref. [6]. It is important to note that the resulting distributions capture only the variability and uncertainty in the feedstock

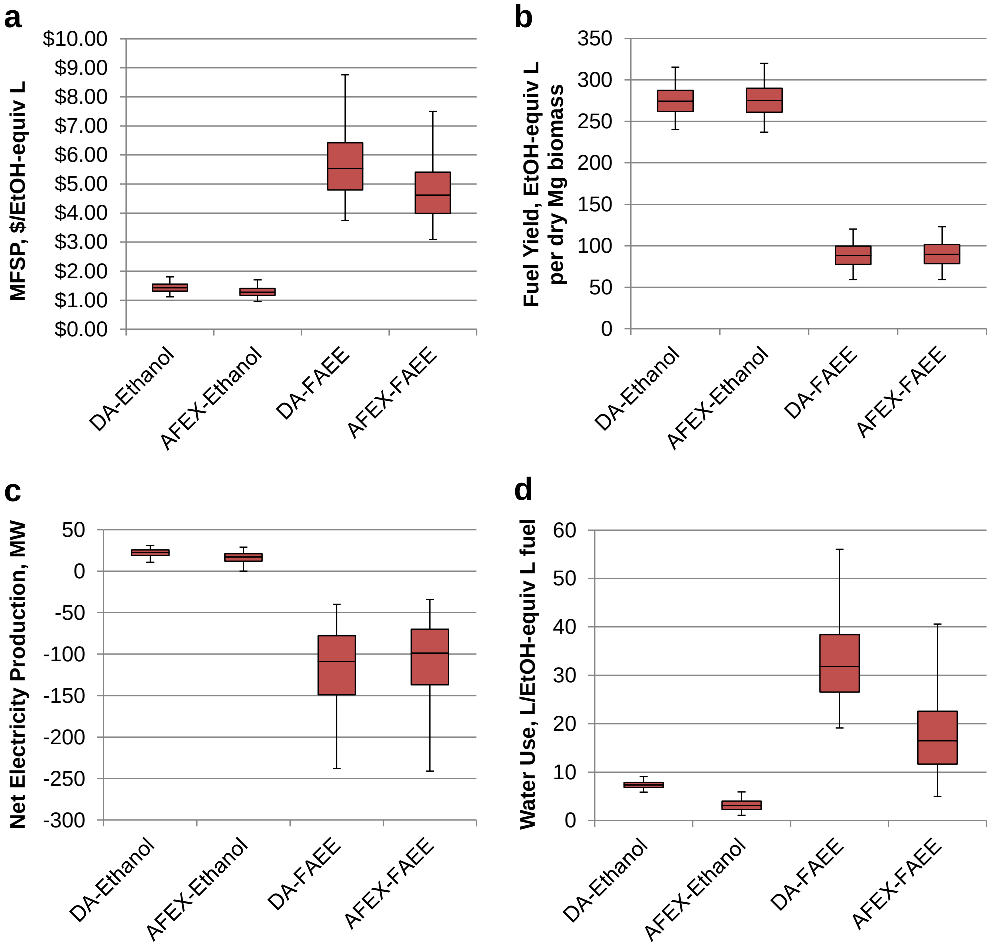

Fig. 2 – (a) Minimum fuel selling price ($ per ethanol-equivalent liter), (b) Fuel yield (ethanol-equivalent liters per metric ton of dry biomass), (c) Net electricity generation (MW) for a 2000 metric ton/day conversion facility, and (d) process water consumption (liters of water per liter of ethanol-equivalent fuel produced) for all pathways to ethanol and FAEE. Stochastic distributions are represented as box plots. The top and bottom edges of each box represent the 75th and 25th percentiles, respectively, the middle line in each box represents the 50th percentile, and the top and bottom whiskers represent the 97.5th and 2.5th percentiles, respectively. Values of each metric at these percentiles are tabulated for all pathways in the supplementary material.

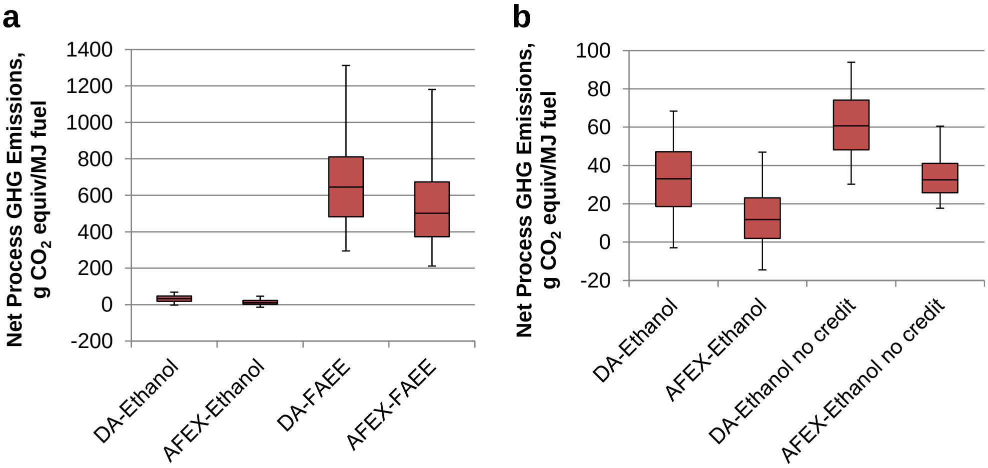

Fig. 3 – Net GHG emissions (g CO_2 equivalents per MJ of fuel produced) for all pathways to ethanol and FAEE. Results in (a) include credits due to co-production of electricity; results for ethanol pathways are presented in (b) both with and without electricity co-production credits. Stochastic distribution data represented by box plots are tabulated for all pathways in the supplementary material.

compositions and process parameters discussed in Section 3.1, and do not represent the complete range of possible performance for a given process. For example, financial and operational parameters – which were fixed for the current analysis – can be expected to have a significant impact on estimated fuel production costs. The value of analyses such as presented here is in comparing similar processes and investigating the relative importance of input parameters to the process model, rather than providing absolute values of performance metrics.

