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Research Article

Economic and Environmental Trade-Offs of Simultaneous Sugar and Lignin Utilization for Biobased Fuels and Chemicals

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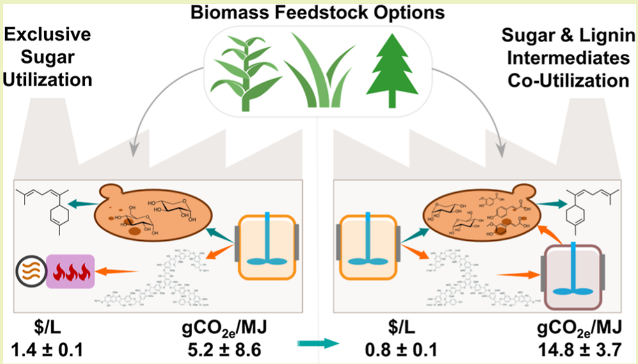

ABSTRACT: Efficient lignin conversion is vital to the production of affordable, low-carbon fuels and chemicals from lignocellulosic biomass. However, lignin conversion remains challenging, and the alternative (combustion) can emit harmful air pollutants. This study explores the economic and environmental trade-offs between lignin combustion and microbial utilization for producing bisabolene as a representative biobased fuel or chemical. Results for switchgrass and clean pine-based biorefineries show that using lignin to increase fuel yields rather than combusting it reduces the capital expenditures for the boiler and turbogenerator if the facilities process more than 1100 bone-dry metric tons (bdt) feedstock/day and 560 bdt/day, respectively. No comparable advantage was observed for lower-lignin sorghum feedstock. Deconstructing lignin to bioavailable intermediates and utilizing those small molecules alongside sugars to boost product yields is economically attractive if the overall lignin-to-product conversion yield exceeds 11−20% by mass. Although lignin-to-fuel/chemical conversion can increase life-cycle greenhouse gas (GHG) emissions, most of the lignin can be diverted to fuel/chemical production while maintaining a >60% life-cycle GHG footprint reduction relative to diesel fuel. The results underscore that lignin utilization can be economically advantageous relative to combustion for higher-lignin feedstocks, but efficient depolymerization and high yields during conversion are both crucial to achieving viability.

KEYWORDS: *lignocellulosic biomass, lignin depolymerization, metabolically accessible aromatics, lignin utilization, sustainable aviation fuel*

■ INTRODUCTION

Lignocellulosic biomass is primarily composed of cellulose (32−52 wt %), hemicellulose (16−33 wt %), and lignin (9−32 wt %).[1] While cellulose and hemicellulose can be deconstructed to sugar monomers and utilized for the production of biofuels or biochemicals in a sugar-first cellulosic biorefinery,[2] lignin remains a low-value coproduct. Consistent with current practices in the pulp and paper industry, lignin can be burned to generate on-site process heat and electricity. However, this generates minimal revenue; for biofuels or biochemicals to be economically viable, all components of the plant biomass must be used to generate the greatest possible value.[1,3] Furthermore, burning moist lignin (∼20 wt %[2]) to generate heat and power is a low-efficiency process that can increase the capital burden for the biorefinery due to the capital-intensive[2] nature of the boiler.

Beyond economic considerations, there are environmental motivations for diverting lignin to non-combustion uses. Burning lignin may release harmful air pollutants at levels exceeding limits set by the Clean Air Act,[4] as discussed further in the Supporting Information S1. This necessitates the implementation of emission-control technologies to ensure continuous biorefinery operation.[4,5] The anticipated cost of mitigating associated pollutants is expected to range from \$0.02 to \$0.4 per gasoline gallon equivalent.[5] Diverting lignin for other non-combustion applications can substantially reduce air pollutant emissions (see details in the Supporting Information S1).[6,7] The focus of this study is to better understand the degree to which non-combustion lignin utilization routes can be economically viable, and what minimum conversion threshold from lignin stream to product must be reached.

Prior studies have explored variations in lignin valorization. For example, one study compared lignin valorization to bioproducts in a corn stover-to-ethanol biorefinery with a

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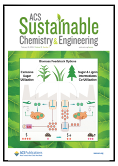

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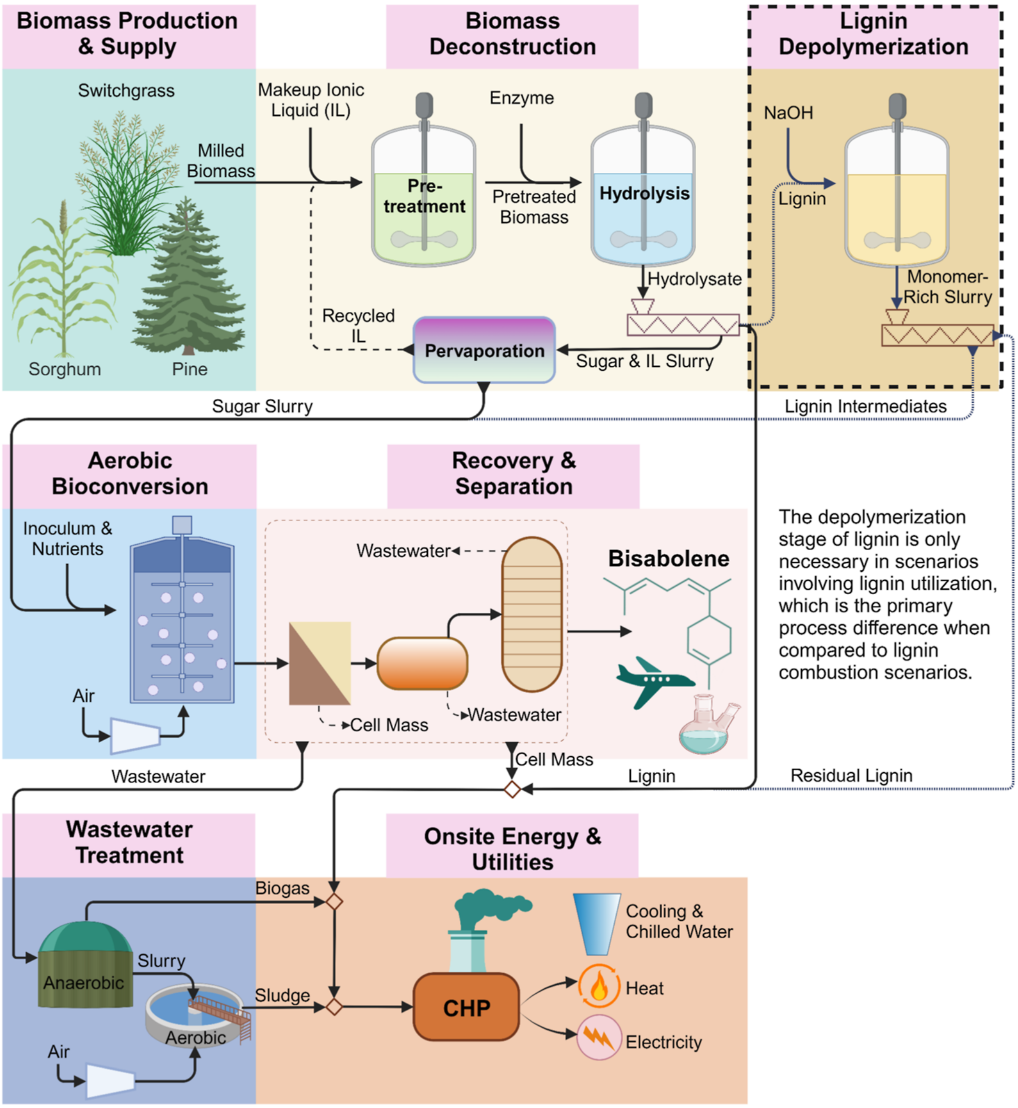


The depolymerization stage of lignin is only necessary in scenarios involving lignin utilization, which is the primary process difference when compared to lignin combustion scenarios.

