**More Rapid and Robust Process Simulation through Phenomena-Oriented Decomposition.**

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**Keywords:**

**Abstract**

Rapid and robust simulation of a chemical production process is critical to address core scientific questions related to process design, optimization, and sustainability. However, efficiently solving a chemical process remains a challenge due to their highly coupled and nonlinear nature. Decoupling of phenomenological nonlinearities (e.g., phase equilibrium, chemical reactions) is widely used to linearize material and energy balances within multistage equilibrium columns and enable more robust simulation. In this work, we developed a phenomena-oriented simulation algorithm for the convergence of the complete flowsheet that expands and generalizes classical decomposition strategies for multistage unit operations. We implemented this new simulation algorithm in BioSTEAM —an open-source process simulation platform implemented in Python— and show how the phenomena-oriented algorithm enables more rapid and robust simulation of large, highly-coupled systems than classical sequential modular simulation. Using the production of glacial acetic acid (99.9% purity) from a dilute mixture of water and acetic acid as a representative case for a large and highly coupled system, the phenomena-oriented algorithm results in a 70% reduction in computation time and a 100x lower offset from the true steady state (computed by optimization) compared to sequential modular simulation.

**TOC Graphic**

**Introduction**

Critical to the design and development of sustainable production processes, chemical process simulation seeks to model the flow rate of material and energy between unit operations and their components. Physical phenomena (e.g., phase equilibrium, chemical reactions, adsorption, and heat exchange) which drive a production process also introduce nonlinearities to the material and energy balances that are tied to the composition and energy content of streams. The presence of this nonlinear coupling between unit operations is the core difficulty in solving for the steady-state solution of a production process in any algorithmic paradigm (e.g., sequential modular simulation, parallel modular simulation, equation-oriented simulation, pseudo-transient modeling, design of surrogate models). Large, highly coupled systems lead to substantially lower computational speed and rate of successful convergence than small, sparse systems. The inability to scale simulations to complex systems limits researchers’ ability rigorously characterize and optimize emerging sustainable production process which attempt to integrate various technologies to minimize waste and environmental impact.

In sequential modular simulation, the most popular approach, each unit operation “module” is solved sequentially and independently using specialized algorithms that take advantage of the topology of the underlying equations. Recycle streams are solved by partitioning and tearing algorithms which determine subsets of modules that are rerun sequentially until all recycle streams converge. While convergence can be accelerated within a numerical solver for better estimates of the tear stream flow rates and temperature, convergence may be hindered by the nonlinear coupling of recycle variables and the accumulation of numerical impression noise from solving individual modules. Equation-oriented simulation, the runner-up in popularity, leverages the sparsity of variables within equations and employes algebraic differentiation to guide convergence within an optimization suite. A key drawback of this approach is that the initial guess must be within the basin-of-attraction (of unknown size) to converge to the correct solution. Equation-oriented simulators may leverage sequential modular simulation to provide an initial guess, which undermines its computational efficiency. More recently, pseudo-transient modeling provides more robust simulation by reformulating steady-state algebraic equations as first-order differential equations to dynamically decouple variables using time scales that reflect the “speed” of different phenomena. For example, separate time scales may be used for material, equilibrium, and heat variables to promote smoother integration. Pseudo-transient modeling has yet to be validated and benchmarked for larger, more complex systems and it is not yet known what is the computational burden compared to sequential modular simulation. Among these approaches, there is no clear winner balancing speed and robustness and, critically, none have been demonstrated to scale in computational effort for large, coupled systems.

Solution strategies for multistage unit operations (e.g., distillation columns, liquid-liquid extraction columns) commonly decouple nonlinear phenomena from material and energy balances, enabling material flows and key energy variables to be solved as systems of linear equations. Although the decomposition of equations is specialized for the type of phenomena (e.g., liquid-liquid equilibrium vs. vapor-liquid equilibrium), these methods have an underlying structure whereby phenomenological nonlinearities, energy balances, and the material balances are solved by sequential substitution. It may be possible to expand and generalize phenomena-based decomposition to linearize the material and energy balances of any set of unit operations, employ the same phenomena-specific strategies to solve for nonlinearities, and iteratively solve the complete flowsheet by sequential substitution or even employ more advanced numerical methods. Such a phenomena-oriented simulation algorithm would consolidate material and energy balances across the flowsheet at every iteration, potentially improving its scalability for large, complex systems.

The goal of this study was to develop a simulation paradigm that can better scale computational time and rate of successful convergence for large, coupled systems. To this end, we completed the following objectives: (i) developed a phenomena-based decomposition scheme that is generalizable to any set of unit operations and flowsheet configuration, (ii) developed a phenomena-oriented simulation algorithm that employes this decomposition scheme, and (iii) benchmarked this algorithm against sequential-modular simulation for representative small, medium, and large systems with highly coupled phenomena. Graph abstractions are used to portray the decomposition scheme and demonstrate how the underlying equations of any unit operation can be reformulated to fit the scheme. We implemented the phenomena-oriented simulation algorithm in BioSTEAM —an open-source process simulation platform implemented in Python—and benchmarked its convergence against sequential modular simulation for various systems with vapor-liquid and liquid-liquid equilibrium stages and recycles, including an industrial separation process for the separation of glacial acetic acid from water using ethyl acetate as a solvent. This separation process consists of 3 recycle loops and over 50 equilibrium stages. Additionally, we developed a decomposition scheme for gradient based optimization which we used to determine the true steady state solution to all systems to the highest numerical precision possible.

maunderlying equations within the flowsheet and solved iteratively

If a process flowsheet could become aware of the connectivity of the governing phenomenological equations, it may be feasible to screen for alternative problem formulations that result in more rapid and robust convergence. The problem formulation and the selection of a suitable solution method, however, is not a simple task.

Decomposition of material, equilibrium, summation and enthalpy (MESH) equations is widely used to reformulate the network of equations within multistage unit operations and enable convergence through sequential substitution.

main limitations to pseudo-transient modeling are the need to define a hierarchy of time-scales, the computational burden overhead of integration, and the need to.

Graph abstractions of the governing equations within a chemical process suggest that it may be possible to expand and generalize the

The ability to perform rapid and robust TEA under uncertainty would empower researchers to address core scientific questions related to technology development, process design, and biorefinery scaling and siting to support the broader goal of establishing resilient, adaptable installations that align goals for economic and environmental sustainability.

Each unit operation “module” is treated as a black-box with only material streams as inputs and outputs. All mass, energy, and thermodynamic equations are formulated and solved independently within each unit operation. While specialized convergence strategies are employed to converge individual units, the convergence of recycle systems can be challenging due to nonlinear coupling between unit operations

The greatest advantage of the sequential mod-

ular approach is its robustness and reliability.

