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Production of Advanced Products from *Sargassum* through Integrated Conversion

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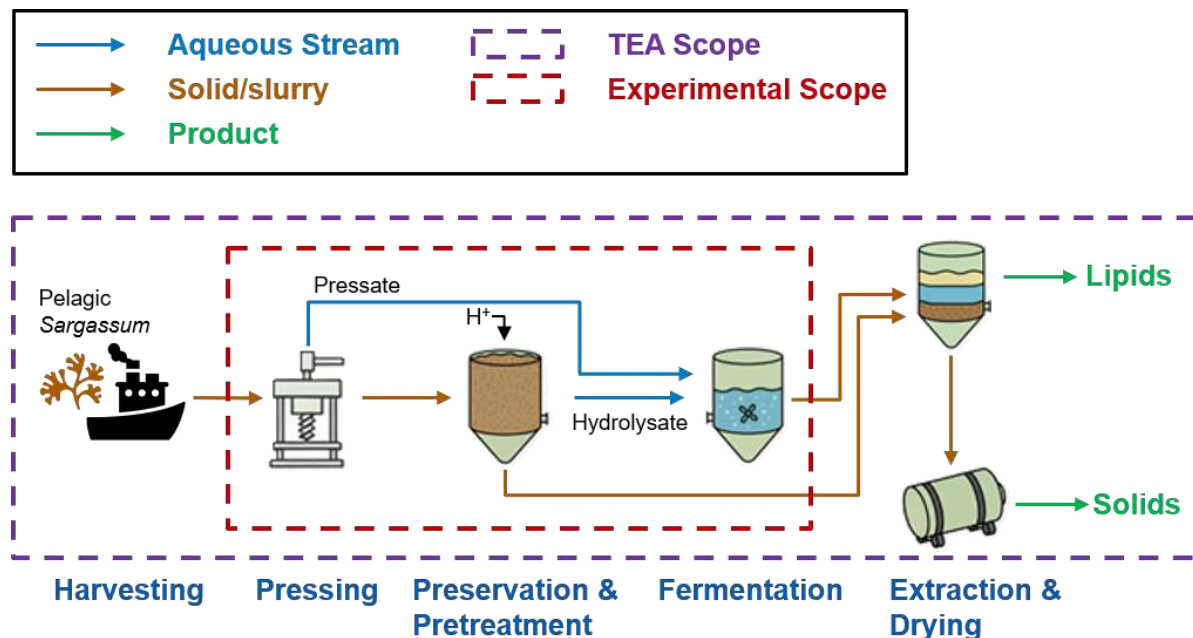
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TOC Graphic



Abstract

Sargassum biomass, a brown macroalgae species whose proliferation in the Atlantic and Caribbean has caused significant environmental and economic challenges, is rapidly

becoming a bioeconomy opportunity through the abundance of biomass. The unique chemical composition and functionalized structural polymers (e.g. alginates and fucoidan polysaccharides) differ significantly from typical biomass sources, requiring custom and sequential conversion processes. We establish here an integrated process and techno-economic analysis for processing *Sargassum* biomass, through hydrolysis into monomeric constituents for biological conversion. Extensive mass and energy balance analysis shows that the valorization of residual solids is a prerequisite for economic viability, and key cost drivers include process scale, feedstock price, preservation, and acid concentration for effective release of organics and fermentable sugars. The recalcitrant nature of the polymers presents unique challenges to this feedstock, necessitating a custom approach to maximize the value recovery from the biomass. We demonstrate release of over 50% of the entrained saccharides into a fermentable sugar stream, enabling downstream conversion by halophilic oleaginous yeast into lipids for oleochemicals or fuels. Optimizing each stage, from preservation and pretreatment to biocatalysis and residue utilization, is critical to transforming *Sargassum* from a coastal nuisance into a valuable resource for biomaterials and bioenergy production.