Figure 1. Overview of the biorefinery process model developed in this study, with major components and inputs. For the lignin combustion scenario, the lignin fraction of biomass is directly routed to the onsite energy generation unit. For the lignin utilization scenario, lignin is first sent to the lignin depolymerization unit and then the lignin-derived intermediates are routed to the aerobic bioconversion reactor while the remaining solids are routed to the onsite energy generation unit.

more conventional configuration and found that lignin valorization resulted in less favorable economics and a need to import fuel to the facility.[8] However, generalizations are difficult to make given the diversity of potential configurations, such as lignin first,[9] lignin last,[8,10] or biomass component fractionation[11,12] (see details in the Supporting Information S2). Corona et al.[10] demonstrated that biologically produced lignin-derived adipic acid could reduce GHG emissions relative to petroleum-derived adipic acid by up to 78%. In contrast to prior studies, the primary focus of this work is the depolymerization of lignin for simultaneous microbial utilization alongside sugars in the production of a single biofuel/bioproduct.

Emerging processes are under development to transform lignin into various products.[1,13] It is now possible to depolymerize lignin to bioavailable intermediates and use microbial hosts to convert those intermediates to products as an alternative to combustion.[14,15] As noted previously, this can either be done in through a process separate from sugar conversion or the lignin-derived intermediates can be coutilized with sugars in the same bioreactor.[3] However, the polyaromatic compounds in lignin have posed challenges for microbial conversion, and lignin degradation products can be toxic to some microbial hosts. Therefore, understanding the performance thresholds needed to make such a process viable is crucial. These thresholds can impact the selection of microbial hosts and molecular targets capable of efficiently utilizing the diverse molecules present in mixed hydrolysates produced from the deconstruction of the cellulose−hemicellulose−lignin matrix.

Bisabolene—a renewable precursor for transportation fuels and chemicals—serves as a useful proxy for a wider variety of biomolecules that can be produced from mixed hydrolysates. Bisabolene can be catalytically hydrogenated to produce bisabolane, which is a potential candidate for use in existing aircrafts, ships, and heavy-duty trucks.[16,17] Bisabolene also has diverse applications across industries such as cosmetics, chemicals, pharmaceuticals, and nutraceuticals.[18,19] Some properties of bisabolane, such as high energy density and a low freezing point, make it a promising candidate as a drop-in

Table 1. Structural Composition of Biomass, Delivered Cost, and GHG Emissions

parameters	units	biomass sorghum	switchgrass	clean pine
biorefinery size[2]	bone-dry metric ton (bdt)/day	2000	2000	2000
feedstock cost	\$/bdt	72.6[33]	78.2[34]	81.7[35−37]
GHG emission	$kgCO_{2e}$/bdt	79.5[33]	69.8[34]	85.2[35−37]
dLUCs	$kgCO_{2e}$/bdt	−77.7[38]	−119.3[38]	−25.1[39,40]
moisture	wt %	20[33]	20[34]	30[35]
		Structural Composition		
cellulose	wt %	40[17]	37.3[1,41]	46[1]
hemicellulose	wt %	29.8[17]	29.4[1,41]	23[1]
lignin	wt %	9.9[17]	18.7[1,41]	28[1]

replacement for conventional diesel and aviation fuels.[16,18] One of the challenges of producing renewable energy-dense diesel and jet fuel blendstocks, such as bisabolane, lies in simultaneously reducing both production costs and greenhouse gas (GHG) emissions.[17] These biofuels are produced using aerobic microbial hosts[18,20,21] and have a volumetric energy density 38−86% higher than that of ethanol (29.2−39.3 MJ/L for advanced biofuels compared to 21.1 MJ/L for ethanol).[17,22] The higher selling price and carbon footprint of advanced biofuels, compared to those of ethanol, are primarily due to a lower bioconversion yield (25−35 wt % from sugars for advanced biofuels[17,22] compared to 51 wt % from sugars for ethanol[2]) and the capital and energy-intensive aerobic bioconversion process.[23] Recent bench-scale experiments have demonstrated bisabolene yields of 19.6 g per 100 g of glucose,[24] 2.2 g per 100 g of mixed sugars (glucose, xylose, and arabinose),[25] 7.6 g per 100 g of organic acids,[26] and 0.77 g per 100 g of bone-dry biomass feedstock[27] based on utilization of an ensiled biomass sorghum hydrolysate that included glucose, xylose, and organic acids.

Different microbial hosts, including *Escherichia coli*,[18] *Saccharomyces cerevisiae*,[18] and *Rhodosporidium toruloides*,[20] have been engineered to produce renewable bisabolene primarily using the plant-derived sugars such as glucose and xylose. Conventionally, the lignin fraction of biomass remains unutilized after bioconversion and is either burned to generate heat and power or discarded as waste. It is currently estimated that only a small portion (∼2%) of available lignin from pulp and paper industries,[28] mainly lignosulfonates, is commercially utilized[29] to produce bioplastics, fuel additives, and chemicals such as vanillin. However, some microbial hosts can utilize a more diverse set of carbon sources beyond sugars. An engineered *R. toruloides* strain can produce bisabolene utilizing biomass or lignin-derived chemical intermediates, thus raising the possibility of coconverting sugars and aromatics together in the same bioreactor.[27]

Diverting the lignin-derived monomers/oligomers to the bioconversion reactor raises two important unanswered questions: (i) what amount of lignin must be utilized by the host microbe to make the strategy of coutilizing sugars and lignin-derived bioavailable intermediates worthwhile from an economic standpoint; and (ii) what fraction of lignin can be utilized while still meeting the Renewable Fuel Standard (RFS) GHG emissions reduction goal of 60% reduction relative to petroleum equivalent. Here, we develop "*n*th plant" biorefinery process models that involve both burning lignin onsite and simultaneous utilization of sugars and lignin-derived intermediates. We identify key thresholds for lignin coutilization, report on the most influential inputs, and discuss process optimization and intensification opportunities.

■ METHODS

Biorefinery Overview. Figure 1 provides an overview of biorefinery process models developed in this study, designed for a nameplate capacity of 2000 bone-dry metric tons (bdt) of biomass feedstock per day. Our analyses encompassed three biomass feedstocks: biomass sorghum, switchgrass, and clean pine. These feedstocks represent a range from herbaceous to woody biomass, each with a varying level of lignin content—specifically, low lignin content (9.9% by mass) for biomass sorghum, medium lignin content (18.7% by mass) for switchgrass, and high lignin content (28% by mass) for clean pine (Table 1). By investigating these feedstocks with diverse initial lignin contents, we can acquire a more comprehensive understanding of the economic and environmental consequences of lignin combustion and the simultaneous utilization of sugars and lignin intermediates. Biomass sorghum and switchgrass are transported to the biorefinery as bales, whereas pine is delivered in the form of chips. It is assumed that the moisture content in sorghum and switchgrass bales is 20 wt %, while pine chips are delivered with a moisture content of 30 wt % (Table 1).

Biorefinery process models are developed in a commercial process modeling software package—SuperPro Designer-V13.[30] Modeled biorefineries are typically situated in resource-rich areas where biomass sorghum, switchgrass, or pine fields are uniformly distributed around the facility. At the biorefinery, either biomass sorghum or switchgrass bales are first conveyed to the shredder and then to the hammer mill to break them up into the desired particle size of 6.35 mm.[2] Alternatively, in the case of a pine-chip-based biorefinery, pine chips are directly conveyed to the hammer mill. The milled biomass is stored for short-term storage and then sent to the biomass deconstruction unit. Data on the structural composition, delivered cost of biomass, and the carbon footprint of biomass production and supply chain were collected from prior studies and are summarized in Table 1.