After many years of work, each unit model has

been carefully studied; therefore, there are pro-

grams which perform the calculations in a very

efficient way. In general, the special properties of

a certain system of equations are used to accel-

erate the modules' convergence. Besides, its physi-

cal behavior is known and used for initialization,

verification of results, consistency, etc. For these

reasons, most of the operations, and in the differ-

ent detail levels, have achieved a remarkable per-

formance and a high development.

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Rapid and robust simulation of a chemical production process is critical to address core scientific questions related to process design, optimization, and sustainability

only a limited set of approaches leverage the mathematical topology of the underlying phenomenological equations across the flowsheet.   
In classical sequential-modular simulation, each unit op-eration is treated as a black-boxseparate model with only material streams as inputs and outputs. All mass, energy, and thermodynamic equations are formulated and solved independently within each unit operation. While specialized convergence strategies are employed to converge individual units, the convergence of recycle systems can be challenging due to nonlinear coupling between unit operations. Equation-based modeling lev-erages the sparsity of the full set of equations and em-ploys algebraic differentiation to guide convergence of the entire system to aid numerical methods in finding the steady state solution. Equation-based modeling may be faster than the sequential modular approach particu-larly when the initial guess is close to the steady state solution. However, highly coupled and complex net-works of equations introduce instabilities and can that lead to convergence failures [3].

Certain unit operations may have tight cluster of components which operate together to achieve a purpose. For example, a liquid-liquid extractor may be composed of multiple mixers and settlers while a distillation column may have multiple trays and heat exchangers.

demonstrated potential of vegetable oil as a feedstock for renewable oleochemicals and oil-based biofuels, the low yield of oil per hectare of land by conventional oilseed crops is limiting our ability to scale production.1,2 Fermentation-derived oil may provide an avenue to improve yields to meet expected demands for oil-based biofuels and oleochemicals in a growing circular bioeconomy.3 While high-yielding sugar crops such as sugarcane and sweet sorghum are traditionally fermented to ethanol, they can also be fermented by oleaginous yeast to produce microbial oil (i.e., single-cell oil), mainly in the form of triacylglycerides (TAG) which are suitable for biodiesel production.4,5 Oleaginous yeasts naturally produce large amounts of oils from simple sugars when an essential nutrient such as nitrogen, phosphorous, or sulfur is limiting. *Rhodosporidium toruloides* (i.e., *Rhodotorula toruloides*), a carotenogenic basidiomycetous yeast, can natively produce oils at titers far greater than many other oleaginous microorganisms (e.g., native *R.* *toruloides* can reach 8 g∙L-1 while *Yarrowia lipolytica*, a model organism for oleaginous yeast, natively reaches 4 g∙L-1) and can natively grow on a wide range of sugars, including glucose, xylose, arabinose, and sucrose.6 A preliminary techno-economic analysis (TEA) on the potential for microbial oil production from *R. toruloides* oil estimated a minimum selling price of microbial oil of 4 to 6 USD∙kg-1 and a carbon intensity of 7.2 to 11.6 kg CO2-eq·kg-1 from glucose.7 Another study using sugarcane juice as the feedstock estimated the production cost of microbial oil to be 1.2 USD∙kg-1 with optimistic assumptions for capacity (48,000 MT∙yr-1 of oil), glucose to oil yield (0.25 g∙g-1), and oil recovery (95 %).8,9 Given that the selling price of soybean oil from 2011 to 2021 was 0.62 to 1.5 USD∙kg-1, microbial oil production from low-value, diluted sugars may be economically feasible with further technological development.10 A recent study by Bonturi *et al.* showed that a sugarcane-to-ethanol biorefinery that diverts the hemicellulosic fraction of bagasse towards biodiesel production using *R. toruloides* can have an economically feasible internal rate of return (IRR) of 14.4%.5 However, under the same set of assumptions on feedstock price and processing capacity, a sugarcane-to-ethanol biorefinery (without microbial oil production) would have a higher (and, thus, more favorable) IRR of 22.1% (evaluated using BioSTEAM’s open-source sugarcane biorefinery model).11 Given the magnitude of market driven uncertainties and continuous improvements in the performance of microbial oil technologies, there is a need to better understand the sustainability implications of the research, development, and deployment landscape of microbial oil production technologies, including their potential to advance goals for financial viability and greenhouse gas emission reduction from traditional and cellulosic sugarcane biorefineries.

In addition to sugar-derived microbial oil, another strategy for improving oil production is through the engineering of highly productive C4 crops (e.g., sugarcane, sweet sorghum) to accumulate vegetative oil within plant tissue. Because oleaginous yeast is limited by a maximum theoretical yield of 0.32 g∙g-1 TAG from glucose,12 diverting carbon flux away from plant sugars directly towards oil accumulation may result in greater oil yields per hectare. Recent studies have reported on the creation of oilcane ─ a sugarcane carrying transgenic alleles designed to accumulate TAG in the vegetative tissues ─ that have been demonstrated to achieve up to 4.3 dw % TAG in the stems and 8.0 dw % TAG in the leaves (a total of 5.2 dw % TAG).13 A detailed TEA suggests that biorefineries could be willing to pay 3.24 [−3.91, 16.42] and 11.7 [−1.9, 32.2] USD∙MT-1 more for oilcane than sugarcane with and without conversion of cellulosic biomass to ethanol, respectively.14 However, oilcane lines with higher oil content have demonstrated lower biomass yields, possibly due to the accumulation of cytotoxic free fatty acids (FFA), which constitute 10 wt % of the total oil.15 For example, oilcane lines 1566 and 1580, with stem TAG contents of 1.8% and 5.4 dw %, have dry biomass yields of 69% and 19% relative to traditional sugarcane, respectively.13 Although oilcane may have a greater value than sugarcane per unit mass, trade-offs in biomass yield may undercut the financial viability of oilcane cultivation.14 It is not yet clear what degree of biomass yield loss can be afforded in favor of higher oil contents and where current lines of oilcane stand in relation to these breakeven points.