1. Background

Sargassum is a brown macroalgal species that has bloomed in the tropical northern Atlantic, including the Caribbean Sea and wider Gulf region, with increasing abundance in recent years.¹ The estimated biomass appearing annually in the Great Atlantic *Sargassum* Belt is over 20 million tonnes and potentially up to 38 million tonnes,² and estimates of beach-cast *Sargassum* are several million tonnes in Puerto Rico alone. This inundation of coastal communities causes significant economic and environmental impacts, reduced access to beaches and ports, and lowers yields for fisheries in Caribbean coastal communities. Nuisance beached *Sargassum* has spurred investments into operations to clean and remove *Sargassum* at an estimated ~\$250k per mile of shoreline per year in direct disposal costs (but much less if collected before it washes ashore)³⁻⁵ to the local municipality or landowners. The estimated cost impacts of *Sargassum* blooms on local economies on shorelines of Florida, Puerto Rico, and the U.S. Virgin islands sum to a total of \$7.6 B per year,⁶ not including impacts in Mexico or other Caribbean islands which also have significant issues with *Sargassum* cleanup.^{7,8} Coastal communities bear these substantial costs purely as waste management burdens with no added value, creating a clear opportunity to recover useful energy products from the biomass while alleviating a persistent burden on coastal regions.

While the large volumes of *Sargassum* from the tropical Atlantic Ocean present a huge challenge for coastal communities, they are also promising as a biomass resource. The abundance of biomass represents a significant opportunity for advanced chemicals and fuels, holding an estimated 6.0-11.4 million tonnes of carbon annually.^{2,9} An analysis by Marx et al. indicated that *Sargassum* could be harvested in the Atlantic ocean at scales of up to 6.3 million wet tonnes per year (nearly 1 million dry tonnes/year at a solids content of 14.3%).¹⁰ Despite this apparent abundance, large-scale harvesting faces logistical and ecological constraints. Blooms are unpredictable, varying in space, time, and volume, making the establishment of a permanent solution challenging. Additionally, environmental regulations tied to the potential for disturbing ecosystems present hurdles to large scale harvesting.¹¹ Alternatively, biomass can be harvested after washing ashore; however, collecting beach-cast *Sargassum* yields lower volumes and also introduces contamination in the biomass, limiting its utility for centralized conversion facilities.¹² Many anticipated commercial uses of *Sargassum* require a thorough understanding of the biomass composition and quality and how the material changes after arriving on beaches or in transit.¹³

A variety of product applications for the biomass have been identified, spanning across industries for bioenergy, food, agriculture, bioremediation, pharmaceuticals, and biomaterials.¹² These products may result from extraction of individual constituents (e.g., alginates, fucoidans, pigments), biological conversion of carbohydrates (e.g., ethanol, biogas, lipids, bioplastic precursors), or valorization of residual solids (e.g., biomaterials, bioremediation, fertilizer, or biochar).

Among the available biomass conversion strategies, developing strategies to maximize the recovery of monomeric sugars remains an attractive proposition to valorize the otherwise waste biomass. One of the most widely studied approaches because of its operational simplicity and relatively low capital cost is acid hydrolysis, where strong acids such as H_2SO_4 and HCl have been the most extensively used for seaweed hydrolysis.¹⁴⁻¹⁷ Acid pretreatment effectively hydrolyzes storage polysaccharides such as laminarin while partially disrupting seaweed cell wall structures, thereby improving sugar accessibility while also offering potential for reduction heavy metals present in the biomass.¹² Several studies have adopted a sequential acid pretreatment and enzymatic hydrolysis strategy to enhance monomeric sugar release from *Sargassum*,¹⁸⁻²⁰ with thermal acid hydrolysis further improving yields.^{21,22} Additionally, emerging strategies have also been explored for *Sargassum*, such as a mild pretreatment approach involving redox-assisted processing conducted by He et al. which showed improved yields compared to conventional pretreatment.²³

Microbial conversion has proven promising in conversion of hydrolyzed saccharides, including limited work on the fermentation or bioconversion of *Sargassum* to products such as bioactive components,²⁴ ethanol,^{20–23} butanol,¹⁷ polyhydroxybutyrate,¹⁸ or biogas.²⁵ Lipid production via oleaginous yeasts offers a promising bioconversion route due to their capacity to utilize diverse carbon sources;²⁶ however, the utilization of *Sargassum* presents significant metabolic hurdles to this process. Beyond its diverse sugar profile, *Sargassum* accumulates high ash and salt concentrations, which can inhibit yeast metabolism and important hydrolytic enzymatic activities. Lipid conversion from *Sargassum* sugars has been demonstrated with solid-state fermentation using *Yarrowia lipolytica*.²⁷ This process was used to both hydrolyze and release the saccharides to immediately produce lipids, with highest activity demonstrated on residual biomass after alginate extraction, reaching titers of 0.35 g/L lipids from a total sugar (monomeric and oligomeric) titer of around 7 g/L. The activity on whole biomass after various treatments proved to be much more challenging, indicating recalcitrance of the biomass. There are no other published examples of using oleaginous yeast with *Sargassum*-derived monosaccharides, underscoring the need for bioconversion research, and in particular organisms capable of metabolizing complex monomeric and oligomeric saccharides in a halophilic environment, while funneling this metabolism to valuable co-products.