The biomass deconstruction process consists of pretreatment, neutralization, and enzymatic hydrolysis. The milled biomass is mixed with a biocompatible ionic liquid, cholinium lysinate, and water to create a slurry that is fed into the pretreatment reactor. The pretreatment is carried out at 140 °C for 1 h.[20,27,31] After pretreatment, the slurry is blended with sulfuric acid to adjust the pH and then sent to the enzymatic hydrolysis reactor. Mixtures of cellulase enzymes, at a concentration of 10 mg of protein per gram of glucan,[23,27] are added to the reactor, and water is added to achieve an initial solid loading of 25 wt %.[23,27,32]

Following hydrolysis, the hydrolyzed slurry is separated into solid and liquid fractions using a vacuum belt filter, which allows for filtration, cake washing with minimal use of water, and pressing. The solid fraction is delivered to the boiler to generate onsite heat and power for the lignin combustion scenario. In the lignin utilization scenario, the solid fraction of the hydrolyzed slurry is routed to the lignin depolymerization unit. The liquid fraction is sent to the ionic liquid-recovery unit.

For the lignin utilization scenario, the recovered lignin after enzymatic hydrolysis is depolymerized and then sent to the bioconversion reactor. In the lignin depolymerization unit, the solid fraction of the hydrolyzed biomass is mixed with NaOH, primarily

Table 2. Modeling Data Inputs Used to Develop Biomass-to-Bisabolene Production System

parameters	units[a]	value
A. Common Parameters		
Biomass Pretreatment		
solid loading[27,52]	wt %	40
ionic liquid loading[27,52]	wt %	5
ionic liquid cost[27]	\$/kg	0.5
pretreatment time[20,31]	h	1
Neutralization		
sulfuric acid loading[27]	kg/kg-IL	0.1
sulfuric acid cost[27]	\$/kg	0.14
Enzymatic Hydrolysis		
enzyme loading[20,27]	mg/g-glucan	10
solid loading[32]	wt %	25
cellulose to glucose[20,27,32,52]	%	95
xylan to xylose[20,27,32,52]	%	90
hydrolysis time[20,27]	h	48
enzyme price[17,27]	\$/kg-protein	5
hydrolysis temperature[32,52]	°C	50
Bioconversion		
inoculum loading[2,17]	wt %	5
corn steep liquor[2,17]	wt %	0.2
DAP[2,17]	g/L	0.3
corn steep liquor cost[2,17]	\$/kg	0.07
DAP cost[2,17]	\$/kg	0.36
ammonia cost[23]	\$/kg	0.42
compressor pressure[23]	kPa	310.3
bioconversion time[2,23]	h	36
Recovery and Separation		
bisabolene recovery[2,17]	wt %	98
ionic liquid recovery[45]	wt %	99
Wastewater Treatment		
nutrient loading[2,17]	wt %	0.05
nutrient cost[2,17]	\$/kg	0.7
organic matter to biogas[2,23]	%	91
Onsite Energy and Utility		
boiler chemicals cost[2,23]	\$/kg	5
natural gas cost[53]	\$/kg	0.22
water cost[2,23]	\$/kg	0.0001
CIP cost[2,23]	\$/kg	0.35
B. Lignin Combustion		
Bioconversion		
ammonia	g/L	1
air supply	m^3/s	9.8
glucose-to-bisabolene	wt %	28.5
xylose-to-bisabolene	wt %	25.6
C. Lignin Utilization		
Lignin Depolymerization		
NaOH loading[23]	wt %	2
lignin to bioavailable intermediates	wt %	50[b]
NaOH cost[23]	\$/kg	0.53
Bioconversion		
ammonia	g/L	0.4
air supply	m^3/s	11.2
glucose-to-bisabolene	wt %	28.5
xylose-to-bisabolene	wt %	25.6
aromatics-to-bisabolene	wt %	28.5

[a] Data reported in percentages (%) represent the conversion percentage, where the yield of the product(s) is determined by balanced chemical equations. The theoretical biological yield of bisabolene production in *R. toruloides* utilizing sugars or aromatics is determined to be 30 wt %. [b] Assumed for analysis in this study, the range of 0−90 wt % is considered to assess its impact on the production cost and carbon footprint of bisabolene.

lignin and a small fraction of unutilized cellulose and hemicellulose, and then fed into the depolymerization reactor. This study considers a mild NaOH loading of 2 wt %[23] to break up lignin into bioavailable intermediates, such as *p*-coumaric acid, ferulic acid, vanillic acid, or any other compounds, which can be consumed by *R. toruloides*. To simplify the modeling process and acknowledge the ongoing development of various lignin depolymerization methods that may yield a range of lignin intermediates from different lignocellulosic biomass feedstocks,[1,9,42−44] this study uses a generic compound, referred to as "lignin-derived bioavailable intermediates", to represent lignin intermediates. This choice avoids specifying individual monomers, dimers, or trimers. It is assumed that these bioavailable intermediates are nontoxic to the host microbes such as *R. toruloides* considered for analysis in this study. Following lignin depolymerization, the lignin-derived bioavailable intermediates and the remaining solids are separated. The lignin-derived bioavailable intermediates are delivered to the bioconversion reactor, and the remaining solids are routed to the boiler.

Ionic liquid is recovered from the hydrolyzed liquid by using a pervaporation system. The performance of pervaporation-based ionic liquid recovery method is documented in a prior experimental study.[45] Following the ionic liquid recovery, the sugar solution is sent to the bioconversion reactor. This early separation of ionic liquid and the remaining solid fraction of biomass allows solid-free bioconversion and reduces the volume of materials going to the bioconversion reactor, resulting in lower energy for agitation and aeration.

The bioconversion unit consists of a series of seed reactors and a main reactor. The seed reactors are used to grow the host microorganism *R. toruloides* to their full capacity. Nitrogen sources, including diammonium phosphate (DAP), ammonia, and corn steep liquor are added in the reactors. An air blower is used to supply air to the seed and main bioreactors. In this study, a maximum volume of bubble column bioreactor of 1000 m^3 is considered to maintain a uniform concentration of dissolved oxygen.[23] In the bioreactor, it is expected that the host microbe, *R. toruloides*, simultaneously utilizes glucose and xylose sugars for the lignin combustion scenario and a mixture of sugars and lignin-derived intermediates for the lignin utilization scenario.

R. toruloides is a native xylose consumer and efficiently utilizes a mixture of glucose, xylose, and lignin intermediates.[46] Previous bench-scale experiments showcased the concurrent utilization of sugars and lignin-derived monomers, including 4-hydroxybenzoic acid,[47] protocatechuic acid,[47] and *p*-coumaric acid,[46] by *R. toruloides*. These experiments highlighted the capability of *R. toruloides* to tolerate and assimilate several lignin-derived aromatics on a gram-per-liter scale. Other recent studies[20,27] have successfully produced bisabolene in *R. toruloides*, with a scale ranging from 30 mL shake flasks to 20 L reactors, by using ionic-liquid-pretreated biomass sorghum hydrolysate. However, ionic liquid pretreatment does not substantially depolymerize lignin and only generates aromatics, including 4-hydroxybenzoic acid, benzoic acid, vanillic acid, ferulic acid, vanillin, and coumaric acid, at a milligram-per-liter scale. Based on the bench-scale experiments,[20,27] the model developed in this study assumes 99% utilization of carbon sources in the bioreactor. If the biological process is inefficient in converting carbon sources to bisabolene, then the remaining sugar is assumed to be equally distributed between cell mass and CO_2. After bioconversion, the entire slurry is routed to the bisabolene recovery and separation unit.