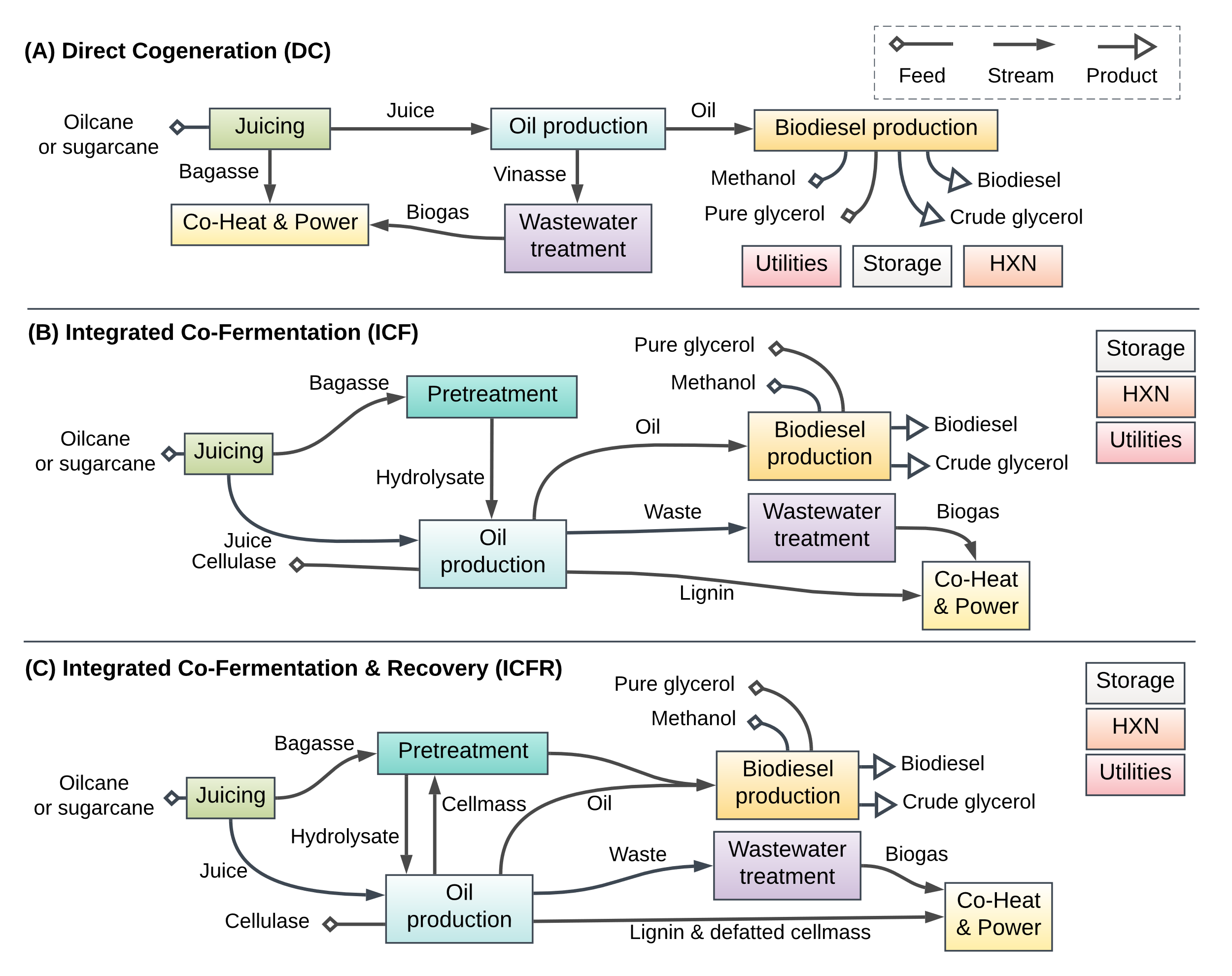
While oil-accumulating crops and microbial oil may appear as competing technologies, integrating microbial oil production within an oilcane and oil-sorghum biorefinery may result in impactful synergies. Due to the capital burden of supporting two biofuel pathways, configurations co-producing biodiesel and ethanol may not be market competitive when oilcane has an oil content below 5% (assuming no losses in biomass yield).14 By converting sugars to microbial oil instead of ethanol, both microbial and vegetative oils can be processed together and converted to biodiesel for better economies of scale. Lastly, the integration of microbial oil also presents opportunities for process intensification in oil recovery from both plant and microbial cells. For example, the high temperature (180°C) and pressure conditions in the liquid hot water pretreatment of bagasse aids the release of up to 70% of the remaining oil within plant cells and can potentially be leveraged to disrupt microbial cells for integrated oil recovery.16 Taken together, integrating microbial oil production at oilcane biorefineries may result in synergies in plant and microbial oil processing for a more sustainable production process.

The objectives of this study were: (i) to characterize the sustainability of various design alternatives for microbial oil production at a sugarcane biorefinery; (ii) to determine the financial viability and carbon intensity of integrated processing of plant and microbial oils and elucidate the most salient features driving sustainability; and, critically, (iii) to establish targets for oilcane and microbial oil market competitiveness. To this end, we developed and evaluated biorefinery models in BioSTEAM —an open-source platform for the design, simulation, and evaluation of biorefineries— for processing sugarcane and oilcane to biodiesel through fermentation-derived oils with and without cellulosic biomass processing (a total of four biofuel pathways). Model calibration was supported by laboratory experiments to characterize oil recovery from co-pretreatment of plant and microbial cells. Each biorefinery was evaluated across a landscape of potential technological performance and market conditions to establish research and development targets for sustainable plant and microbial oil production. In particular, the minimum oilcane biomass yield for market competitiveness (as compared to traditional sugarcane) was elucidated across potential oil contents. Cradle-to-grave greenhouse gas emissions were quantified by leveraging BioSTEAM simulation results coupled with inventory data from the Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation (GREET)17 and ecoinvent 3.718. Three different allocation methods (displacement, economic, and energy) were employed to compare the carbon intensity (reported as 100-year global warming potential, GWP100) among the multi-product biorefinery configurations. The minimum biodiesel selling price (MBSP) was used as the main profitability indicator. The sensitivity of the MBSP and carbon intensity to feedstock oil content, product prices, and other techno-economic parameters were evaluated using Spearman’s rank-order correlation coefficients, facilitated by Monte Carlo uncertainty analysis.

**Materials & Methods**

**Biorefinery Configurations and Process Design**

Three biorefinery configurations for biodiesel production from plant and microbial oils were developed in this study: (i) fermentation of juice and direct cogeneration (DC) of heat and power from bagasse, (ii) integrated co-fermentation (ICF) of both juice and bagasse hydrolysate, and (iii) integrated co-fermentation and recovery (ICFR) of plant and microbial oil (**Figure 1**). [Note: The term *fermentation* is used here in a way that is consistent with chemical engineering literature, which represents microbial processes –including aerobic processes– for substrate conversion to a specific product in a bioreactor.] Both the DC and ICF configurations closely follow the ethanol/biodiesel-based DC and ICF configurations from our previous work, including the mass balances related to vegetative oil recovery.14 However, instead of neglecting the cost of managing the vinasse or using a conventional, resource-intensive wastewater treatment process, the new DC and ICF cane-to-biodiesel configurations implement a high-rate wastewater treatment design calibrated using sugarcane and oilcane wastewaters.19 Both the DC and ICF configurations employ a drum drier and a screw press to mechanically recover the microbial oil, a conventional strategy for oil recovery from algae with an efficiency of 70% (Figure S1A and S1B, respectively, in the Supporting Information, SI).20 The ICFR configuration sends the cell mass to be pretreated together with the bagasse and then recovers the oil by centrifugation (Figure S1C). The microbial oil recovery from hydrothermal pretreatment at a solids loading of 50 w/v % was experimentally determined to be 50 wt %. This value was used as the baseline microbial oil recovery for the ICFR configuration. The impact of oil recovery efficiencies and microbial oil production yields on the final yield of biodiesel were calculated and depicted as Sankey diagrams (**Figure 2**). Details on the experimental procedure and results are available in Section S8 of the SI. All configurations were modeled in BioSTEAM ─the Biorefinery Simulation and Techno-Economic Analysis Modules─ which automates the design, simulation, and techno-economic analysis of thousands of potential scenarios.11,21