Techno-economic analyses of various *Sargassum* biorefinery concepts have been published, often employing recovery of a high-value product (e.g., alginate, fucoidan, pigments, or phenols) to demonstrate economic viability^{12,28–31} alongside commodity products such as fuels. For example, Dussan et al. presented an approach of extracting high-value alginate and fucoidan prior to anaerobic digestion to biomethane,³¹ while Thompson et al. showed that viability of electricity generation via anaerobic digestion was challenging unless also selling residual solids into high-value international fertilizer markets.²⁸ Marx et al. presented a unique exception, outlining a hydrothermal liquefaction-based approach which did not employ high value coproducts, albeit at higher biorefinery scales than are considered here. Across all examples, no dedicated report is available on tracking the specific *Sargassum* saccharide utilization through an integrated conversion process accompanied and informed by a thermodynamically rigorous techno-economic analysis (TEA).

To address these knowledge gaps, we present the first systematic evaluation of integrated *Sargassum* biorefining that demonstrates (1) reliable biomass storing methods, (2) an pretreatment approach that is scalable and applicable to continuous processing in a biorefinery setting, and (3) continuous biological conversion of biomass into lipid intermediate precursors, while (4) developing a rigorous techno-economic framework for the conversion to fuels and products in a commercial-scale biorefinery.

2. Methods

2.1: *Sargassum* biomass sourcing

Sargassum biomass (~110 kg) was collected in Puerto Rico along the Southern coast, during an inundation event in late summer of 2023, frozen and shipped to NLR. After the thawed biomass was thoroughly mixed, representative samples were collected for compositional analysis and solids assessment. The thawed biomass was processed with a screw press (Vincent Corporation, FL), set at 15 psi (103 kPa) or 40 psi (276 kPa), generating a pressate (liquid product generated during pressing operations of wet material) and residual solids. A total of 34 kg of wet material was processed, generating 14.7 kg of residual solids and 18.5 kg of liquid pressate.

2.2: Biomass compositional analysis

All compositional analyses were performed on lyophilized biomass derived from representative samples of the large shipment described above, following previously documented and published procedures summarized here briefly.^{32–35} For moisture content, an aliquot of the sample was dried at 40 °C for 2 days and a percent moisture was determined.³⁶ All subsequent values were determined on a moisture-free (dry weight) basis. Dry oxidation (ashing) overnight at 575°C was performed on the oven dried sample to determine the ash content of the biomass.³⁶ Protein content of the biomass was calculated based on elemental nitrogen content determined via combustion CHN-analysis, and multiplied by 4.92 as a macro-algae specific N-to-protein conversion factor.³⁷ For carbohydrate content, a two-step sulfuric acid hydrolysis was used to hydrolyze the polymeric forms of carbohydrates in whole biomass into monomeric subunits in a hydrolysate. The monomers in the analytical hydrolysate and in the pressate liquid were then quantified by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD).^{34,35} The separation and detection method used to characterize the biomass follows a minor deviation from the published procedure; after hydrolysis, samples were filtered through 0.2 µm nylon filters. Additional details are provided in the Supplemental Information.

2.3: Screw press biomass densification and particle size reduction

A total of approximately 110 kg of frozen, wet *Sargassum* feedstock was divided into two groups: 46 kg were kept frozen to serve as a control, while the remaining 64 kg were thawed overnight and subsequently dewatered using a Vincent screw press. Two different pressures were applied to the screw tip, resulting in two distinct dewatered *Sargassum* samples and two distinct pressate liquids.