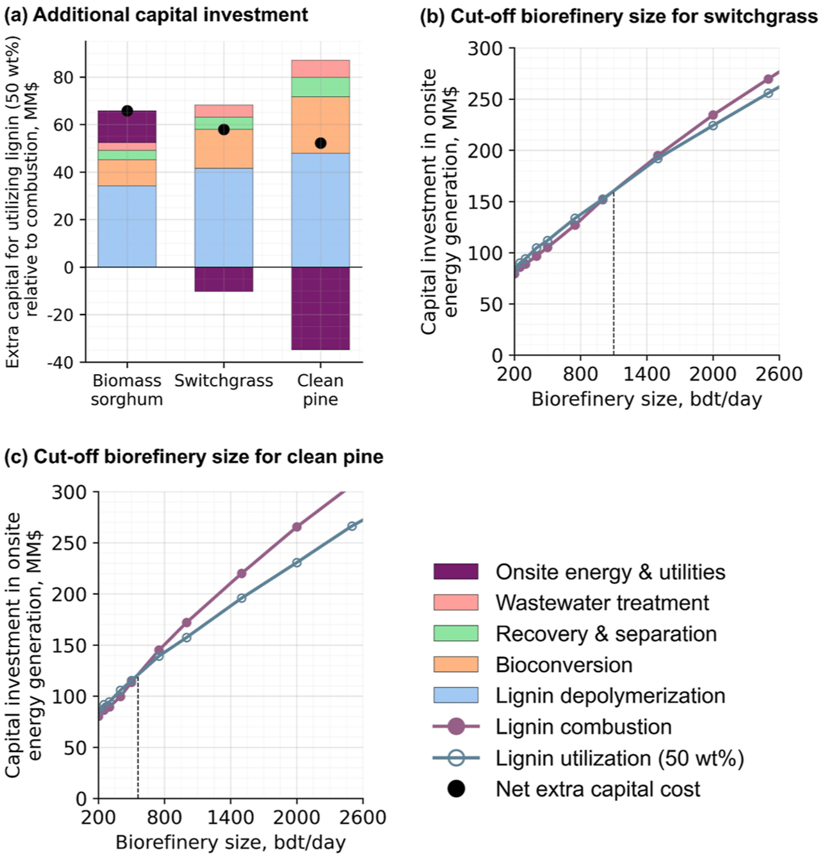


Figure 2. Differences in capital investment between lignin utilization (50 wt %) and lignin combustion scenarios for the baseline biorefinery size of 2000 bdt/day (a) and capital investment in the onsite generation stage as a function of biorefinery size for switchgrass- (b) and clean pine- (c) based bisabolene biorefineries. The biomass sorghum-based bisabolene biorefinery does not exhibit a cutoff biorefinery size in capital investment for onsite energy generation. Detailed results are listed in Supporting Information Figure S1. Negative values indicate that lignin utilization decreases the capital investment.

The recovery and separation unit includes systems for cell mass separation and bisabolene recovery. The cell mass is separated using subsequent centrifugation and ultrafiltration units. Bisabolene is recovered using decantation followed by distillation, as it has a low solubility in water of 0.008994 mg/L.[48] The overall bisabolene recovery is assumed to be 98%. The recovered cell mass is sent to the boiler, and the wastewater and any remaining materials are delivered to the wastewater treatment unit.

The wastewater produced at the bisabolene biorefinery undergoes subsequent anaerobic and aerobic treatment processes, which align with the assumptions from previous studies.[2,23] The biogas generated from the anaerobic digestion is directed to the boiler for reuse, while the treated water is recycled and utilized onsite.

In the on-site energy generation and utility section, the unutilized solid biomass residues, cell mass, and biogas are burned in a boiler to generate steam. However, these biogenic energy sources may not always be sufficient to meet the heat and power demands of the facility, particularly when lignin is diverted to bioconversion reactors. In these cases, natural gas is used as a supplemental fuel source for the boiler. Any remaining steam after allocation for the upstream processes is used to generate electricity through steam turbines. This section also includes a groundwater pumping system, a cooling water tower, a chilled water system, and a clean-in-place (CIP) system. These units, respectively, provide makeup process water, cooling water, chilled water, and hot cleaning and sterilization chemicals. The assumptions and system layout for this stage are consistent with previously published works.[2,23]

Table 2 summarizes the key data inputs used in the modeling for this study. Most of these inputs are consistent with optimal future scenarios that have been considered in prior studies.[2,17,23] However, some of the data inputs, such as achieving above 90% of the theoretical sugar yield at a low enzyme loading of 10 mg of protein per g of glucan, combined with a high solid loading exceeding 25 wt %, continue to be out of reach in the near term. Achieving this target would require several decades of experimental work, as most experimental studies still use two to five times higher enzyme loading with solid loading of 20 wt % or lower, to achieve similar sugar yields.[20,27,32,49,50] It may be that researchers are more often seeking to report the highest possible sugar yields in published studies and err on the side of using excess enzymes rather than focusing on process intensification and lower enzyme loading. This highlights the need for scale-up and industry-led optimization, where minimizing costs will be a central priority. Another potential challenge lies in the conversion of sugars and lignin-derived intermediates into bisabolene.

The demonstrated titer, rate, and yield of bisabolene in *R. toruloides*, using ionic liquid-pretreated hydrolysate from ensiled and dry biomass sorghum,[20,27] range from 1.3 to 2.2 g/L, 0.007 to 0.01 g/L/h, and 1.6 to 2.6 g per 100 g of carbon sources present in the hydrolysate, respectively. These carbon sources include glucose, xylose, acetic acid, lactic acid, 4-hydroxybenzoic acid, and a small amount (approximately 40 mg/L) of other lignin monomers, such as benzoic acid, vanillic acid, ferulic acid, vanillin, and coumaric acid. As observed from our metabolic modeling results, the maximum biological yield is approximately 30 g per 100 g of a mixture consisting of glucose, xylose, and *p*-coumaric acid as a representative lignin monomer. The maximum biological yields were previously calculated[51] utilizing genome-scale metabolic models. These models serve as mathematical representations of a specific host's metabolism, accounting for metabolic reaction stoichiometry as well as cellular energy and redox requirements essential for cellular maintenance. The gap between experimentally demonstrated and theoretical yields is not surprising considering that these are early-stage experiments. This