**Figure 1.** Simplified flowsheets of the **(A)** direct cogeneration (DC), **(B)** integrated co-fermentation (ICF), and **(C)** integrated co-fermentation and recovery (ICFR) configurations with microbial oil production. In all configurations, either sugarcane or oilcane is crushed to release the juice, the oil in the fermentation effluent is recovered through a 3-phase decanter centrifuge, and a heat exchange network (HXN) integrates heating and cooling of process streams to decrease utility demand. In the DC configuration, the bagasse is sent directly to the boiler to produce heat and power. In the ICF and ICFR configurations, the bagasse is pretreated with liquid hot water, hydrolyzed with cellulases, and co-fermented with the juice. In the DC and ICF configurations, the oil in the cell mass is recovered mechanically with a screw press after drying. In the ICFR, the oil in the cell mass is recovered by centrifugation after cellulosic pretreatment with the bagasse.

A diagram of a diagram of a variety of liquids

Description automatically generated with medium confidence

**Figure 2.** Sankey diagrams of carbon flow (as % C of oilcane line 1566 containing 1.8 dw% oil) of the **(A)** direct cogeneration (DC), **(B)** integrated co-fermentation (ICF), and **(C)** integrated co-fermentation and recovery (ICFR) configurations with microbial oil production. The ICF configuration has a greater biodiesel yield than the ICFR configuration due to greater oil recovery of microbial oils.

**Feedstock Composition and Oil Content**

Previously published biorefinery designs co-producing ethanol and biodiesel were envisioned to process oilcane with oil contents between 5 to 15 dw % to overcome trade-offs in capital investment that stem from the equipment necessary for ethanol and biodiesel production.14,22 The biorefinery configurations presented here are alternative designs based on our previous work that eliminate ethanol production in favor of microbial oil production. Because both plant and microbial oils are processed together for biodiesel production, the new biorefinery configurations can process oilcane at any oil content, including 0 to 5 dw %, without trade-offs in capital investment.

We intentionally varied the oil content in oilcane for uncertainty and sensitivity analyses, specifically examining the impacts of market conditions, design decisions, and potential long-term technological performance. To characterize the implications across the spectrum of observed oil content and yields, we varied oil content uniformly from 1.8 to 5.4 % and biomass yield uniformly from 4.87 to 25.62 dry MT∙ha-1 (the low yield from oilcane 1580 and the high yield from sugarcane).13 We modeled the effect of oil content on the overall composition in manner that was consistent with previously published work.23,24 Briefly, an energy balance approach was used to balance an increase in oil content with a decrease in sucrose content. Because oil is about 2.5 times as energy-dense as sucrose, the loss of biomass from increasing oil content was compensated by fiber. In the uncertainty analysis of oilcane lines at the current state of technology, the biomass yield and the oil, sugar, fiber, and moisture content were sampled from a truncated normal distribution with mean and standard deviation extracted from published data (Table S6, SI).13

**Accessing Biorefinery Models and Detailed Results**

All biorefinery source code and excel results are available in the BioSTEAM Bioindustrial-Park GitHub repository,25 an official repository for complete biorefinery models and results to foster accessibility and deeper communication within the biorefinery simulation community. The repository also includes flowsheets, stream tables, utility requirements, design requirements, itemized costs, cash flow analysis, and reaction stoichiometries for each biorefinery. Detailed results of all oilcane and sugarcane biorefinery configurations are available in the Bioindustrial Park at <https://github.com/BioSTEAMDevelopmentGroup/Bioindustrial-Park/blob/master/biorefineries/cane/README.md>. Note that the full repositories can be downloaded directly from Github without the need to create an account. Alternatively, the biorefinery models can be installed using the command “pip install biorefineries==2.28.0” from your terminal (Mac/Linux) or command line (Windows) with a working Python 3.10 installation. The documentation also features details on how to leverage the model: [https://biosteam.readthe  
docs.io/en/latest/API/biorefineries/cane/index.html](https://biosteam.readthedocs.io/en/latest/API/biorefineries/cane/index.html).

**Thermodynamic Property Package**

The thermodynamic property package estimates phase equilibrium using modified Raoult’s law with activity coefficients estimated through Dortmund UNIFAC interaction parameters. Modified Raoult’s law is suitable to estimate phase equilibria of nonideal mixtures at low to moderate molecular weights and pressures up to 10 atm.26 Therefore, this phase equilibrium model can accurately predict the interactions of the highly polar, low molecular weight compounds present in the biorefinery (e.g., water, methanol, glycerol). The lignocellulosic components are modeled following the pure component property values used by NREL27 (heat of formation, molecular weight, etc.), with the exception that liquid densities and heat capacities for lignocellulosic components are held constant at 25 ⸰C. Pure component properties (heat capacity, density, etc.) of fluids are estimated using higher order polynomial fits to the fundamental Helmholtz equation of state (a state-of-the-art property prediction model),28,29 recommended correlations from critical reviews on thermodynamic properties,30 and equations from well-established public references such as the CRC handbook of chemistry and physics and the NIST Webbook.31 Mixture properties are estimated using a molar weighted average of the pure chemical properties. This property package has been validated against a cellulosic biorefinery model by NREL implemented in Aspen Plus and both sugarcane and oilcane biorefinery models in SuperPro.11

**Aerated Bioreactor Design and Modeling**

Aerated bioreactors, including heat exchangers, compressors, and agitators, constitute a major capital investment and utility expenditure in a bioprocess.32 For example, the cost of the microbial oil bioreactor system alone has been estimated to cost 17.5 million USD at 3x the baseline fermentation productivity considered in this study and at a production capacity comparable to the DC configuration.7 Because the economic design of an aerated bioreactor is critical for financial viability, a full discussion on the design and costing algorithm is warranted.

The production of microbial oil is an aerobic process that can be modeled as the sum of three stoichiometric reactions: oil production from sugar, respiration, and cell growth. The aerobic fermenter is assumed to have a high heat-production rate of 110 kcal per mol of O2 consumed, consistent with published literature.32,33 The nitrogen present is a complex mixture of nucleic acids, protein, and other reduced forms of nitrogen; as such, the conversion of these components to cell mass is neglected. Magnesium sulfate is provided in the bioreactor model to provide Mg and S to support cell growth. These are also excluded from the growth reaction because the Mg and S contribution to the cell mass is minimal. Any substrate not converted to cell mass or oil is assumed to be consumed for respiration. Given the yield of TAG, *Yp* (g∙g-1), and the fraction of biomass produced over the total substrate not consumed for oil production (*Yb*;g∙g-1), the extent of reaction for TAG production (*Xp*),growth (*Xb*), and respiration (*Xr*), can be calculated (Table 1).