4.1.1. *Minimum fuel selling price (MFSP)*

A comparison of the break-even price per ethanol-equivalent liter across the four biomass conversion pathways under consideration is given in Fig. 2a. The most striking feature of the results is the large disparity between the costs for ethanol and FAEE for both pretreatment technologies. These results are driven to a large degree by the respective fermentation parameters for each fuel (see Table 3). The fermentation yield of ethanol from glucose is assumed to be roughly three times that of FAEE, and an even greater advantage for ethanol is assumed for xylose fermentation; thus, for roughly the same cost in terms of feedstock, biorefinery infrastructure, and other raw materials, a significantly lower annual output of FAEE will be obtained compared to ethanol. It is recognized that the fermentation of FAEE is at a relatively early stage of development, and there is potential for significant improvements in the fermentation process that will narrow the gap in cost with ethanol. The impacts of such improvements are explored in Section 4.2.

The costs for pathways employing different pretreatment strategies to produce a given fuel are much more closely aligned, with significant overlap in the stochastic distributions in the case of both ethanol and FAEE production. This is consistent with the results of Tao and coworkers [76], who estimated similar costs for producing cellulosic ethanol via a range of pretreatment technologies. The cost of dilute acid pretreatment is slightly higher than that for AFEX pretreatment, a trend that will be observed regardless of the fuel being produced since the choice of pretreatment technology has no explicit influence on the fermentation step in the conversion process model. Ethanol costs shown in Fig. 2a are higher than those reported in previously published studies due to the more conservative performance and raw materials (biomass and enzymes) cost assumptions in the current analysis; recent estimates of the cost of producing ethanol from herbaceous biomass using dilute acid or AFEX pretreatment range from \$0.27/L to \$1.21/L [7,76–80], with Humbird and coworkers [6] reporting a value of \$0.57/L (dilute acid pretreatment).

4.1.2. *Fuel yield*

Fig. 2b presents fuel production per metric ton of dry biomass for the conversion process pathways of interest. Fuel production is given in terms of ethanol-equivalent liters on an energy basis, allowing direct comparison of the ethanol and FAEE processes. The estimated yield of ethanol via both pretreatment pathways is lower than those reported by Humbird et al. [6] (dilute acid, 330 L/Mg) and Laser et al. [8] (AFEX, 318 L/Mg) due to our selection of less aggressive conversion parameters. The production of FAEE is significantly lower than that of ethanol, due to the low fermentation yields assumed for the FAEE processes.

The maximum fuel production per metric ton of switchgrass is limited by the sugar oligomer content of the feedstock and by the maximum theoretical fermentation yield of fuel from sugar based on the relevant metabolic pathways. A key factor in the current analysis is the fact that the maximum theoretical fermentation yield of ethanol is over 10% higher than that of FAEE on an energy basis: the maximum yield is 97% for ethanol versus 88% for FAEE, based on a recent analysis of the relevant metabolic pathways [58]. Thus, assuming the baseline values of switchgrass composition from Table 1, the maximum yield of ethanol is 394 ethanol-equivalent liters per metric ton of biomass, whereas for

FAEE the maximum yield is 358 ethanol-equivalent liters per metric ton of biomass.

4.1.3. *Electricity production*

In the cellulosic ethanol processes that serve as the basis for the model used in this work, electricity generated by the combustion of lignin and other residual organics is sufficient to meet the power needs of the process with some excess amount available for export to the grid. The only ethanol scenarios in which residual biomass may not be sufficient to provide excess power involve AFEX pretreatment with high water content and low reaction temperatures, necessitating large amounts of steam for ammonia recovery [80] and thus reducing the amount that is available for power generation. However, the production of FAEE from cellulosics as modeled here will likely require electricity inputs, as indicated by the net electricity generation results presented in Fig. 2c. In this case, the reason for the poor performance of the FAEE process can be traced to the use of aerobic fermentation, which requires a considerable amount of power to aerate and agitate the fermentation vessels (2 HP per 100 gallons of fermentor volume). The result is an electricity demand that is expected to approach 12 kWh per liter of ethanol-equivalent fuel produced for fermentation alone. In addition, residual sugars not utilized for fuel production are converted primarily to CO_2 by the FAEE-producing microbes, and are thus unavailable for conversion to electricity.

To put this electricity demand in perspective, net consumption of 12 kWh for a single ethanol-equivalent liter of fuel produced would require 67 MJ of fuel at an electricity generation efficiency of 55%, which is more than three times the energy content (HHV) of a liter of ethanol. Clearly, a biofuel production process with a negative energy balance – even without considering energy inputs to produce and deliver biomass and other raw materials – is not likely to be a candidate for commercialization on a large scale.

4.1.4. *Process water consumption*

Detailed accounting of water use in each unit operation yields the total water consumption of the process, given in terms of liters of water consumed per liter of ethanol-equivalent fuel produced (Fig. 2d). Nearly 90% of the water present in the conversion process is recovered in the wastewater treatment section for recycle. The main source of water losses is cooling tower evaporation associated with cooling water use, with most of the remaining losses occurring via the combustor stack during the steam and electricity generation stage. As was the case for the minimum fuel selling price, the primary driver of high water consumption in the FAEE processes is low fuel yield; a secondary driver is the large cooling load of the fermentation system, attributable to power dissipation in compressors and agitators as well as heat generated by the oxidation of sugars that are not converted to fuel. The higher water consumption of dilute acid pathways reflects the higher pretreatment temperatures and lower solids loading – and thus the increased cooling requirements downstream – associated with that technology.

The estimates of water consumption for the dilute acid–ethanol pathways in Fig. 2d are higher than that reported by Humbird and coworkers [6] (5.4 L water/L ethanol) due to the less aggressive conversion parameter and pretreatment solids loading ranges chosen for the current study. Only the AFEX-Ethanol pathway approaches the water consumption of 3.0 L/L reported for corn-derived ethanol production processes; however, irrigation of corn acreage results in total water consumption of between 14 and 236 L/L ethanol depending on the agricultural region [81], exceeding the total consumption of both FAEE processes (assuming no irrigation of switchgrass). Although irrigated switchgrass is not considered here, for cases in which irrigation is employed it is important to note that reduced biomass conversion efficiency will result in incrementally higher water consumption – both upstream and in the process – per unit of fuel. The production of gasoline is more water-efficient than all of the biofuels pathways under consideration, consuming between 1.9 and 2.5 L water/ethanol-equivalent L [81].