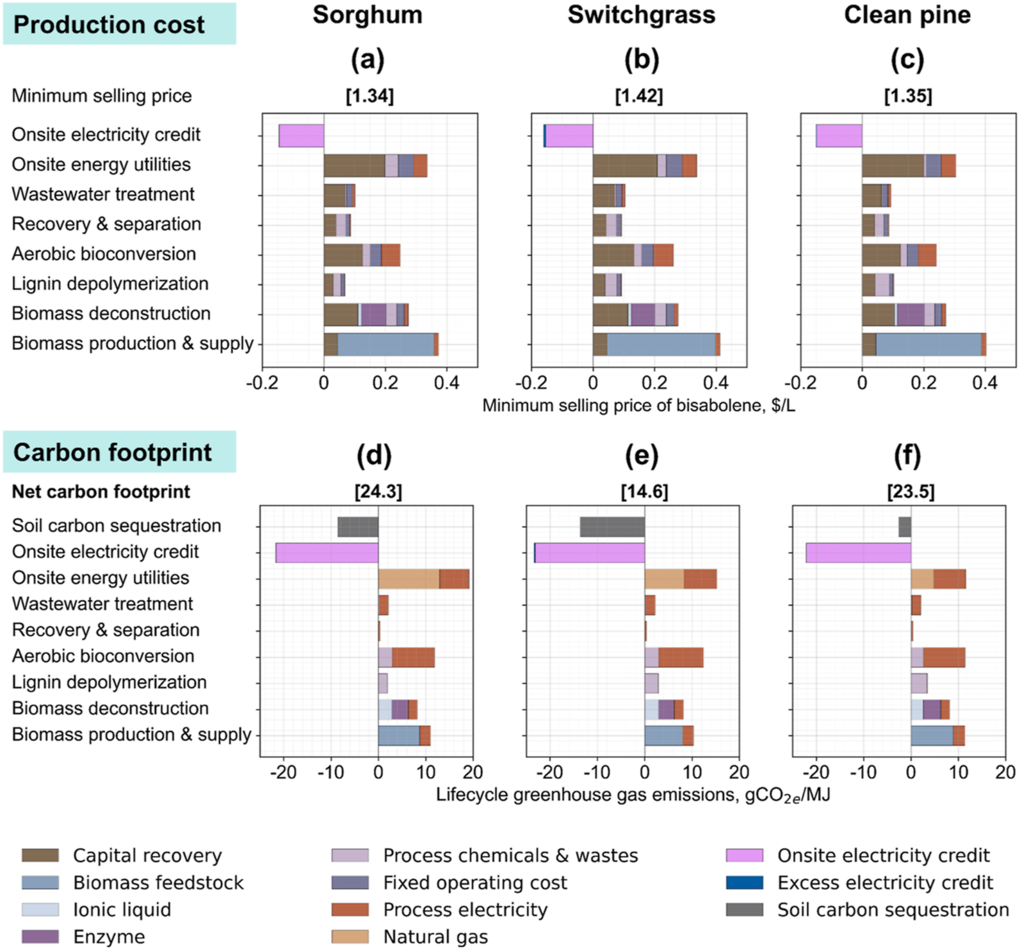


Figure 3. Minimum selling price of bisabolene with a 50 wt % lignin utilization scenario for biomass sorghum (a), switchgrass (b), and clean pine (c), as well as corresponding life cycle GHG emissions with biomass sorghum (d), switchgrass (e), and clean pine (f). The detailed minimum selling price and GHG emissions results for lignin combustion and lignin utilization (90 wt %) scenarios are documented in Supporting Information Figures S5 and S12, respectively.

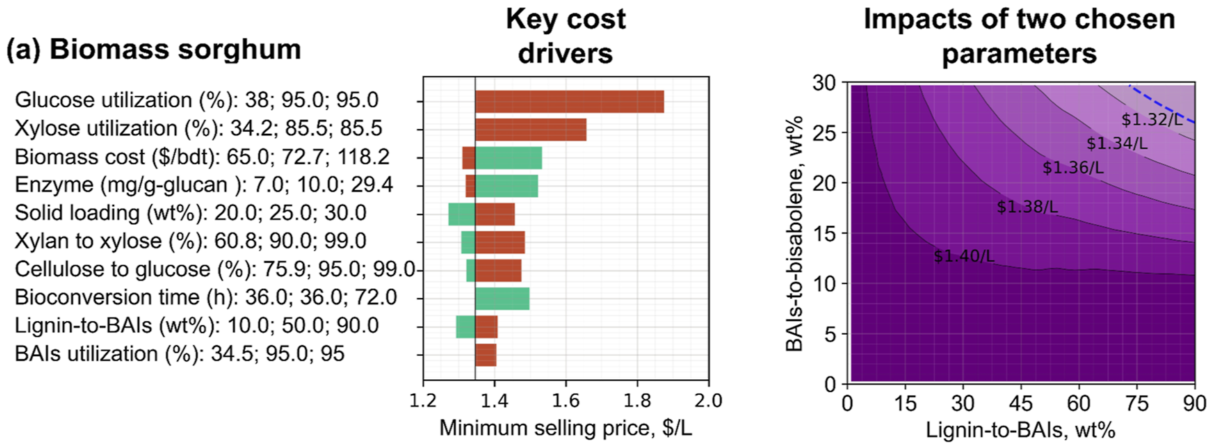
suggests that substantial research efforts will be required in the future to efficiently utilize the entire plant hydrolysate, which includes sugars and bioavailable intermediates derived from lignin. Despite this gap between reported performance and idealized systems, previous studies[17,23,27] have targeted similar optimal data inputs, as considered in this study, to achieve market-competitive biofuels selling prices with or without considering carbon mitigation credits.

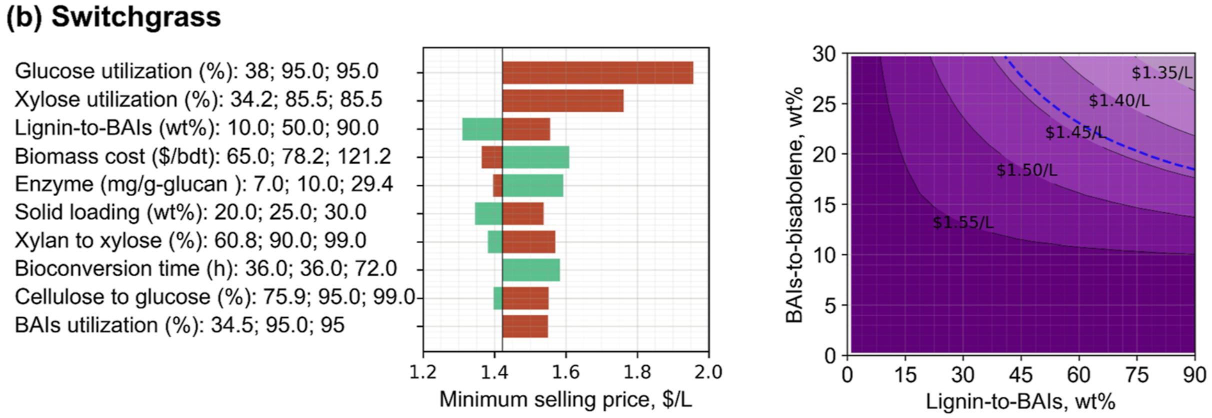
Production Cost Estimation. The method used to estimate the minimum selling price of bisabolene is consistent with prior studies.[2,23] Briefly, material and energy balance analyses are performed by using built-in functions available in the process modeling software—SuperPro Designer V13. The material and energy balance results are used to determine equipment sizes and specifications. The equipment size and specifications are used to determine the purchase cost and quantity of process equipment. Equipment purchase cost and installation factor of each piece of process equipment are used to determine the installed equipment cost. Once the installed equipment cost was determined, several direct and indirect costs are added to determine the total capital investment. Following the capital cost calculation, the annual operating cost is determined by adding annual costs of biomass feedstock, process chemicals, maintenance, insurance, property taxes, consumables, waste disposal, labor, and utilities. Assumptions for land cost and working capital are consistent with prior studies.[2,23] We perform the discounted cash flow rate of return analysis to determine the minimum selling price of bisabolene required to obtain a zero net present value with an internal rate of return of 10%.[23] We considered a plant lifetime of 30 years, plant operating hours of 7920 h (330 days/year and 24 h/day), and income tax of 21%.[17,23]

Estimation of Life Cycle GHG Emissions. We constructed a comprehensive life cycle inventory for a bisabolene production system by systematically gathering material and energy inputs and outputs for each stage of the production process from field to bisabolene. These data inputs were obtained by performing rigorous material and energy balance analyses in the process modeling software SuperPro Designer. We used a physical units-based input−output matrix and GHG impact vectors gathered from widely used life cycle assessment databases, including GREET LCA model,[36] ecoinvent,[54] and US LCI[55] database to determine the direct and indirect GHG emissions. The approach we used for life cycle assessment has been thoroughly discussed in a prior study.[56] In cases where the biorefinery generates excess electricity, we used the system expansion method to account for excess electricity credit while considering the GHG emissions footprint of the U.S. average electricity mix. We assumed a higher heating value of bisabolene of 45.7 MJ/kg,[17] which was estimated theoretically using bond energy, and a functional unit of 1 MJ.