Dewatering and particle size reduction was completed via a continuous dewatering Vincent screw press. The press operates by a screw rotating inside a stainless-steel screen and forcing the liquid through pores. The screw has a graduated pitch with interrupted flighting to maximize dewatering and prevent co-rotation when paired with resistor teeth. The pressed cake moisture content is controlled by a discharge cone, actuated by an air cylinder providing an additional level of dewatering control. The air cylinder's pressure is controlled manually and can be set to pressures up to 40 psi. The pressate liquid is collected separately from the pressed solids and analyzed for monomeric saccharides as described in 2.2.

Following processing in the screw press, optional secondary particle size reduction was completed via a 12" mechanical disk refiner. The disk refiner is equipped with a 5 hp motor and type C2976 NH plates on the stationary and rotating disks operating continuously. Motor speed is controlled via variable frequency drive, and the gap is manipulated manually. The clearance between the rotating and stationary disks can be adjusted based on the output material property requirements of the refining process. A 0.03-0.05 mm gap size was selected for *Sargassum* processing. Approximately 2 kg of wet solids were processed through the continuous disk refiner at 50 Hz motor speed and recovered at the outlet. No liquid stream was generated during disk refiner operation; however, some liquid was lost to vaporization as the disk refiner generates significant heat during operation.

2.4: Preservation at small scale

Triplicate sterile 50 ml plastic syringes were packed with 10-15 g 40 psi pressed 2x washed *Sargassum* biomass treated with 3% (dry weight basis) 72% H₂SO₄, adding 5 ml of deionized water per syringe to facilitate acid integration. To create an anaerobic environment, the material in the syringe was pressed with the plunger to complete compaction and sealed with a Luer-Lok cap. Syringes were incubated in a dark 30°C incubator and sacrificially sampled at T₀ and three months incubation. The entire syringe was lyophilized for carbohydrate composition.

2.5: Pretreatment optimization: time, temperature, and acid concentration

Small-scale microwave pretreatment experiments were executed in sealed 10 mL microwave pressure vials with self-sealing septa. A 20-point parameter optimization sweep was conducted on pressed *Sargassum* biomass (Table S-1). A microwave digester (CEM, Discover) was used to screen pretreatment conditions. A response surface, Central Composite Design (CCD) with three factors (time, temperature, and acid loading) featured factorial, axial, and replicated center points to provide insight into the behavior of the novel feed while limiting the burden on experimental set. Agitation was provided by stir bars to ensure homogenous heating and prevent the formation of any hot spots during hydrolysis.

Sulfuric acid (10% w/w) was added to the reaction according to the experimental design and well-mixed prior to processing. Following the 20-point CCD, three reaction conditions were down-selected for confirmation of pretreatment conditions (Table S-2).

Larger-scale pretreatment was conducted in a 2-gal stirred tank Parr Series 4552 Floor Stand Reactor with temperature control via direct high-pressure steam injection. Reactants were added into a 1.75-gal Hastelloy insert and placed in the Parr reactor at 100 rpm. High pressure steam was added to reach the desired temperature set point and held at temp +/- 2°C for the desired residence. Steam was vented and the pretreated slurry was quenched in an ice bath. Solid-liquid-separation was conducted first through a cotton fabric for a coarse filtration followed by vacuum filtration through a 0.2-micron filter to provide clarified liquor for fermentation.

2.6: Bioconversion

Cutaneotrichosporon oleaginosum (ATCC 20509) was used for bioconversion experiments due to its wide substrate profile and superior lipid production.³⁸ Bioreactor experiments were performed in Sartorius Q-plus, 0.5 L bioreactor, outfitted with a MidGee, 0.2 µm, hollow fiber filter (Cytiva Cat# CFP-2-E-MM01A). Once inoculated to an initial OD of 0.8, the reactor was run in a discontinuous, fed-batch mode. Briefly, once carbohydrates were consumed, the reactor broth was circulated through the hollow fiber filter at approx. 30 ml/min with trans membrane pressure maintained via a needle valve at approximately 3 psi (21 kPa). Approximately 2/3rd of the spent media was expelled as permeate, while cells were retained in the reactor. Fresh media of the same volume as the permeate was then fed to the reactor and the batch reaction continued.