4.1.5. *GHG emissions*

The estimated GHG emissions associated with producing switchgrass-based ethanol are dominated by contributions from the production of biomass feedstock, ammonia, and enzymes. Lime and sodium hydroxide inputs are also significant sources of emissions for dilute acid pathways, resulting in higher estimated emissions relative to AFEX pathways. For the ‘most likely’ parameter values selected for the current analysis, GHG emissions due to the chemical and enzyme inputs exceed those due to the production of switchgrass; even at the lower catalyst and enzyme loadings assumed by MacLean and Spatari [39], the contribution of the chemical and enzyme inputs represents 30–35% of the total well-to-tank emissions. If the avoided emissions associated with co-production of electricity are allocated to switchgrass-based ethanol, the net process emissions become negative for at least some of the Monte Carlo trials, as shown in Fig. 3. The lower fuel yields of the FAEE processes result in higher emissions per MJ of fuel produced based on the same raw materials. However, the primary driver of GHG emissions for the FAEE processes is the increased electricity demand, accounting for approximately 80% of the total in both cases.

The GHG emissions results for ethanol production are similar to those reported by Spatari and coworkers [9], who estimate 22.1–29.5 g CO_2 equiv/MJ for AFEX-based processes and 28.8–39.2 g CO_2 equiv/MJ for dilute acid-based processes without electricity credits. Lower values between 6.5 and 14.4 g CO_2 equiv/MJ (excluding credits for electricity generation) have been reported by other authors [40,82,83], due to the fact that emissions associated with process chemicals and enzymes were neglected. Spatari and MacLean [84] and Mullins et al. [85] have conducted stochastic studies which include emissions due to indirect land-use changes, resulting in higher estimates of GHG emissions with wider distributions. The median GHG emissions presented here for the production of ethanol with no co-product credit represent a 31–63% reduction compared to gasoline (88 g CO_2 equiv/MJ [40]), whereas the median emissions for the FAEE pathways indicate an increase of 470–630%. Previous studies have estimated GHG emissions associated with the production of corn-based ethanol ranging from 34 to 111 g CO_2 equiv/MJ [82,86–88].

4.2. *Potential improvements to the FAEE process*

Microbial production of FAEE was demonstrated at bench-scale only recently, and the fermentation performance can be expected to improve as resources are devoted to this goal. It is instructive to investigate the specific improvements that would be necessary to approach or exceed the performance of cellulosic ethanol processes. The obvious starting point for such an investigation is the fermentation step itself, as poor yields and the use of aerobic fermentation were shown to negatively affect the performance of the FAEE processes. There may also be possibilities for enhancing the separation of FAEE following fermentation, which was modeled as occurring via centrifugation. Use of a less capital- and energy-intensive unit operation such as a gravity settler may be possible, given the immiscibility of FAEE with water. The potential impacts of improvements in these areas are analyzed through comparisons of the following scenarios:

Ethanol baseline scenario: Production of ethanol from switchgrass employing dilute acid pretreatment, based on the parameter ranges described in Section 3.1.

FAEE baseline scenario: Production of FAEE from switchgrass employing dilute acid pretreatment, based on the parameter ranges described in Section 3.1.

FAEE high yield scenario: Identical to FAEE baseline scenario, but assuming ethanol fermentation parameter ranges for FAEE production (see Table 3).

FAEE anaerobic high yield scenario: Identical to the FAEE high yield scenario, but assuming anaerobic fermentation of sugars to FAEE.

FAEE best case scenario: Identical to the FAEE anaerobic high yield scenario, but assuming zero cost and zero energy use for FAEE recovery operations.

4.2.1. *Minimum fuel selling price*

Break-even fuel prices for each of the five scenarios are compared in Fig. 4. An improvement in the FAEE fermentation parameters – yield and residence time – to match those of ethanol leads to a 65% reduction in the median fuel cost, whereas the switch to anaerobic fermentation and elimination of centrifugation costs yield more modest reductions. The improvement of fermentation yields is the main potential driver of cost reduction, as it would enable increased fuel production – and hence greater revenue – for essentially the same total production cost. Switching from aerobic to anaerobic fermentation reduces capital costs due to less stringent sterilization requirements and the elimination of aeration equipment, and also reduces electricity demand for agitation (see Section 4.2.2). Eliminating the costs of separating FAEE yields the smallest incremental cost reduction.

Although the advances that would be necessary to attain the FAEE best case scenario in practice present an enormous challenge, even the combination of these three changes does not result in a median fuel cost that is lower than that for ethanol. Because the only difference between the ethanol baseline process and the FAEE best case scenario – other than the fuel being produced – is the lack of separation costs in the FAEE scenario, one might expect the FAEE costs to be lower; however, the cost reductions that accompany the "free" separation of FAEE are offset by reduced absolute sugar conversion efficiencies that are intrinsic to the metabolic pathways utilized by the microbes to produce FAEE. This conclusion is emphasized in Fig. S-3 in the Supplementary Material, showing a comparison of the costs associated with the ethanol baseline scenario and the FAEE best case scenario. A significant negative contribution due to reduced FAEE separation costs is offset by smaller increases across all other process areas and inputs due to the reduced fuel output. Thus, even in the most optimistic case of technology development for the FAEE production process, the best that can be expected is to match the performance of cellulosic ethanol technology. It is assumed that all improvements in feedstock production practices and pretreatment and hydrolysis technology will apply equally to the production of both fuels.