RESULTS AND DISCUSSION

Capital Investment and Minimum Selling Price. Figure 2 depicts the differences in capital investment between lignin utilization (50 wt %) and lignin combustion scenarios as well as the capital investment in the onsite generation stage relative to biorefinery size. This study explores the differences in capital costs because such costs can, independent of expected future


(a) Biomass sorghum


(b) Switchgrass

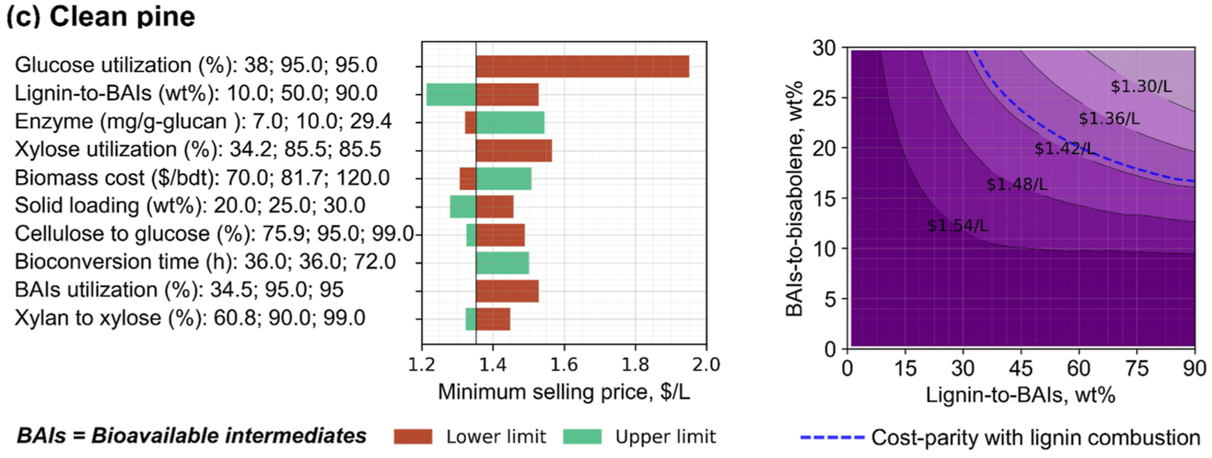

(c) Clean pine

Figure 4. Most influential input parameters for bisabolene cost with biomass sorghum (a), switchgrass (b), and clean pine (c) along with thresholds for lignin utilization to achieve cost-parity with lignin combustion for each feedstock.

cash flow, serve as the first barrier to raising sufficient funds to construct a new facility. Thus, minimizing capital costs can be a high priority for companies. Detailed capital investments relative to biorefinery size required for biomass sorghum, switchgrass, and clean pine-based bisabolene biorefineries are documented in Supporting Information Figures S1–S4. Our results reveal how total capital costs vary with scale for both lignin-combustion and lignin-utilizing facilities. However, the results did not establish a specific cutoff size for the total capital investment (Supporting Information Figure S1). For the selected biomass feedstocks (Supporting Information Figure S1), when biorefineries utilize less than 300 bdt of biomass per day, the difference in total capital investments between the lignin combustion and lignin utilization scenarios is below $30 million. This difference more than doubles when the biorefinery size is increased to 3000 bdt/day (Supporting Information Figure S1).

Upon examining the difference in capital cost for each bisabolene production stage in more detail (Figure 2a), we found that a biorefinery with a high lignin feedstock, such as switchgrass and pine, can offset the total capital investment for lignin utilization by reducing the capital cost required for the on-site energy generation stage. This is because large-scale biorefineries that utilize either switchgrass or pine generate surplus electricity by burning lignin, which requires a larger boiler and turbine. However, diverting lignin for bioconversion enables the selection of a smaller boiler and turbine by generating only the heat and electricity required to meet the facility's demands. For high lignin feedstocks such as switchgrass (Figure 2b) and clean pine (Figure 2c), there is a specific facility scale at which the onsite energy generation stage for lignin utilization becomes the less capital-intensive option. For switchgrass, this point is at 1100 bdt/day, and the onsite energy generation stage with lignin utilization becomes the less capital-intensive option at 560 bdt/day for clean pine feedstocks. This threshold was not observed for low lignin feedstocks like biomass sorghum (Supporting Information Figure S1a), where the onsite energy generation stage with lignin utilization is always the more capital-intensive option. This underscores the greater importance of lignin utilization for high lignin feedstocks, which benefit by reducing the capital requirements for boiler and turbo-generator systems.

To develop a more holistic understanding of facility economics, we also calculated the minimum selling price for bisabolene. Figure 3 depicts the minimum selling price of bisabolene with a 50 wt % lignin utilization scenario. The detailed minimum selling price results for lignin combustion and lignin utilization (90 wt %) scenarios are documented in Supporting Information Figure S5. We find a decreasing trend in the minimum selling price of bisabolene as lignin utilization increases relative to lignin combustion, and the reduction rate increases with an increasing initial lignin content in the biomass feedstock (Supporting Information Figure S5). For low lignin feedstocks, such as biomass sorghum, lignin intermediates yield of only 50 wt % of the initial lignin is insufficient to reduce the price below the lignin combustion scenario (Figure 3a and Supporting Information Figure S5a). In such cases, a higher lignin intermediates yield (i.e., 90 wt %) is required to achieve a reduction of 1% in the minimum selling price of bisabolene (Supporting Information Figure S5a). This suggests that increasing the volume of bisabolene with a lower lignin utilization could not offset the corresponding increased capital and operating costs, requiring a higher lignin utilization to reach cost-parity with the lignin combustion scenario. High lignin feedstocks, such as switchgrass and pine, provide a more substantial reduction in the selling price relative to the lignin combustion scenario. For instance, in the case of clean pine feedstock, a 50 wt % lignin-derived bioavailable intermediates yield reduces the minimum selling price of bisabolene by 4% compared to the lignin combustion scenario (Supporting Information Figure S5c). This reduction increases to 13% with a lignin-derived bioavailable intermediates yield of 90 wt %. Similarly, for switchgrass feedstock, the reduction rate in the minimum selling price of bisabolene increases from 1 to 8% as the lignin-derived bioavailable intermediates yield increases from 50 to 90 wt % (Supporting Information Figure S5b). These results indicate that high lignin biomass feedstocks provide a better opportunity to offset increased capital and operating costs due to lignin utilization, even at a lower lignin-to-biofuel yield.

Although a 90 wt % lignin utilization scenario shows an economic advantage over lignin combustion, the decrease in the minimum selling price of bisabolene from \$1.3−1.5/L with lignin combustion to \$1.2−1.3/L with 90 wt % lignin utilization is modest. The modest difference in the minimum selling price occurs because a substantial quantity of other materials, primarily water (68−80 wt %, depending on the yield of bioavailable intermediates during lignin depolymerization), enters the bioconversion reactor along with the lignin-derived bioavailable intermediates. This dilution effect caused by the additional materials entering the bioconversion reactor decreases the concentration of sugars, hindering the increase in biofuel titer that would be expected with the utilization of both sugars and lignin-derived bioavailable intermediates. Indeed, in the case of biomass sorghum with a 50 wt % lignin utilization, the titer of bisabolene is decreased by roughly 1% (58.8−58.4 g/L), which negatively impacts on the production cost of bisabolene. A low concentration of carbon sources in the bioconversion reactor is a key challenge for achieving an industrially relevant product titer. Substantial improvements in upstream biomass hydrolysis and lignin depolymerization are required to provide concentrated carbon sources that can be metabolized by microbes. Additionally, the results indicate that optimizing other key cost drivers (Figure 4 and Supporting Information Figures S6−S8), including fertilizer use in biomass production, biomass transportation, ionic liquid and enzyme use for biomass deconstruction, and nitrogen sources and energy use for bioconversion, is even more important for lignin utilization biorefineries. Nonetheless, clear price gaps between lignin utilization and lignin combustion, as demonstrated in the Supporting Information Figure S5, encourage future research to identify lignin-to-bioavailable intermediates conversion technologies that are more efficient and produce higher yields, as well as further improvements in engineering host microbes to more efficiently convert these intermediates to biofuels. Alternatively, even in the presence of dilute whole-plant hydrolysate, the value of lignin utilization can be enhanced by carefully choosing biomass feedstock, lignin depolymerization methods, microbial hosts, and high-yielding bioproducts.