**Table 1.** Stoichiometry and reaction extent of microbial oil production, cell growth, and respiration.

|  |  |  |
| --- | --- | --- |
| Reaction | Stoichiometry (by mol) | Reaction extent |
| TAG Production: |  |  |
| aGrowth: |  |  |
| Respiration: |  |  |

a The molecular formula of yeast is assumed to be CH1.61O0.56.34

While fed-batch and draw-fill operation modes lead to higher yields and titers compared to standard batch operation,35 the mode of operation in this study is limited to batch due to the high nitrogen content of the substrates. Nitrogen serves as the limiting nutrient for growth and its absence drives microbes to accumulate oil during the production phase. The C:N ratio (by weight) of the oilcane bagasse hydrolysate and juice were measured to be 40 and 54, respectively, which are far lower than the optimal for the production phase (~120 C:N ratio): as a result, continuous feeding would stop the microbes from entering the production phase.36,37 The starting C:N ratios of the substrates are closer to the optimal starting C:N ratio for batch operation (70).36–38 It may be possible to supplement with hydrolysates from other feedstocks with lower C:N ratios (e.g., wood chips or pure sucrose) to enable other modes of operation, but these were not considered in this study due to the complexity of the design and logistics.

The total power consumed by the fermentation systems was minimized numerically by varying the flow rate of air provided by the compressor and the power consumed by the agitators under the constraint that the oxygen uptake rate is equal to the oxygen transfer rate at a dissolved oxygen concentration of 50% saturation.32,33 The power consumed by the agitator was 0.06 kW∙m-3 under the baseline fermentation performance, which is sensible compared to the heuristic value recommended for industrial homogeneous reactions (0.3 kW∙m-3).32,39,40 The overall mass transfer coefficient, kLa (lumped together with the specific interfacial area), was estimated using Van’t Riet’s non-viscous mass transfer correlation.41 The design procedure accounts for the pressure gradient across the vessel due to the liquid head using the log-mean driving force for mass transfer.33,40

The uncertainty analysis considers a range of titers, productivities, and product yields from the baseline performance on hydrolysate (13.2 g∙L-1 titer, 0.17 g∙L-1∙h-1 productivity, 0.132 g∙g-1 yield)42 to an optimistic batch performance achieved on pure glucose substrate (27.4 g∙L-1 titer, 0.31 g∙L-1∙h-1 productivity, 0.18 g∙g-1yield)43. Multi-effect evaporation of the juice and hydrolysate is used to achieve the required titer in each scenario. At the lower and upper ends of performance, *Yb* does not change significantly (0.39 and 0.42 g∙g-1, respectively). To achieve a continuous design space, we assumed this parameter has an average value of 0.405 g∙g-1. Assuming a yield of 0.132 g∙g-1, the overall stoichiometric reaction on a molar basis for microbial oil production from oilcane hydrolysate becomes:

The maximum volume of each aerated bioreactor was 500 m3, a “world’s largest” class that exists in small numbers.32 The number of vessels is computed based on the influent flow rate, reaction time, and cleaning time (3 h).

**Feedstock Price and Carbon Intensity as a Function of Land Availability and Biomass Yield**

Previous TEAs have assumed the price of oilcane to be the same as the price of sugarcane with identical growth and harvest requirements.22,23 However, the yield per hectare is lower for oilcane lines than for sugarcane and the price of oilcane by mass will be higher.13 The *Sugarcane Production in the Louisiana* worksheet is a decision tool for the estimation of the cost of sugarcane cultivation that incorporates key assumptions for field preparation, planting, harvesting, and other operations.44 This tool has been leveraged for estimating the price of energy cane by adjusting the biomass yield of plant cane and stubble crop (i.e., ratoon) rotations.45 A key assumption embedded in the tool and used in this study is that the cost of farming sugarcane – including field expenses and variable production expenses – per unit land is independent of biomass yield. Assuming the cost of transporting sugarcane has a linear relationship with distance, the cost of transportation per unit biomass was estimated to be proportional to the square root of the total area.46 In the same spirit, the carbon intensity of oilcane production was estimated by summing the contributions from cultivation and transportation using extracted impacts for cultivation and transportation of sugarcane from GREET17. Details on feedstock price and carbon intensity calculations are available in Section S9 (SI).

**Setting Targets for Competitive Biomass Yield**

To inform continued oilcane development, critical targets for biomass yield as a function of oil content were determined to enable oilcane to become market-competitive with sugarcane. These targets represent the competitive biomass yield, defined as the biomass yield required to achieve the same return on investment (ROI) as sugarcane under the same assumptions related to available agricultural land, fuel prices, and technological performance. In each simulation, the competitive biomass yield was solved numerically.

**Life Cycle Assessment (LCA)**

The LCA was performed following the same procedure used in our previous study.14 Briefly, the system boundary extends from cradle to biorefinery-gate for the operational phase and does not include the construction phase of the biorefinery. All operational inventory data originated from the biorefinery models leveraged in this study (Table S7; SI). Life cycle inventory data for each raw material and ancillary input were adapted from the GREET 2020 model17 and the ecoinvent 3.7 life cycle inventory database.18 The life cycle impact assessment methodology used was the Intergovernmental Panel on Climate Change (IPCC) 2013,47 with a focus on GWP100 as the primary indicator (reported herein as carbon intensity). Three different allocation methods were employed to compare carbon intensity across biorefinery configurations: displacement, economic, and energy allocation (a detailed breakdown of the LCA results using each allocation method is provided in Section S4 of the SI). Because the biorefinery configurations presented here mainly produce biofuel, we focus our discussion on results using energy-based allocation of carbon intensity. The only co-product with alternative end uses is crude glycerol. While crude glycerol is produced in significant quantities, the choice of allocation factor under either economic or energy allocation (minimally 1.18% and maximally 4.53%; Tables S9 and S10) does not change the conclusions of this study (Figures S4 A, B, C, and D; SI), in agreement with the low energy content (12 MJ∙kg-1) and low price (0.1 USD∙kg-1)23 of crude glycerol as compared to biodiesel (37 MJ∙kg-1 and 0.50 USD∙kg-1, minimally).