Despite this seemingly discouraging conclusion, it is important to note that other factors beyond the production cost will play an important role in determining the economic competitiveness of FAEE and other water-immiscible biofuels. Although the distribution and final use of fuels is beyond the scope of the current analysis, other researchers have documented the obstacles to expanding the use of ethanol

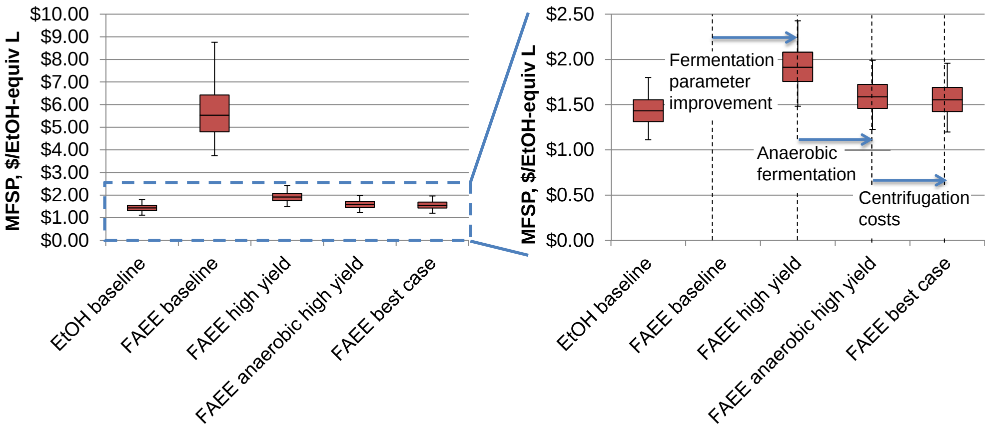

Fig. 4 – Minimum fuel selling price ($/ethanol-equivalent liter) for the ethanol (EtOH) and FAEE scenarios. Stochastic distribution data are tabulated for all scenarios in the supplementary material.

significantly beyond current levels [89] due to fuel distribution infrastructure and vehicle incompatibilities; in contrast, the widespread use of FAEE or FAEE-diesel blends would be possible with little additional investment in these areas. Use in diesel engines will also enhance the overall efficiency of FAEEs as a fuel due to their higher energy conversion efficiency (translating to a ~20% advantage in fuel economy vs. gasoline engines [90]). Looking beyond road transportation, energy dense water-immiscible biofuels may also be developed for use as jet fuel, an application for which ethanol is unsuited. This last point highlights the fact that ethanol and water-immiscible biofuels are not competing with each other, but rather with petroleum-derived fuels. Given these considerations, it may not be necessary to match the production costs of ethanol in order to justify the large-scale commercialization of FAEE and other advanced biofuels.

4.2.2. *Electricity production*

The net electricity production for each of the scenarios under consideration is displayed in Fig. 5. In contrast with the results for fuel cost, an enhancement in the fermentation performance (represented in the difference between the FAEE baseline scenario and the FAEE high yield scenario) does not result in a significant increase in electricity production, since fuel yield has little effect on the total electricity use. Some improvement is observed due to reduced fermentation residence times assumed in the FAEE high yield scenario; however, it is apparent from Fig. 5 that the major driver of electricity consumption in the FAEE process is aerobic fermentation, and in particular the aeration and agitation requirements of the fermentation vessels. In comparison, the electricity consumption of the centrifuges is minor (also see Table 4), as reflected in the very slight rise in net electricity production in moving from the FAEE anaerobic high yield scenario to the FAEE best case scenario.

Although the elimination of distillation and ethanol drying operations has been offered as a potential justification for pursuing the development of water-immiscible biofuels, the estimated contribution of ethanol separation and purification to total biorefinery energy consumption (and fuel cost) is relatively minor, as seen in Table 4 for the 'most likely' ethanol baseline scenario. Thus, only modest energy and cost reductions for the overall biorefinery can be expected from changes to the product recovery step.

4.2.3. *Process water consumption*

Water losses in the processes under consideration occur primarily through cooling tower evaporation and loss through the flue stack with combustion gases. Since the composition of the materials sent to the combustor is similar across all pathways, the main source of variability in total water consumption is in the cooling load. Inspection of Fig. 6 reveals a trend similar to that of fuel cost in Fig. 4; the main driver of excessive water consumption on a per-fuel-liter basis is low fuel output. Modest reductions in water use accompany the switch from aerobic to anaerobic fermentation, as the cooling load of the fermentation vessels is significantly reduced, and the cooling of centrifuges accounts for the nearly imperceptible decrease in water use between the FAEE anaerobic high yield and best case scenarios.

4.2.4. *GHG emissions*

The net GHG emissions associated with each of the scenarios under consideration are shown in Fig. 7. As the fermentation yield increases in moving from the FAEE baseline scenario to the FAEE high yield scenario, more fuel is produced as the process inputs which drive GHG emissions – switchgrass, chemicals, and enzymes – remain fixed and process electricity demands are reduced by shorter fermentation residence times. The GHG intensity of the FAEE product is further reduced as the electricity requirements are slashed with the conversion to anaerobic fermentation. The GHG emissions associated with the FAEE anaerobic high yield and best case scenarios are both comparable to those for the ethanol

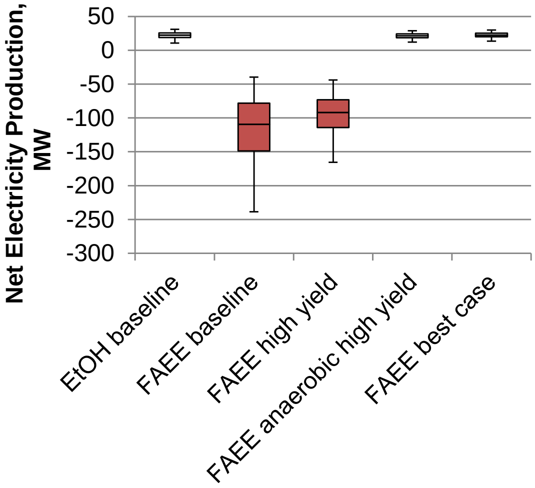

Fig. 5 – Net electricity production (MW) for the ethanol (EtOH) and FAEE scenarios. Based on a conversion facility receiving 2000 dry metric tons of switchgrass per day. Stochastic distribution data are tabulated for all scenarios in the supplementary material.