The substrate for this work was comprised of 75% by volume clarified acid hydrolysate and 25% by volume pressate. In the clarified hydrolysate, glucose was supplemented to 3.5 g/l to represent anticipated levels of glucose released via enzyme treatment currently being researched. This was then pH adjusted to pH 6.5 with solid Ca(OH)₂ and subsequently sterile filtered to remove excess Ca(OH)₂ and CaSO₄ formed during neutralization. Finally, this was combined with 25% volume sterile filtered pressate (containing 12 g/L mannitol).

2.7: Techno-economic analysis

Techno-economic analysis (TEA) was carried out consistent with approaches taken in previous assessments of microalgae conversion;^{39,40} a process model of a commercial scale conversion facility, built in Aspen Plus, is used to generate a mass and energy balance of the process, which informs capital and operating costs in a discounted cash flow rate of return (DCFROR) analysis. The DCFROR determines the minimum selling price of the primary product required to maintain a 10% internal rate of return over the lifetime of facility. Here, we designate the residual solid coproduct as the primary product, as it is the

main revenue driver, and use the minimum solid selling price (MSSP) as the main TEA metric. A list of financial assumptions used in the DCFROR is included in Table S-3.

2.7.1: Feedstock Availability and Cost

The base case in this analysis assumes that biomass is harvested by dedicated ocean vessels, allowing for adequate collection scales and lower costs compared to off-shore farming. *Sargassum* is harvested from the floating mats within 40 km of shorelines. A feed scale of 3.0 million wet tonnes per year (1,306 dry tonnes/day) is assumed for the TEA, approximately half of the scale assumed by Marx et al. for ocean-harvested *Sargassum*,¹⁰ though we emphasize that feedstock availability is a key uncertainty for the analysis.

The harvesting cost of biomass is equally uncertain and depends highly on the distance boats must travel to the bloom. We have estimated that pelagic biomass could be harvested at \$195/dry tonne (see additional details in the Supplemental Information). Other colleagues at Princeton University have estimated lower costs for a floating facility, on the order of \$5/wet tonne (\$35/dry tonne).⁴¹ This estimate has not yet been published in peer-reviewed form; we report it here as an illustrative lower bound pending independent verification. Alternatively, oceanic harvest of *Sargassum* near beach communities could warrant a credit in scenarios where harvest of oceanic *Sargassum* reduces the cost burden of beach cleanup. Here, we assume a biomass cost of \$195/tonne (delivered to the dock) and add a ground transportation cost of \$58/tonne, calculated based on a trucking cost of \$3.43 per truck-mile,⁴² a truck volume of 100 cubic yards (76.5 m³),⁴³ and an average transportation distance of 50 miles (80.5 km); resulting in a feedstock cost of \$253/tonne delivered to the conversion facility. Given the wide range in estimates for feedstock availability and cost, these are further investigated in the sensitivity analysis section.

2.7.2: Seasonality and Preservation

Regardless of the harvesting method, *Sargassum* availability is seasonally variable due to fluctuations in temperature and solar intensity. In addition to seasonal variability, there is significant monthly variability in the amount of biomass that is available for processing, likely due to weather patterns in the regions that are prone to *Sargassum* blooms. The ratio of the maximum monthly rate to the average annual rate in Puerto Rico ranged from 2.9 to 12 during the years 2011-2023 (mean = 4.85, median = 4.43; see Figure S-1).⁴⁴ Seasonal variability increases the capital cost burden of a biorefinery, as conversion operations must be sized for peak feedstock availability, leading to underutilization of equipment during periods of lower biomass availability. Alternatively, biomass can be stored throughout the year to mitigate seasonal variability. Here, we have included biomass diversion and preservation with sulfuric acid in peak months to ensure a consistent flow of biomass throughout the year. Biomass flow by month is modeled according to the average annual

availability profile from data referenced above.⁴⁴ Storage occurs after pressing, so that the pressate is always utilized immediately, while solids may be diverted to storage in peak months. A dry matter loss (DML) of 5% is assumed, a conservative assumption consistent with experimental observations.

2.7.3: Biomass Conversion Approach

The biomass conversion approach represented in Figure 1 was selected based on the experimental results presented here. A summary of the assumptions used in the process model is presented in Table S-4. These assumptions are generally reflective of targeted performance which has been deemed to be reasonably achievable with optimization but has not been demonstrated experimentally in an integrated manner. Where applicable, the demonstrated experimental parameters are also included in Table S-4.