Lignin Utilization Thresholds. Identifying performance thresholds for lignin utilization is crucial in setting targets for future research and prioritizing strategies that are most likely to be viable in the long term. To demonstrate these cutoff points where lignin utilization economically outperforms lignin combustion, we varied two important metrics: the lignin-to-bioavailable intermediates yield and the bioavailable intermediates-to-bisabolene yield while holding other modeling inputs constant. Figure 4 illustrates the resulting thresholds for the selected biomass feedstocks, such as biomass sorghum, switchgrass, and clean pine. Similar results for GHG emissions are documented in Supporting Information Figures S9−S11. Recent studies[42,57−62] have shown that the lignin-to-monomers conversion typically ranges from 20 to 50 wt %. Assuming that all lignin monomers are efficiently consumed by microbes along with sugars, the yield of these monomers falls at least 1.7 times short of reaching cost-parity with lignin combustion for biomass sorghum. Although successful bisabolene production has been demonstrated by utilizing the whole plant biomass (both sugars and lignin monomers),[27] the lignin monomers-to-bisabolene conversion still needs to be demonstrated at a large-scale. These results suggest that substantial improvements in lignin-to-bioavailable intermediates conversion and the engineering of microbial hosts that efficiently consume such intermediates are required to make lignin coutilization feasible for low lignin biomass feedstocks, such as biomass sorghum. These are being actively pursued in academic research, with examples including the bioconversion of lignin intermediates into compounds such as muconic acid[10] or 2-pyrone-4,6-dicarboxylic acid.[11]

Medium and high lignin biomass feedstocks, such as switchgrass and clean pine, require a lignin-to-bioavailable intermediates conversion of at least 41 and 33 wt %, respectively, to make the lignin utilization strategy economically viable over lignin combustion. These yields can be achieved, particularly with reductive or oxidative catalytic depolymerization methods.[42,57−62] The average monomer yield is approximately 30 wt % for hardwood and herbaceous crops,[42,58,59,61,62] and yields exceeding 50 wt % are regularly reported for hardwood[61,62] with the reductive catalytic depolymerization method. Monomer yields from oxidative depolymerization methods are mostly reported in the range of 20−40 wt %,[57,60−62] while the lowest average monomer yield mostly in the range of 10−20 wt %[61] is reported for the acid- or base-catalyzed depolymerization process. These findings highlight the necessity of selecting appropriate biomass feedstocks and depolymerization methods to achieve the targeted monomer yields, although each of these processes requires different levels of capital and operating expenses. These results also emphasize that further improvements in the lignin depolymerization will provide flexibility to achieve cost-parity with lignin combustion at a lower lignin-derived intermediates-to-bisabolene conversion.

For the low-lignin biomass sorghum considered in this work, the economics of conversion of lignin to bisabolene are challenging. Even with a 90 wt % lignin-to-bioavailable intermediate conversion, it is very challenging to reach cost-parity with lignin combustion. The host microbes would need to convert bioavailable lignin-derived intermediates to bisabolene in a biological theoretical yield of or above 87% of the biological theoretical yield. However, it is worth noting that the lignin content can vary considerably among different sorghum lines. The average lignin content of nonengineered biomass sorghum is about 21 wt %,[17,27] which is twice the value assumed in this study. To enable economic utilization of engineered low-lignin biomass sorghum feedstock in biorefineries, substantial improvements in other process parameters, particularly titer and rate of bisabolene, are required. At the same lignin-derived bioavailable intermediate yield of 90 wt %, switchgrass and clean pine only require 62 and 56% of the biological theoretical lignin-derived bioavailable intermediates to bisabolene conversion in order to achieve cost-parity with lignin combustion. These results imply that while some processes may be flexible in the feedstocks with which they are compatible, it may make sense to tailor lignin utilization strategies to specific feedstocks. The economics of depolymerizing and microbially converting lignin are less attractive for low-lignin feedstocks.

Impacts of Lignin Utilization on the Carbon Footprint of Bisabolene. Figure 3 illustrates the impact of lignin coutilization (50 wt %) on the GHG emissions of bisabolene. The detailed GHG emissions results for lignin combustion and lignin utilization (90 wt %) scenarios are documented in Supporting Information Figure S12. The use of lignin coutilization increases the GHG emissions for the biorefinery configuration considered in this study, where natural gas is used as a supplemental energy source. The difference in the carbon footprint between lignin coutilization and lignin combustion becomes more pronounced when there is an increase in the lignin content in biomass feedstocks, primarily due to the reduction in excess electricity generated from burning lignin (Supporting Information Figure S12). The primary reason for the increased carbon footprint with lignin coutilization is the rise in natural gas consumption for heat and power generation, as all of the initial lignin is not available for onsite energy production. However, biomass feedstocks with a high lignin content, such as switchgrass and clean pine, can largely offset the carbon footprint contribution of supplemental natural gas through an increase in the biofuel production volume. Low lignin feedstocks, such as biomass sorghum, can offset only a small portion of GHG emissions generated by the supplemental natural gas. Regardless of the selected biomass feedstocks, lignin coutilization reduces GHG emission contributions from biomass feedstock, enzyme, ionic liquid, and process electricity of most bisabolene production stages (Figure 3 and Supporting Information Figure S13). More importantly, these GHG emission reductions (2−10 gCO_{2e}/MJ) are sufficient to offset the GHG emissions generated from the lignin depolymerization process (1.9−3 gCO_{2e}/MJ). However, in order to maximize the GHG emissions reduction benefits of lignin coutilization, it is necessary to develop a more energy- and carbon-efficient lignin depolymerization process than the NaOH-based process considered in this study (Supporting Information Figures S14−S16).

Supplemental natural gas alone can increase the GHG emissions of bisabolene by 5−9 gCO_{2e}/MJ with 50 wt % of initial lignin utilization and 13−17 gCO_{2e}/MJ with 90 wt % of initial lignin utilization (Supporting Information Figure S13). These results are substantial when compared to the GHG emissions of bisabolene with lignin combustion, which range from −2.7 to 14.3 gCO_{2e}/MJ (Supporting Information Figure S12). Therefore, it is important for biorefineries to consider using less carbon-intensive energy sources than natural gas to generate heat and electricity when lignin is not available. However, biomass or other renewable sources that have an energy value less than that of lignin and a higher delivered cost than that of biomass feedstock are not preferred, as they would increase the cost by requiring a larger boiler and raise the raw material cost. Alternatively, biorefineries could generate process steam only on-site and source electricity directly from the grid when lignin is not available. This strategy foregoes some of the efficiency advantages inherent in combined heat and power systems. However, it may still be preferable in cases where deep decarbonization of the electricity grid has been achieved.[63,64]

The results indicate that it is possible to divert 100% of lignin to a bioreactor while still meeting the RFS GHG emissions reduction mandate for cellulosic biofuel, which requires a 60% reduction relative to petroleum equivalent.[65] This has prompted recent research efforts focused on lignin depolymerization and conversion to biofuels and high-value commodity chemicals. While a 60% reduction in GHG emissions for cellulosic biofuels could be achieved without including soil organic carbon (SOC) sequestration credits, the utilization of biomass with high SOC sequestration, such as switchgrass, offers greater potential for reducing the increased GHG emissions resulting from lignin utilization. This suggests that engineering annual crops that can store more below-ground biomass or transitioning annual crops to perennials is critical to reducing GHG emissions when lignin is not available for onsite energy generation.