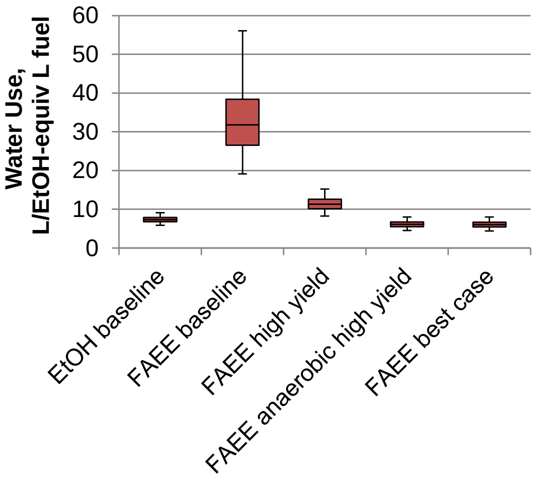

Fig. 6 – Process water use (liters per ethanol-equivalent liter of fuel) for the ethanol (EtOH) and FAEE scenarios. Stochastic distribution data are tabulated for all scenarios in the supplementary material.

Table 4 – Electricity and steam use and total capital costs for distillation and ethanol drying operations in the 'most likely' ethanol baseline scenario, and for centrifugation costs in the 'most likely' case of the FAEE anaerobic high yield scenario.

	Ethanol baseline scenario		FAEE anaerobic high yield scenario	
	% of plant total	% of feedstock HHV	% of plant total	% of feedstock HHV
Electricity use	3.1%	0.20%	4.1%	0.27%
Steam use	49.6%	8.5%	–	–
Capital costs	3.7%	–	4.2%	–

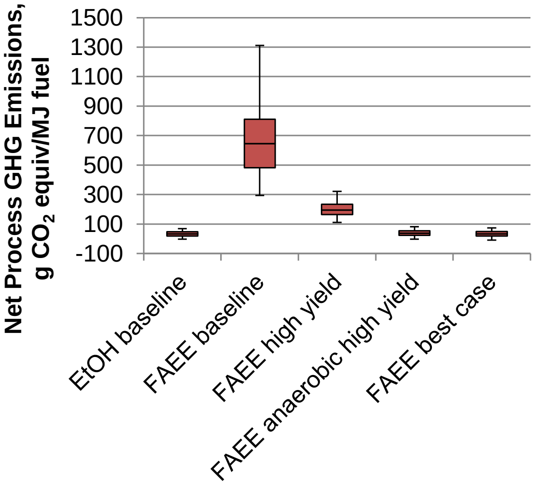

Fig. 7 – Net process GHG emissions (g CO_2 equivalents per MJ fuel produced) for the ethanol (EtOH) and FAEE scenarios. Stochastic distribution data are tabulated for all scenarios in the supplementary material.

baseline scenario, as the electricity demand of the centrifuges does not significantly impact the results.

5. Conclusions

The results of the foregoing analysis indicate that biochemical processes to produce FAEE from switchgrass are uncompetitive with similar processes to produce ethanol under the current state of technology development, in terms of both economic and environmental metrics. This may be an unsurprising result, given the short development history of FAEE production technology. However, an investigation of potential improvements to the FAEE production processes highlighted the significant challenges that confront efforts to close the gap in performance with cellulosic ethanol processes.

The two primary areas for potential improvement to the FAEE processes are 1) an enhancement in fermentation parameters (yield on sugars and fermentation time) and 2) a switch from aerobic to anaerobic fermentation. The main path to process improvement is to increase the fermentation yield, and thus the production of fuel from a given amount of biomass. By improving the fermentation parameters to be on par with those for ethanol, the FAEE processes can make up most of the differences in cost, fuel production, GHG emissions, and water use relative to the ethanol pathways. The switch to anaerobic fermentation will offer modest gains in cost and water use, but will significantly decrease electricity consumption such that the processes become net electricity producers rather than net electricity consumers. Further improvements in FAEE separation efficiency offer only minor cost and energy reductions. Ultimately, FAEE production processes will approach intrinsic limitations in the metabolic pathways utilized by the host microorganisms, which place FAEE at a disadvantage versus ethanol in terms of the maximum potential fuel production from sugars (15.2 MJ of ethanol per kg of sugar vs. 13.8 MJ of FAEE per kg of sugar). This disadvantage in maximum energy conversion efficiency appears to offset any potential gains resulting from the elimination of distillation and dehydration steps in the cellulosic ethanol processes.

Parameters related to the feedstock, pretreatment, and hydrolysis unit operations did not affect fermentation in the model, and thus did not influence the relative performance of the processes used to produce different fuels. However, due to the relatively high yields achievable for ethanol fermentation, these parameters were observed to be major drivers of the performance of the ethanol pathways. Although the AFEX-based processes exhibited slightly lower median fuel costs, GHG emissions, and water consumption than the dilute acid processes, there was a significant overlap in the performance of processes utilizing different pretreatment strategies to produce the same fuel. Improvement in these areas will be crucial to reducing the cost of all cellulosic biofuels in relation to petroleum-derived fuels.

The authors acknowledge that the comparison between processes to produce ethanol and FAEE is an imperfect one, as ethanol is a gasoline additive/replacement and FAEE is appropriate for diesel engines. However, the results of our analysis are broadly applicable to other water-immiscible biofuels, most if not all of which suffer from similarly low maximum theoretical fermentation yields (see Ref. [58] for a discussion and examples). This point also underscores the need to extend the scope of such comparisons beyond the biorefinery to the distribution of the fuel to end users and ultimately to combustion in engines. Further study is needed to explore the question of whether potential advantages in infrastructure compatibility and combustion efficiency will be sufficient to overcome the apparent process limitations of producing FAEE for fuel via biochemical pathways.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biombioe.2012.11.029.