The conversion approach begins with an initial densification step via screw press, which yields an aqueous pressate containing soluble sugars and uronic acids (sent directly to bioconversion), and residual solids at 20% solids (experimental work achieved higher solids content on the order of 24%; however, we have modeled a lower value of 20% here to ensure pumpability). Following the screw press, solids are washed and a portion of the solids may be diverted to seasonal storage to mitigate the seasonality of *Sargassum* blooms, depending on the time of year. In preservation, biomass is subjected to a sulfuric acid loading of 0.03 w/w dry biomass, assumed to contribute to the total acid loading of 0.2 w/w in pretreatment following storage. In seasons with low *Sargassum* availability, biomass is withdrawn from storage to maintain a constant flow rate of biomass.

In all seasons, the biomass then proceeds to dilute acid pretreatment to yield further solubilization of sugars and uronic acids. The aqueous stream is then separated with a vacuum belt filter press before proceeding to aerobic bioconversion alongside the pressate. Following bioconversion to lipids by ATCC 20509, the lipid-enriched product stream is subjected to another milder dilute acid pretreatment^{45,46} and solvent extraction to isolate lipids. Unconverted *Sargassum* solids from the first pretreatment step also undergo solvent extraction to maximize lipid yield. Lipids are saponified to reduce impurities and sold as a renewable lipid feedstock for biofuels or bioproducts at a price of \$1100/tonne.

The residual solids from solvent extraction are dried and sold as a generic solid coproduct. The biomass may be suitable for use as a soil amendment if challenges associated with salts and heavy metals can be managed,⁴⁷⁻⁴⁹ which might fetch a value of \$20-100/tonne based on typical bulk compost values (or potentially higher if more aligned with premium seaweed fertilizer products). Alternatively, the solids may be used as a supplemental aquaculture^{50,51} or animal feed⁵² (assuming mitigation of concerns associated with contaminants are addressed); estimates for value as a fish feed are approximately

\$400/tonne⁵³, and displacement of soybean meal may warrant a selling price of \$275-550/tonne based on historical market values.

These solids may also be suitable for use as a co-feed in producing partially bio-based thermoplastics (\$900/tonne, aligned with the assumed value of residual microalgae solids destined for thermoplastic co-feeds^{39,54}); however, this has not been verified experimentally and may be challenging based on the relatively low protein, high cellulose, and high aromatics content of the residual solids. Other potential higher-value applications include further conversion of the residual solids to biochar or graphite, leveraging the high content of polyaromatic components (quantification of this fraction is identified here as a priority for future work); however, these are out of scope for this analysis.