**Techno-Economic Analysis (TEA)**

All capital cost correlations and parameters used in the discounted cash flow analysis follow the assumptions made in our previous study.14 Because all biofuel configuration pathways were found to achieve over 60% reduction in carbon intensity relative to a 2016 petroleum benchmark established by the EPA48 (see the Results and Discussion section), the return on investment included the value of renewable identification numbers (RINs) of biomass-based diesel (i.e., D4 RIN), and cellulosic biofuel (i.e., D3 RIN) pathways following the guidance of 75 FR 14863.43 As specified by 40 CFR 80.1451(b)(1)(ii)(U),49 the fraction of fuel (biodiesel) produced from the planted crop feedstock (sugarcane juice) received D4 RINs while the remaining fraction originating from the crop residue feedstock (bagasse) received D3 RINs. RIN credits were treated as a co-product and decreased the minimum biodiesel selling price (MBSP). A breakdown of the estimated revenue and capital and operating expenditures can be found in Tables S1–S3 and Figure S2 (Section S2; SI).

**Uncertainty and Sensitivity**

A total of 40 economic and technological performance parameters were varied in Monte Carlo simulations (Section S3; SI). Parameter distributions for oil composition, cellulosic pretreatment performance, ethanol production performance, operating days, IRR, co-product prices, and feed prices follow the same assumptions as our previous study.14 Fuel and electricity prices were updated to include the latest market prices from the USDA Economic Research Service,10 the U.S. Energy Information Administration,50 and the U.S. EPA.51 Latin hypercube sampling was used to generate 5,000 shared scenarios which were used to evaluate each oilcane line and configuration. At each oil content, the competitive biomass yield was characterized across 200 scenarios. Halving the number of scenarios resulted in no significant change in uncertainty and sensitivity results. The sensitivity of the MBSP and carbon intensity to each input parameter was characterized by Spearman’s rank-order correlation, a measure of monotonicity between input and output parameters.

Data for each contour plot was generated by evaluating the biorefinery configurations across a spectrum (20 x 20; 400 points) of potential scenarios for technological performance. Parameters not explicitly varied were set to the baseline values (Tables S4 and S5**,** Section S3; SI). A Gaussian filter was applied to contour plots to smooth discontinuities originating from automated discrete design decisions (e.g., heat exchanger network configurations, number of multi-effect evaporators in series).

**Results and Discussion**

**Sustainability of Designs for Microbial Oil Production from Sugarcane**

The ICFR configuration makes use of the high temperature and pressure conditions in the cellulosic pretreatment of bagasse to disrupt the cells and release microbial oil, removing the need for energy and capital-intensive drying and extrusion of the cell mass (which are required in the ICF configuration). Although integrating oil recovery constitutes a process intensification by reducing the number of unit operations, the pretreatment reactor itself is energy and capital-intensive and results in comparable costs under the same assumptions for oil recovery (Figure 3). Considering the lower oil recovery of the ICFR configuration, the MBSP (6.06 USD∙L-1) is substantially higher than the MBSP of the ICF configuration (4.34 USD∙L-1) under baseline assumptions. Other oil recovery strategies such as solvent extraction, supercritical CO2, or enzymatic cell lysis may potentially result in more economical alternatives.52,53 Because the ICFR configuration was less financially viable, we focus only on the DC and ICF configurations in our remaining discussion.

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**Figure 3**. Contour plots of the MBSP with sugarcane as the feedstock, analyzed across potential microbial oil recoveries and fermentation yields for **(A, D)** direct cogeneration, **(B, E)** integrated co-fermentation, and **(C, F)** integrated co-fermentation and recovery. **(A-C)** represent results under baseline productivity (0.17 g∙L-1∙h-1) and titer (13.2 g∙L-1) performance, and **(D-F)** represent results under a potential target productivity (0.31 g∙L-1∙h-1) and titer (27.4 g∙L-1). The orange circles represent the baseline assumptions for oil recovery and fermentation yield and the yellow circles represent a potential target for fermentation performance.

The MBSP from all configurations were sensitive to microbial oil yield and microbial oil recovery, the latter of which was dampened (i.e., made less sensitive) through the burning of unrecovered oil for heat and power cogeneration. The cellulosic configurations (i.e., ICF and ICFR) produce larger quantities of microbial oil and exhibit greater sensitivity to oil recovery than the DC configuration. In addition to oil recovery and microbial yield, the productivity and the maximum achievable titer are also key technological drivers of sustainability (Figures S5 and S6; SI). At the baseline case, which represented conservative assumptions for fermentation performance which have been achieved at lab-scale using oilcane hydrolysate,42 the MBSP of the DC and ICF configurations are 3.50 and 4.34 USD∙L-1, respectively. These results are comparable than the estimated price of microbial-oil-based biodiesel from glucose (3.36 USD∙L-1).54 At the target case, representing potentially feasible achievements which have been demonstrated on glucose feedstocks,43 the MBSP of the DC and ICF configurations (1.99 and 2.23 USD∙L-1, respectively) are still higher than the upper bound of biodiesel market prices (1.11 USD∙L-1)10,51.

Although biodiesel production from microbial oil may not be financially lucrative under these assumptions, it results in greater yields and a lower carbon intensity than biodiesel from soybean oil. Even under uncertainty in potential operating days, microbial oil yields, and oil recoveries, this result holds true for 100 % of evaluated scenarios (**Figure 4**). Specifically, the DC and ICF yields were 1780 [1310, 2360] and 2750 [2050, 3640] L·ha–1·yr–1, respectively, which is 2 to 6 times greater than the yield of biodiesel from soybean oil (617 L·ha–1·yr–1).55 Additionally, DC and ICF had estimated carbon intensities of 0.531 [0.49, 0.575] and 0.659 [0.594, 0.804] kg·CO2e·L–1, approximately half that of conventional biodiesel from soybean oil (1.29 kg·CO2e·L–1)17. Biofuel policy incentives do not yet distinguish between more sustainable oil-based biofuel pathways and conventional oil seed pathways. Under EPA guidelines,49 the fraction of the microbial oil produced from non-cellulosic sugars qualifies for biomass-based diesel RIN (D4; which is also credited to soybean-based biodiesel). With further validation of the modeled land usage and greenhouse gas benefits from our study, policy makers may aim to incentivize fuel production from microbial oil over conventional oil seeds to spur additional investment and scale-up.

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**Figure 4.** Kernel density scatter plot of the biodiesel yield and carbon intensity under energy allocation for the **(A)** DC and **(B)** ICF biorefinery configurations processing sugarcane and producing microbial oil. Points in regions with higher probability are bright yellow, while points in regions with lower probability are dark purple. The box and whisker plots of the biodiesel yield (blue) and the carbon intensity (yellow) represent median values (dark, solid lines), 25th to 75th percentiles (shaded region), 5th and 95th percentiles (whiskers), and minimum and maximum values (triangles). The grey horizontal and vertical lines represent the biodiesel yield and carbon intensity of biodiesel production from soybean oil. Thus, relative to biodiesel production from soybean oil, points above the horizontal grey line achieved higher biodiesel yields and points to the left of the vertical grey line had lower carbon intensities.