REFERENCES

[1] Perlack RD, Stokes BJ. U.S. billion-ton update: biomass supply for a bioenergy and bioproducts industry. Oak Ridge, TN: Oak Ridge National Laboratory; 2011. p. 227.
[2] Carroll A, Somerville C. Cellulosic biofuels. Annu Rev Plant Biol 2009;60:165.
[3] Blanch HW, Simmons BA, Klein-Marcuschamer D. Biomass deconstruction to sugars. Biotechnol J 2011;6:1086.
[4] Fischer CR, Klein-Marcuschamer D, Stephanopoulos G. Selection and optimization of microbial hosts for biofuels production. Metab Eng 2008;10:295.
[5] Bacovsky D, Dallos M, Worgetter M. Status of 2nd generation biofuels demonstration facilities in June 2010. IEA Bioenergy; 2010.
[6] Humbird D, Davis R, Tao L, Kinchin C, Hsu D, Aden A, et al. Process design and economics for biochemical conversion of lignicellulosic biomass to ethanol. National Renewable Energy Laboratory; 2011.
[7] Klein-Marcuschamer D, Oleskowicz-Popiel P, Simmons BA, Blanch HW. Technoeconomic analysis of biofuels: a wiki-based platform for lignocellulosic biorefineries. Biomass Bioenerg 2010;34:1914.
[8] Laser M, Jin H, Jayawardhana K, Lynd LR. Coproduction of ethanol and power from switchgrass. Biofuel Bioprod Bior 2009;3:195.
[9] Spatari S, Bagley DM, MacLean HL. Life cycle evaluation of emerging lignocellulosic ethanol conversion technologies. Bioresour Technol 2010;101:654.
[10] Dutta A, Dowe N, Ibsen KN, Schell DJ, Aden A. An economic comparison of different fermentation configurations to convert corn stover to ethanol using *Z. mobilis* and *Saccharomyces*. Biotechnol Prog 2010;26:64.
[11] Piccolo C, Bezzo F. A techno-economic comparison between two technologies for bioethanol production from lignocellulose. Biomass Bioenerg 2009;33:478.
[12] Martín M, Grossmann IE. Energy optimization of bioethanol production via hydrolysis of switchgrass. Aiche J 2012;58:1538.
[13] Datta R, Maher MA, Jones C, Brinker RW. Ethanol—the primary renewable liquid fuel. J Chem Technol Biotechnol 2011;86:473.
[14] Lee SK, Chou H, Ham TS, Lee TS, Keasling JD. Metabolic engineering of microorganisms for biofuels production: from bugs to synthetic biology to fuels. Curr Opin Biotech 2008;19:556.
[15] Atsumi S, Hanai T, Liao JC. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature 2008;451:86.
[16] Renninger NS, McPhee DJ. Fuel compositions including farnesane and farenesene derivatives and methods of making and using same; 2008.
[17] Schirmer A, Rude MA, Li X, Popova E, del Cardayre SB. Microbial biosynthesis of alkanes. Science 2010;329:559.
[18] Steen EJ, Kang YS, Bokinsky G, Hu ZH, Schirmer A, McClure A, et al. Microbial production of fatty-acid-derived fuels and chemicals from plant biomass. Nature 2010;463:559.
[19] Huang W-D, Percival Zhang YH. Analysis of biofuels production from sugar based on three criteria: thermodynamics, bioenergetics, and product separation. Energ Environ Sci 2011;4:784.
[20] Haas MJ, McAloon AJ, Yee WC, Foglia TA. A process model to estimate biodiesel production costs. Bioresour Technol 2006;97:671.
[21] Sanderson MA, Reed RL, McLaughlin SB, Wullschleger SD, Conger BV, Parrish DJ, et al. Switchgrass as a sustainable bioenergy crop. Bioresour Technol 1996;56:83.
[22] Mitchell R, Vogel KP, Sarath G. Managing and enhancing switchgrass as a bioenergy feedstock. Biofuel Bioprod Bior 2008;2:530.
[23] Lemus R, Brummer EC, Moore KJ, Molstad NE, Burras CL, Barker MF. Biomass yield and quality of 20 switchgrass populations in Southern Iowa, USA. Biomass Bioenerg 2002;23:433.
[24] Lemus R, Charles Brummer E, Lee Burras C, Moore KJ, Barker MF, Molstad NE. Effects of nitrogen fertilization on biomass yield and quality in large fields of established switchgrass in Southern Iowa, USA. Biomass Bioenerg 2008;32:1187.
[25] Sladden SE, Bransby DI, Aiken GE. Biomass yield, composition and production costs for eight switchgrass varieties in Alabama. Biomass Bioenerg 1991;1:119.
[26] Lee D, Owens VN, Boe A, Jeranyama P. Composition of herbaceous biomass feedstocks. Brookings, SD: Sun Grant Initiative, North Central Center; 2007.
[27] Biomass feedstock composition and property database. U.S. Department of Energy, Office of Energy Efficiency & Renewable Energy, Office of the Biomass Program.
[28] Monti A, Di Virgilio N, Venturi G. Mineral composition and ash content of six major energy crops. Biomass Bioenerg 2008;32:216.
[29] Yan J, Hu Z, Pu Y, Charles Brummer E, Ragauskas AJ. Chemical compositions of four switchgrass populations. Biomass Bioenerg 2010;34:48.
[30] David K, Ragauskas AJ. Switchgrass as an energy crop for biofuel production: a review of its ligno-cellulosic chemical properties. Energ Environ Sci 2010;3:1182.
[31] Demirbas A. Relationships between lignin contents and heating values of biomass. Energ Convers Manag 2001;42:183.
[32] Sanderson MA, Egg RP, Wiselogel AE. Biomass losses during harvest and storage of switchgrass. Biomass Bioenerg 1997;12:107.
[33] Hess JR, Wright CT, Kenney KL, Searcy EM. Uniform-format solid feedstock supply system: a commodity-scale design to produce an infrastructure-compatible bulk solid from lignocellulosic biomass. Idaho National Laboratory; 2009.
[34] Haynes WM, editor. CRC handbook of chemistry and physics. 92nd ed. Boca Raton, FL: CRC Press/Taylor and Francis; 2011–2012.
[35] Knothe G. "Designer" biodiesel: optimizing fatty ester composition to improve fuel properties. Energ Fuel 2008;22:1358.
[36] Oliveira MB, Varanda FR, Marrucho IM, Queimada AJ, Coutinho JAP. Prediction of water solubility in biodiesel with the CPA equation of state. Ind Eng Chem Res 2008;47:4278.