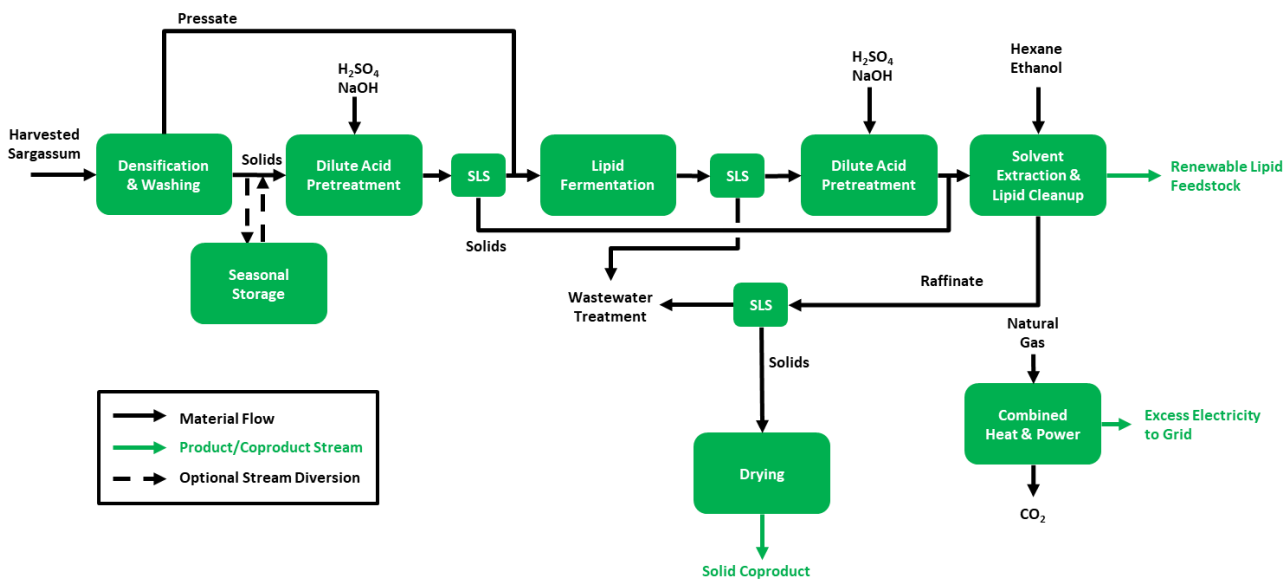


Figure 1. Process flow diagram for the conversion process modeled in the TEA.

3. Results & Discussion

3.1: *Sargassum* biomass composition

A custom compositional analysis approach was developed for *Sargassum*. *Sargassum* is rich in polysaccharides, like alginates (uronic acid polymers), sulfated fucans, and glucans of unique structure. The polysaccharides are embedded in a biomass matrix alongside a polyphenolic residue that resembles terrestrial biomass lignin. The compositional analysis was conducted on the original (non-pressed) *Sargassum*, as well as the solids pressed at 15 psi and 40 psi, covering ash, fatty acid methyl esters (FAME), protein (nitrogen by

combustion), and CHN (see Table 1; carbohydrate and uronic speciation data are provided in Table S-5 and Table S-6). Additionally, the corresponding pressates from 15 and 40 psi were analyzed for monomeric and total carbohydrates (including polymeric forms released after further hydrolysis) and uronic acids (Table S-7). In both the original biomass and pressed solids, glucose and mannitol are the primary carbohydrates, while the pressate is dominated by mannitol (accounting for 99% of the carbohydrates), with only trace quantities of other sugars and uronic acids detected. Pressing the original sample led to a slight reduction in ash content, decreasing by 4% in the 40 psi sample. Ash-free data for the pressed solids show a minor increase in carbohydrates and uronic acids following pressing.

Table 1: Overview of biomass composition of Sargassum, expressed on a dry weight basis, data are mean $\pm$ stdev of triplicate composition measurements for each treatment, comparisons shown are for (1) freshly harvested and frozen Sargassum, (2) biomass after milling for size-reduction and washing, and (3) solids retained after pressing harvested biomass (both 15 and 40 psi pressure pressing is shown)

Sample ID	Ash (%)	FAME (%)	Protein (%)	Carbohydrates (%)	Uronic acid (%)	Sum (%)	C (%)	H (%)	N (%)
Fresh	36.1 $\pm$	0.96 $\pm$	6.18 $\pm$	16.09 $\pm$ 0.56	15.74 $\pm$	75.08 $\pm$	26.94 $\pm$	3.34 $\pm$	1.26 $\pm$
Sargassum (frozen)	1.00	0.01	0.37		0.51	1.31	0.69	0.13	0.08
Wet-Milled	13.66 $\pm$	1.27 $\pm$	3.97 $\pm$	19.89 $\pm$ 0.25	29.4 $\pm$	68.18 $\pm$	39.57 $\pm$	4.88 $\pm$	0.81 $\pm$
and washed	0.07	0.04	0.15		0.68	0.74	0.07	0.04	0.03
Pressed Solids (15 psi)	32.07 $\pm$	1.02 $\pm$	5.71 $\pm$	14.89 $\pm$ 1.27	17.74 $\pm$	65.72 $\pm$	31.36 $\pm$	3.88 $\pm$	1.16 $\pm$
	1.03	0.02	0.26		2.13	2.70	0.70	0.14	0.05
Pressed Solids (40 psi)	28.54 $\pm$	0.99 $\pm$	6.25 $\pm$	15.52 $\pm$ 0.74	19.12 $\pm$	70.41 $\pm$	31.76 $\pm$	3.89 $\pm$	1.27 $\pm$
	2.38	0.04	0.55		1.75	3.09	0.45	0.11	0.11

3.2: Densification and mechanical refining

Initial biomass conditioning was completed by densification via Vincent screw press and 12” disk refiner, which both condensed the biomass and reduced the particle size distribution (Figure 2). Pressing increased the solids content from 14.3% to 24–27%, substantially reducing the amount of water introduced into the pretreatment and downstream processes (Table 2). Additionally, the bulk density increased by up to 2.6-fold. The pressing operation also produced a stream of pressed liquor containing nearly 10 g/L of mannitol, which is suitable for fermentation.