Pathways to Reach a Market-Competitive Selling Price of Bisabolene. As discussed in the previous section, biomass feedstocks with a high lignin content are most attractive for lignin coutilization. To achieve a market-competitive price for bisabolene and meet the GHG emissions reduction goal of 60% relative to petroleum equivalent for cellulosic biofuels, it is crucial to determine the necessary amount of lignin utilized by the host microbe. This lignin utilization involves both the conversion of lignin to

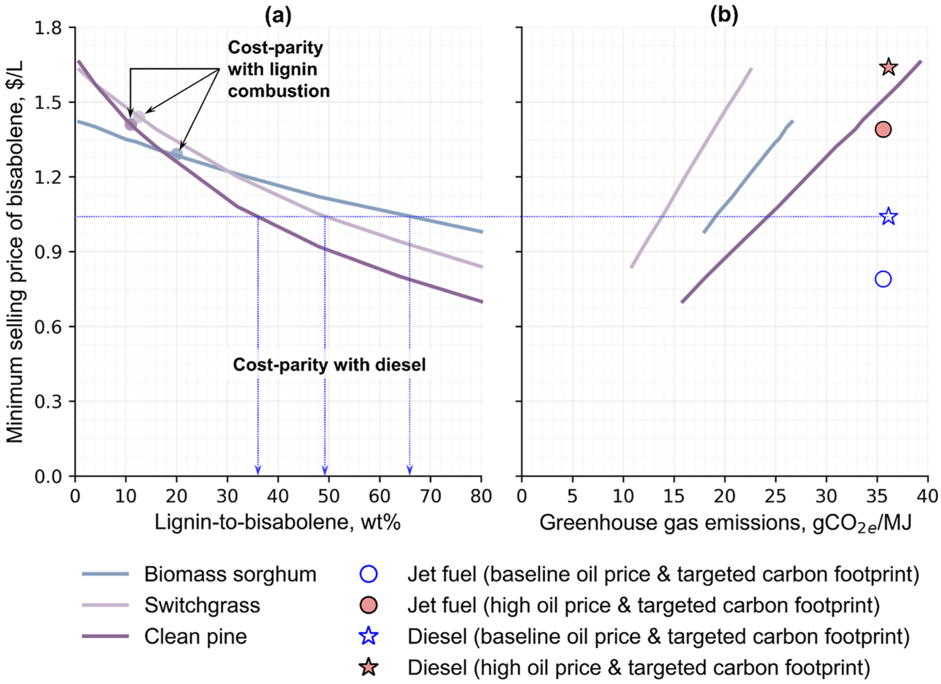


Figure 5. Minimum selling price of bisabolene as a function of (a) lignin to bisabolene conversion and (b) GHG emissions of bisabolene. The targeted carbon footprint of bisabolene aims for a 60% reduction relative to petroleum. All prices are presented in 2022$. Projected petroleum prices in 2050 (2022$) were gathered from Annual Energy Outlook 2023.[73]

bioavailable intermediates and the subsequent conversion of these intermediates to bisabolene. In this study, a fully optimized lignin depolymerization system is considered that achieves a 90% conversion of initial lignin into bioavailable intermediates by mass. The results of this analysis are depicted in Figure 5.

The results show that biomass sorghum would need a lignin-to-bisabolene conversion of 20 wt % to reach cost-parity with lignin combustion, which represents a substantial improvement compared to the current yield of approximately 1.5 wt % (Figure 5a).[27] Additionally, in order to achieve a 60% reduction in GHG emissions relative to petroleum equivalent, it is necessary to convert at least 9 wt % lignin into bisabolene (Figure 5a,b). It is worth noting that high-lignin biomass feedstocks, like clean pine and switchgrass, require lower lignin-to-bisabolene conversions at 11 and 13 wt %, respectively. Nevertheless, irrespective of the lignin content in biomass crops, achieving economic benefits over lignin combustion necessitates a substantial enhancement in the bioconversion yield.

Host microbe optimization is one of the key areas that needs improvement. Although lignin-to-monomers conversion yields mostly in the range of 20−50 wt % have been demonstrated,[42,57−62] the development of high-performance microbes that can simultaneously catabolize sugars and lignin-derived intermediates is still in its very early stages. Most prior biological lignin upgrading work has focused on converting lignin monomers into commodity chemicals and high-value products, including indigoidine,[66] vanillin,[67] muconic acid,[68] polyhydroxyalkanoates,[69] methane,[70] and lipids,[71] using different host organisms such as *Pseudomonas putida* and *Rhodococcus opacus*. To avoid additional costs and energy associated with potential upgrading processes, a focus on bioconversion pathways that directly convert lignin monomers to targeted biofuels or biochemicals is essential. More importantly, there is a need to develop host organisms that can metabolize not only lignin monomers but also aromatic oligomers and other lignin-derived intermediates.

Moreover, it is important to engineer carbon-efficient host microbes that can efficiently utilize diverse lignin intermediates and achieve near-stoichiometric theoretical yields, as this can further reduce both the production cost and the carbon footprint of biofuels. For instance, the host strain *R. toruloides* considered in this study achieves a biological theoretical yield of 30 wt % for *p*-coumaric acid, which is about two times lower than the stoichiometric theoretical yield of 56.3 wt %. The stoichiometric theoretical yield is determined following the method reported in prior work, which involves the reduction of carbon sources to products using H_2 and excludes biological constraints. Lignin depolymerization leads to a range of aromatic compounds suitable for conversion. Aromatic compounds typically also have higher yield ratios to final products, especially for reduced compounds. For instance, guaiacols, such as methyl-, ethyl-, and propyl-guaiacol, show possible stoichiometric theoretical yields of bisabolene in the range of 66.9−73.1 wt %, 4-vinylphenol exhibits a possible bisabolene yield of 76.9 wt %, and many other monomers show possible bisabolene yields of above 50 wt %. One key challenge in the use of aromatics is their toxicity. Toxicity to the microbial conversion platform will vary from strain to strain, where microbes such as *R. toruloides* have an innate high tolerance to such compounds due to native degradation pathways. Using engineering routes, it may be possible to integrate such existing catabolism or degradation steps into the conversion pathway,[66,72] thus maintaining (or enhancing) the microbe's innate tolerance while also providing biosynthetic routes to convert the aromatic to the final product. Our findings indicate that a lignin-to-bisabolene conversion of 36−66 wt % is necessary to achieve cost-parity with the baseline market price of diesel in 2050, which is $1.1/L (2022$).[73] This emphasizes the importance of appropriately selected lignin depolymerization methods to obtain high-yielding bisabolene monomers and the use of host microbes that can efficiently metabolize such monomers into bisabolene. This approach is crucial for the production of market-competitive low-carbon bisabolene. However, if the bioconversion yield is not substantially improved, low-lignin substrates do not justify an extensive lignin valorization process, especially for the production of low-yielding biofuel molecules.

The results show that bisabolene could be an economic precursor for biobased diesel and jet fuel blendstocks and could be more competitive in high-oil scenarios. However, the process parameters considered in this study still need to be demonstrated at a pilot or large scale, and an additional cost of about 6−10 cents is required to upgrade per liter of bisabolene into bisabolane,[17] which is an equivalent renewable diesel or jet fuel molecule. Alternatively, bisabolene can be utilized as a commodity chemical, which typically holds a higher value than fuel and is more competitive with incumbent products.

CONCLUSIONS

This study examines the economic and environmental implications of using lignin coutilization strategies to produce bisabolene. The results indicate that the use of low-lignin biomass feedstocks, such as biomass sorghum, for lignin coutilization requires substantial improvements in the conversion of lignin to bioavailable intermediates and microbial engineering to become an economic option. Conversely, medium- to high-lignin biomass feedstocks, such as switchgrass and clean pine, are relatively more attractive for lignin coutilization in the short term but still require additional improvement in bioconversion yields of bisabolene to achieve cost-parity with lignin combustion. While lignin coutilization can reduce GHG emissions, further progress is necessary to fully capture its benefits and achieve the desired impact on the carbon footprint of bisabolene. The study highlights the importance of reaching the lignin utilization thresholds to make this approach economically viable and provides valuable insights for future research on lignin utilization and plant engineering. The results underscore the need for ongoing research and development efforts to establish lignin coutilization as a sustainable and effective strategy for biofuel production.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.3c05541.

> Variation in capital investment with biorefinery size, detailed capital investment for lignin combustion and utilization scenarios, detailed sensitivity analysis, heat maps illustrating the impact of lignin utilization on the carbon footprint of bisabolene, and key carbon footprint contributors (PDF)

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Notes

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