[37] Ladisch MR, Mosier NS, Kim Y, Ximenes E, Hogsett D. Converting cellulose to biofuels. Chem Eng Prog 2010;106:56.
[38] Aden A, Ruth M, Ibsen K, Jechura J, Neeves K, Sheehan J, et al. Lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis for corn stover. Golden, CO: National Renewable Energy Laboratory; 2002.
[39] MacLean HL, Spatari S. The contribution of enzymes and process chemicals to the life cycle of ethanol. Environ Res Lett 2009;4.
[40] Wang MQ. GREET 1 2011, transportation fuel-cycle model. Argonne, IL: Argonne National Laboratory; 2011.
[41] Hsu DD, Inman D, Heath GA, Wolfrum EJ, Mann MK, Aden A. Life cycle environmental impacts of selected US ethanol production and use pathways in 2022. Environ Sci Technol 2010;44:5289.
[42] Vose D. Quantitative risk analysis: a guide to Monte Carlo simulation modelling. Chichester, UK: Wiley and Sons; 1996.
[43] Kim Y, Mosier NS, Ladisch MR, Pallapolu VR, Lee YY, Garlock R, et al. Comparative study on enzymatic digestibility of switchgrass varieties and harvests processed by leading pretreatment technologies. Bioresour Technol 2011;102:11089.
[44] Wyman CE, Balan V, Dale BE, Elander RT, Falls M, Hames B, et al. Comparative data on effects of leading pretreatments and enzyme loadings and formulations on sugar yields from different switchgrass sources. Bioresour Technol 2011;102: 11052.
[45] Jensen JR, Morinelly JE, Gossen KR, Brodeur-Campbell MJ, Shonnard DR. Effects of dilute acid pretreatment conditions on enzymatic hydrolysis monomer and oligomer sugar yields for aspen, balsam, and switchgrass. Bioresour Technol 2010;101:2317.
[46] Morinelly JE, Jensen JR, Browne M, Co TB, Shonnard DR. Kinetic characterization of xylose monomer and oligomer concentrations during dilute acid pretreatment of lignocellulosic biomass from forests and switchgrass. Ind Eng Chem Res 2009;48:9877.
[47] Li C, Knierim B, Manisseri C, Arora R, Scheller HV, Auer M, et al. Comparison of dilute acid and ionic liquid pretreatment of switchgrass: biomass recalcitrance, delignification and enzymatic saccharification. Bioresour Technol 2010;101:4900.
[48] Garlock RJ, Balan V, Dale BE, Pallapolu VR, Lee YY, Kim Y, et al. Comparative material balances around pretreatment technologies for the conversion of switchgrass to soluble sugars. Bioresour Technol 2011;102:11063.
[49] Garlock RJ, Balan V, Dale BE. Optimization of AFEX™ pretreatment conditions and enzyme mixtures to maximize sugar release from upland and lowland switchgrass. Bioresour Technol 2012;104:757.
[50] Alizadeh H, Teymouri F, Gilbert T, Dale B. Pretreatment of switchgrass by ammonia fiber explosion (AFEX). Appl Biochem Biotechnol 2005;124:1133.
[51] Selig M, Weiss N, Ji Y. Enzymatic saccharification of lignocellulosic biomass. Golden, CO: National Renewable Energy Laboratory; 2008.
[52] Pallapolu VR, Lee YY, Garlock RJ, Balan V, Dale BE, Kim Y, et al. Effects of enzyme loading and beta-glucosidase supplementation on enzymatic hydrolysis of switchgrass processed by leading pretreatment technologies. Bioresour Technol 2011;102:11115.
[53] Lau MW, Gunawan C, Dale BE. The impacts of pretreatment on the fermentability of pretreated lignocellulosic biomass: a comparative evaluation between ammonia fiber expansion and dilute acid pretreatment. Biotechnol Biofuel 2009;2.
[54] Kumar R, Wyman CE. Effect of enzyme supplementation at moderate cellulase loadings on initial glucose and xylose release from corn stover solids pretreated by leading technologies. Biotechnol Bioeng 2009;102:457.
[55] Gao DH, Uppugundla N, Chundawat SPS, Yu XR, Hermanson S, Gowda K, et al. Hemicellulases and auxiliary enzymes for improved conversion of lignocellulosic biomass to monosaccharides. Biotechnol Biofuel 2011;4.
[56] Bals B, Rogers C, Jin MJ, Balan V, Dale B. Evaluation of ammonia fibre expansion (AFEX) pretreatment for enzymatic hydrolysis of switchgrass harvested in different seasons and locations. Biotechnol Biofuel 2010;3.
[57] Dien BS, Ximenes EA, O'Bryan PJ, Moniruzzaman M, Li XL, Balan V, et al. Enzyme characterization for hydrolysis of AFEX and liquid hot-water pretreated distillers' grains and their conversion to ethanol. Bioresour Technol 2008;99:5216.
[58] Rude MA, Schirmer A. New microbial fuels: a biotech perspective. Curr Opin Microbiol 2009;12:274.
[59] Hahn-Hagerdal B, Karhumaa K, Fonseca C, Spencer-Martins I, Gorwa-Grauslund MF. Towards industrial pentose-fermenting yeast strains. Appl Microbiol Biotechnol 2007;74:937.
[60] Sedlak M, Ho NWY. Production of ethanol from cellulosic biomass hydrolysates using genetically engineered *Saccharomyces* yeast capable of cofermenting glucose and xylose. Appl Biochem Biotechnol 2004;113:403.
[61] Lau MW, Dale BE. Cellulosic ethanol production from AFEX-treated corn stover using *Saccharomyces cerevisiae* 424A(LNH-ST). Proc Natl Acad Sci U S A 2009;106:1368.
[62] Lau MW, Gunawan C, Balan V, Dale BE. Comparing the fermentation performance of *Escherichia coli* KO11, *Saccharomyces cerevisiae* 424A(LNH-ST) and *Zymomonas mobilis* AX101 for cellulosic ethanol production. Biotechnol Biofuel 2010;3.
[63] Hawkins GM, Doran-Peterson J. A strain of *Saccharomyces cerevisiae* evolved for fermentation of lignocellulosic biomass displays improved growth and fermentative ability in high solids concentrations and in the presence of inhibitory compounds. Biotechnol Biofuel 2011;4.
[64] Hector R, Dien B, Cotta M, Qureshi N. Engineering industrial *Saccharomyces cerevisiae* strains for xylose fermentation and comparison for switchgrass conversion. J Ind Microbiol Biotechnol 2011;38:1193.
[65] Ha S-J, Galazka JM, Rin Kim S, Choi J-H, Yang X, Seo J-H, et al. Engineered *Saccharomyces cerevisiae* capable of simultaneous cellobiose and xylose fermentation. Proc Natl Acad Sci U S A 2011;108:504.
[66] Agrawal M, Mao Z, Chen RR. Adaptation yields a highly efficient xylose-fermenting *Zymomonas mobilis* strain. Biotechnol Bioeng 2011;108:777.
[67] Mohagheghi A, Evans K, Chou YC, Zhang M. Cofermentation of glucose, xylose, and arabinose by genomic DNA-integrated xylose/arabinose fermenting strain of *Zymomonas mobilis* AX101. Appl Biochem Biotechnol 2002;98:885.
[68] Matsushika A, Inoue H, Kodaki T, Sawayama S. Ethanol production from xylose in engineered *Saccharomyces cerevisiae* strains: current state and perspectives. Appl Microbiol Biotechnol 2009;84:37.
[69] Cai Z, Zhang B, Li Y. Engineering *Saccharomyces cerevisiae* for efficient anaerobic xylose fermentation: reflections and perspectives. Biotechnol J 2012;7:34.
[70] Dien BS, Cotta MA, Jeffries TW. Bacteria engineered for fuel ethanol production: current status. Appl Microbiol Biotechnol 2003;63:258.
[71] Jain AK, Khanna M, Erickson M, Huang H. An integrated biogeochemical and economic analysis of bioenergy crops in the Midwestern United States. GCB Bioenerg 2010;2:217.
[72] Perrin R, Vogel K, Schmer M, Mitchell R. Farm-scale production cost of switchgrass for biomass. Bioenerg Res 2008;1:91.