**Financial and Environmental Impact of Integrated Plant and Microbial Oil Processing**

Across all parameters, both the economic and environmental sustainability of oilcane is most sensitive to the dry biomass yield (Figure S5 and S6; SI). Although increasing oil content at the expense of a modest reduction in biomass yield can result in a net financial benefit, the best performing oilcane line (balancing oil content and biomass yield; Oilcane 1566) and the oilcane line with the highest oil content (Oilcane 1580) have such large reductions in dry biomass yield (relative to sugarcane) that they have similar or greater MBSPs and carbon intensities compared to sugarcane (**Figure 5**). The reduced biomass yield of current oilcane prototypes is a result of high constitutive expression of lipogenic genes WRI and DGAT, which lead to either elevated levels of toxic free fatty acids (caused by high WRI expression) or depletion of phosphatidylglycerol needed for thylakoid membranes (caused by high DGAT expression).13 Higher biomass yields and lipid contents may be achieved if alternative regulatory elements are implemented to fine-tune the spatial and temporal expression of lipogenic genes.56 Research in the development of oil-accumulating feedstocks may direct efforts towards regulating lipogenic gene expression to sustain both high biomass yield and oil contents. In addition to oilcane, researchers are currently developing new lines of oil-accumulating sweet-sorghum57 (up to 3 dw % oil in stems) which have not exhibited growth penalties (field trials still on-going) and oil-accumulating energycane (up to 1.5 dw % TAG in leaves)58–60 which natively have greater biomass yield than sugarcane. Once field trials are completed for these new feedstocks, future assessments may seek to evaluate their sustainability for the production of biofuel and bioproducts.

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**Figure 5**. Contour plots of the impact of feedstock oil content and biomass yield on **(A)** the MBSP of the direct cogeneration (DC) configuration, **(B)** the MBSP of the integrated co-fermentation (ICF) configuration, **(C)** carbon intensity of the DC configuration, **(D)** carbon intensity of the ICF configuration. The orange, blue, and purple circles represent cane lines WT, 1566, and 1580, respectively. While increasing oil content has the benefit of decreasing the MBSP and carbon intensity, current lines of oilcane have greater MBSP and carbon intensity than sugarcane due to significantly lower biomass yields.

**Oilcane Yield Targets for Plant Oil Competitiveness with Microbial Oil**

Given that vegetative oil is 1.9 times as carbon-dense as glucose per unit mass, we can expect that producing biodiesel directly from an oil crop can afford reductions in biomass yield compared to a process producing biodiesel from a sugar crop via fermentation. To better inform research and development on critical targets for biomass yield as a function of oil content, we determined the competitive biomass yield under uncertainty across potential feedstock oil contents (**Figure 6**). Under assumptions for the best theoretical performance of microbial oil production (i.e., 100% of glucose carbon converted to microbial oil, minimal fermentation costs, and a microbial oil recovery as high as plant oil recovery), a 1% increase in oilcane oil content can be accompanied by (on average) a 1.21 % reduction in biomass yield (relative to the baseline yield of sugarcane) to achieve equivalent profitability. In reality, due to the need for cell growth and respiration (not all carbon is converted to oil) and the significant costs of aerated bioreactor conditions in microbial oil production, the actual affordable loss in oilcane biomass yield can be much greater. While the biomass yield for oilcane 1566 (17.7 DMT∙ha-1∙y-1) is 31 % lower than the sugarcane wildtype, this biomass yield is just 0.56 % lower than the 5th percentile of the competitive biomass yield (21.6 [17.8, 22.6] DMT∙ha-1∙y-1 in the ICF configuration; **Figure 6B**). Therefore, reasonable improvement in either biomass yield or oil content may enable this oilcane line to present economic advantages over sugarcane at a biorefinery producing microbial oil. On the other hand, the oilcane line with the highest oil content (oilcane 1580) has a biomass yield (4.9 DMT∙ha-1∙y-1) that is 62.0 % lower than the 5th percentile of the competitive yield (16.0 [12.9, 18.6] % in the ICF configuration). At such a low biomass yield, only improving the biomass yield may result in financial competitiveness.

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**Figure 6**. Target and observed biomass yield versus oil content for processing via **(A)** the direct cogeneration configuration and **(B)** the integrated co-fermentation configuration of the microbial oil biorefineries. The green lines and shaded regions represent target biomass yield for market competitiveness under uncertainty. Results are presented with median values (dark, solid lines), 25th to 75th percentiles (shaded region), and 5th and 95th percentiles (dash-dotted lines). The orange, blue, purple, and green circles represent cane lines WT, 1566, 1580, and proposed target, respectively. The dashed grey line represents the theoretical maximum competitive target under the assumption that microbial oil production can achieve the highest theoretical performance. Oilcane 1566 (as labeled in the figure) has a biomass yield comparable to the 5th percentile of the market competitive yield.

Assuming a target of engineering highly productive C4 crops with 10 dw % oil content by 2027, we propose to establish a lower limit in biomass yield to conservatively dictate whether the engineered oil crop is more financially viable than its non-engineered counterpart. A prospective “target” oilcane line could have an oil content of 10 dw % and biomass yield of 18.0 DMT∙ha-1∙y-1 (the maximum competitive biomass yield in the DC configuration; **Figure 6A**). The target oilcane line would result in biodiesel yields of 247 [-305, 754] and 559 [-162, 1187] L∙ha-1∙y-1 greater than sugarcane as well ascapital investments of 255 [125, 435] and 286 [100, 523] 106∙USD lower than sugarcane for the DC and ICF configurations, respectively (Figure S3, SI). Additionally, the target oilcane line would result in MBSPs (1.48 [1.0, 2.05] and 1.7 [1.25, 2.22] USD∙L-1 for the DC and ICF configurations, respectively) that are generally higher than the upper limit of the market price range (0.45 –1.11 USD∙L-1)10,51.

**Critical Targets for Integrated Plant and Microbial Oil Biorefineries**

The oil content and yield of oilcane lines along with the titer, productivity, and yield of microbial oil production are the main economic bottlenecks that require improvements to achieve market competitiveness. Greater oil recoveries would also be impactful. However, it would require further research and development of new oil recovery configurations (e.g., supercritical CO2 and solvent extraction) to assess trade-offs in capital and operating expenditures. For this reason, we focus our discussion on the implications of the fermentation performance and feedstock development targets set forth in this study. Future studies may seek to perform detailed analyses on alternative oil recovery methods.