[73] Mooney DF, Roberts RK, English BC, Tyler DD, Larson JA. Yield and breakeven price of 'Alamo' switchgrass for biofuels in Tennessee. Agron J 2009;101:1234.

[74] Hess JR, Kenney KL, Ovard LP, Searcy EM, Wright CL. Commodity-scale production of an infrastructure-compatible buld solid from herbaceous lignocellulosic biomass. Uniform-format bioenergy feedstock supply system design report series. Idaho National Laboratory; 2009.

[75] Klein-Marcuschamer D, Oleskowicz-Popiel P, Simmons BA, Blanch HW. The challenge of enzyme cost in the production of lignocellulosic biofuels. Biotechnol Bioeng 2012;109:1083.

[76] Tao L, Aden A, Elander RT, Pallapolu VR, Lee YY, Garlock RJ, et al. Process and technoeconomic analysis of leading pretreatment technologies for lignocellulosic ethanol production using switchgrass. Bioresour Technol 2011;102:11105.

[77] Kazi FK, Fortman J, Anex R, Kothandaraman G, Hsu D, Aden A, et al. Techno-economic analysis of biochemical scenarios for production of cellulosic ethanol. Golden, CO: National Renewable Energy Laboratory; 2010.

[78] Huang H-J, Ramaswamy S, Al-Dajani W, Tschirner U, Cairncross RA. Effect of biomass species and plant size on cellulosic ethanol: a comparative process and economic analysis. Biomass Bioenerg 2009;33:234.

[79] Sendich E, Laser M, Kim S, Alizadeh H, Laureano-Perez L, Dale B, et al. Recent process improvements for the ammonia fiber expansion (AFEX) process and resulting reductions in minimum ethanol selling price. Bioresour Technol 2008;99:8429.

[80] Bals B, Wedding C, Balan V, Sendich E, Dale B. Evaluating the impact of ammonia fiber expansion (AFEX) pretreatment conditions on the cost of ethanol production. Bioresour Technol 2011;102:1277.

[81] Wu M, Chiu Y. Consumptive water use in the production of ethanol and petroleum gasoline – 2011 update. Argonne, IL: Argonne National Laboratory; 2008. p. 100.

[82] Farrell AE. Ethanol can contribute to energy and environmental goals. Science 2006;311:506.

[83] Schmer MR, Vogel KP, Mitchell RB, Perrin RK. Net energy of cellulosic ethanol from switchgrass. Proc Natl Acad Sci 2008;105:464.

[84] Spatari S, MacLean HL. Characterizing model uncertainties in the life cycle of lignocellulose-based ethanol fuels. Environ Sci Technol 2010;44:8773.

[85] Mullins KA, Griffin WM, Matthews HS. Policy implications of uncertainty in modeled life-cycle greenhouse gas emissions of biofuels. Environ Sci Technol 2011;45:132.

[86] Pimentel D, Patzek T. Ethanol production using corn, switchgrass, and wood; biodiesel production using soybean and sunflower. Nat Resour Res 2005;14:65.

[87] Patzek TW. Thermodynamics of the corn–ethanol biofuel cycle. Crit Rev Plant Sci 2004;23:519.

[88] Liska AJ, Yang HS, Bremer VR, Klopfenstein TJ, Walters DT, Erickson GE, et al. Improvements in life cycle energy efficiency and greenhouse gas emissions of corn–ethanol. J Ind Ecol 2009;13:58.

[89] Wakeley HL, Hendrickson CT, Griffin WM, Matthews HS. Economic and environmental transportation effects of large-scale ethanol production and distribution in the United States. Environ Sci Technol 2009;43:2228.

[90] Wang M. Development and use of GREET 1.6 fuel-cycle model for transportation fuels and vehicle technologies. Argonne, IL: Argonne National Laboratory; 2001.