We evaluated the impact of stepwise improvements to feedstock and fermentation technologies for the DC configuration along four key indicators of sustainability: MBSP, total capital investment (TCI), carbon intensity, and biodiesel yield (**Figure 7**). The MBSP serves as an indicator for profitability while the TCI serves as an indirect measure of financial feasibility due to the inherent difficulties of financing capital-intensive projects with emerging technologies. We focus our discussion on the DC configuration because not only is it the most financially viable, but it also has a lower carbon intensity than the ICF configuration (Figure S9; SI). Taking oilcane 1566 and the demonstrated oilcane fermentation performance as the baseline, all scenarios at the current state of technology have MBSPs significantly above the market price range (**Figure 7A**). Evaluated improvements in both fermentation technologies and feedstock oil content and yield would reduce the MBSP such that 20% of evaluated scenarios lie within the market price range of biodiesel (**Figure 7B-C**). Further improvements to the biomass yield (up to 25.6 DMT∙ha-1∙y-1; equivalent yield to sugarcane) would bring down the MBSP such that 87% of evaluated scenarios fall within or below the market price range (**Figure 7D**). Therefore, assuming key improvements to oilcane biomass yield and oilcane fermentation, biorefineries with integrated plant and microbial oil processing can be market competitive at 10 dw % oil content. The TCI decreases with improvements to fermentation performance and cane oil content (**Figure 7A-C**) and increases with cane biomass yield under the assumption of constant land availability (as expected from greater feedstock processing capacity; **Figure 7D**). Regardless of technological improvements, the TCI remains 3 – 7 times as large as the TCI of soybean biodiesel (83.9 106∙USD; **Figures 7A-D**).

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**Figure 7**. Kernel density scatter plots of the DC configuration’s **(A-D)** MBSP and TCI and **(E-H)** biodiesel yield and carbon intensity for **(A, E)** oilcane 1566 under baseline assumptions on fermentation performance (0.17 g∙L-1∙h-1 productivity, 13.2 g∙L-1 titer, 13.2 g∙g-1 yield), **(B, F)** oilcane 1566 under improved fermentation assumptions (0.31 g∙L-1∙h-1 productivity, 27.4 g∙L-1 titer, 18.0 g∙g-1 yield), **(C, G)** proposedoilcane target (10 dw % oil content and 18.0 DMT∙ha-1∙y-1 biomass yield) under improved fermentation assumptions, and **(D, H)** oilcane at 10 dw % with no reduction in biomass yield compared to sugarcane (25.6 DMT∙ha-1∙y-1) under improved fermentation assumptions. Points in regions with higher probability are bright yellow, while points in regions with lower probability are dark purple. The box and whisker plots of the biodiesel yield (blue) and the carbon intensity (yellow) represent median values (dark, solid lines), 25th to 75th percentiles (shaded region), 5th and 95th percentiles (whiskers), and minimum and maximum values (triangles).

Compared to conventional oil seeds, integrated microbial and vegetative oil pathways have decreased land usage and lower carbon intensity even at conservative assumptions for feedstock and fermentation technologies (**Figure 7E**). As fermentation technologies mature and greater feedstock oil content and biomass yields are achieved, both the carbon intensity and the land usage can decrease significantly (**Figures 7E-H**), resulting in a carbon intensity of 0.51 [0.472, 0.55] kg·CO2e·L–1 and a biodiesel yield of 2140 [1870, 2410] L·ha–1·yr–1 for the scenario with improved fermentation and the target oilcane (**Figure 7G**). Compared to biofuel production from soybean, this outcome is equivalent to 3.0 to 3.9 as much biofuel per hectare of land and a 57 to 63% reduction in carbon intensity. If biofuel policy would incentivize the land usage and environmental benefits of integrated microbial and vegetative oil-based biofuel pathways (as compared to conventional oil seed pathways), a greater fraction of scenarios would readily fall within the market price range of biodiesel. Furthermore, these policy incentives could be implemented as tax exemptions and deductions to reduce the capital investment and, in turn, lower the economic barrier for deployment.

The integrated plant and microbial oil processing pathway developed in this study (available as installable open-source applications in Python) could be modified to produce a wide range of oleochemicals (e.g., thermoset plastics through ozonolysis and plasticizers through epoxidation)61 and biofuels (e.g., green diesel and sustainable aviation fuel) with lower environmental impact and land usage than soybean oil-based pathways. Biomass-based pathways are critical for achieving the Sustainable Aviation Fuel (SAF) Grand Challenge to supply 3 billion gallons of SAF per year by 2030 and replace 100% of conventional jet fuel by 2050.62,63 However, the use of feedstocks such as corn (through the alcohol-to-jet pathway) and soybean (through hydroprocessed esters and fatty acids) for SAF production is linked with high land use expansion that limits our ability to scale.64 The configurations for plant and microbial oil processing developed in this study may provide an avenue for even greener and more scalable biomass-based SAF. Further techno-economic and life cycle assessment studies are needed to quantify the potential benefits of plant/microbial oil pathways to oil-based biofuels and oleochemicals other than biodiesel.

**Conclusion**

Microbial oil-based biodiesel production at a sugarcane biorefinery leads to half the carbon intensity of soybean oil-derived biodiesel and 2-6 times greater biodiesel yield per unit land. While microbial oil production can be more environmentally sustainable than traditional vegetable oil from oil seeds, it faces challenges in financial viability with all evaluated scenarios having MBSPs above the incentivized market price range. Further research and development in microbial oils may seek to improve the fermentation performance and evaluate other oleochemical and oil-based biofuel pathways from microbial oil that may be more lucrative than biodiesel production.

While integrating recovery of both plant and microbial oil through cellulosic pretreatment constitutes a process intensification by reducing the number of unit operations, the additional utility and capital expenses from a larger pretreatment reactor results in comparable costs to traditional mechanical oil recovery. Other oil recovery strategies such as solvent extraction, supercritical CO2, or enzymatic cell lysis may potentially result in more economic alternatives.

Due to trade-offs in biomass yield with increasing feedstock oil content, current oilcane prototypes were estimated to have MBSPs and carbon intensities higher than sugarcane. With reasonable improvements in oil content and sustained biomass yield (relative to sugarcane), however, combined processing of plant oils with microbial oil would result in greater economic and environmental sustainability. A biomass yield of 18.0 DMT∙ha-1∙y-1 (i.e., a loss in biomass yield of 30 % relative to sugarcane) at 10 dw % oil content was established as the minimum biomass yield needed to guarantee both greater financial viability and biofuel yield than sugarcane-based microbial oil. This target would also decrease MBSPs to be close to or within the market price range of conventional biodiesel. With additional incentives that support decreased land use and environmental impact, a future oilcane biorefinery may be financially viable with the proposed improvements to biomass yield, oil content, and fermentation performance. Ultimately, this study characterized how further development of oil-accumulating C4 feedstocks and microbial oil technologies would enable the wider production of oleochemicals and oil-based biofuels.

**Associated Content**

**Supporting Information.** The Supporting Information is available online with the published manuscript, including:

Detailed overview of the biorefinery configurations and assumptions, detailed breakdown of biorefinery capital and operating expenditures, guidelines to access biorefinery models, assumptions on input parameter distributions for uncertainty and sensitivity analyses, detailed breakdown of LCA results, uncertainty and sensitivity results, and supplementary description of experimental materials and methods.

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