To create industrially relevant conditions for downstream operations, the biomass was added wet after mechanical refining; however, the biomass heterogeneity (specifically, a $\pm$ 4% variation in solids content) added uncertainty to measurements used within the

dataset and complicated downstream calculations. This phenomenon is commonly observed when treating and converting wet biomass and was addressed by measuring variation of dry matter of the biomass and considering this variation in the TEA sensitivity analysis.

Densification and mechanical refining provide methods of homogenization that can improve feed consistency and density of the biomass material, improving its capacity to be stored and reducing the burden on transportation of feeds. This allows for a more reliable feedstock, leading to positive cascading effects on several downstream processes. It is also important to note that there is no significant loss of carbohydrates or uronic acid in either process; the intrinsic value of the feed is improved by removing unnecessary water and improving particle size for downstream needs. Naturally, increased severity comes with a trade-off in energy consumption; while screw pressing is implemented in all scenarios, milling is considered as an optional step and investigated further in TEA sensitivity analysis but was left out of experimental validation work.



Figure 2: Visual representation of biomass (l to r) as received, after screw press densification, and after milling for further particle size reduction

Table 2: Summary of screw press mass balance of pressate and pressed solids, along with solids content and estimated bulk density. To maximize solids recovery from the screw press, 200-500g of ice was passed through the system post-processing to displace any residual material from the press. Discrepancies in final mass balance can be reconciled by the added water in this mass recovery step.'

Pressing Pressure (psi)	Starting material wet (kg)	Final Pressed solids (kg)	Pressate (kg)	Solids (%)	Bulk density (g/L)
n/a	64	n/a	n/a	14.3%	259
15	30.36	17.86	12.8	23.7%	620
40	34.04	14.78	18.45	27.0%	678

3.3: Preservation of biomass compositional quality

To rapidly test dry matter loss over the duration of up to three months of biomass storage, a scaled down preservation method was developed. Conditioning the biomass with acid (sulfuric acid, 3% w/w) and dropping pH < 4 was the most effective method at preserving the biomass and the composition with at most a 1.1% dry matter loss as calculated after biomass incubation and lyophilization in syringe set up (Table S-8). This indicates that a pre-incubation at low pH was able to stabilize the biomass and protect against biomass degradation.

3.4: Pretreatment to release monosaccharides

To understand how Sargassum polysaccharides can be released, we pretreated small quantities of biomass with either acid (H₂SO₄) or base (NaOH), heated the biomass between 140 °C and 170 °C between 15 and 30 min, and tracked the hydrolysis of monomeric saccharides, both neutral sugars and uronic acids (Figure S-2). The results indicate that hydrolysis effectiveness is primarily driven by acid concentration and time and temperature of incubation. Base pretreatment did not release appreciable quantities of monomers and was not investigated further.

Glucans like laminarin are highly recalcitrant to acid pretreatment, and require increased severity, which simultaneously accelerates degradation of more labile polymers like alginate and fucoidan. Higher acid loading and elevated temperature were shown to increase monomeric sugar yields, and longer pretreatment times further enhance sugar release (Figure 3). ANOVA results indicate that acid loading (p = 0.0005) has a more significant effect on monomeric sugar yield than temperature (p = 0.16) or time (p = 0.24). In contrast, uronic acid recovery favors higher acid loading at lower temperatures, likely due to its thermal instability. ANOVA analysis shows that acid concentration (p = 0.0081) is more influential than temperature (p = 0.30) and time (p = 0.76). Additionally, prolonged pretreatment leads to increased degradation of uronic acids. These trends highlight a trade-off in optimizing pretreatment conditions: conditions that maximize monomeric

sugar yield may compromise uronic acid recovery. Therefore, the optimal pretreatment strategy should be determined based on the relative content of monomeric sugars and uronic acids in the biomass, as well as their respective conversion efficiencies in downstream fermentation processes.

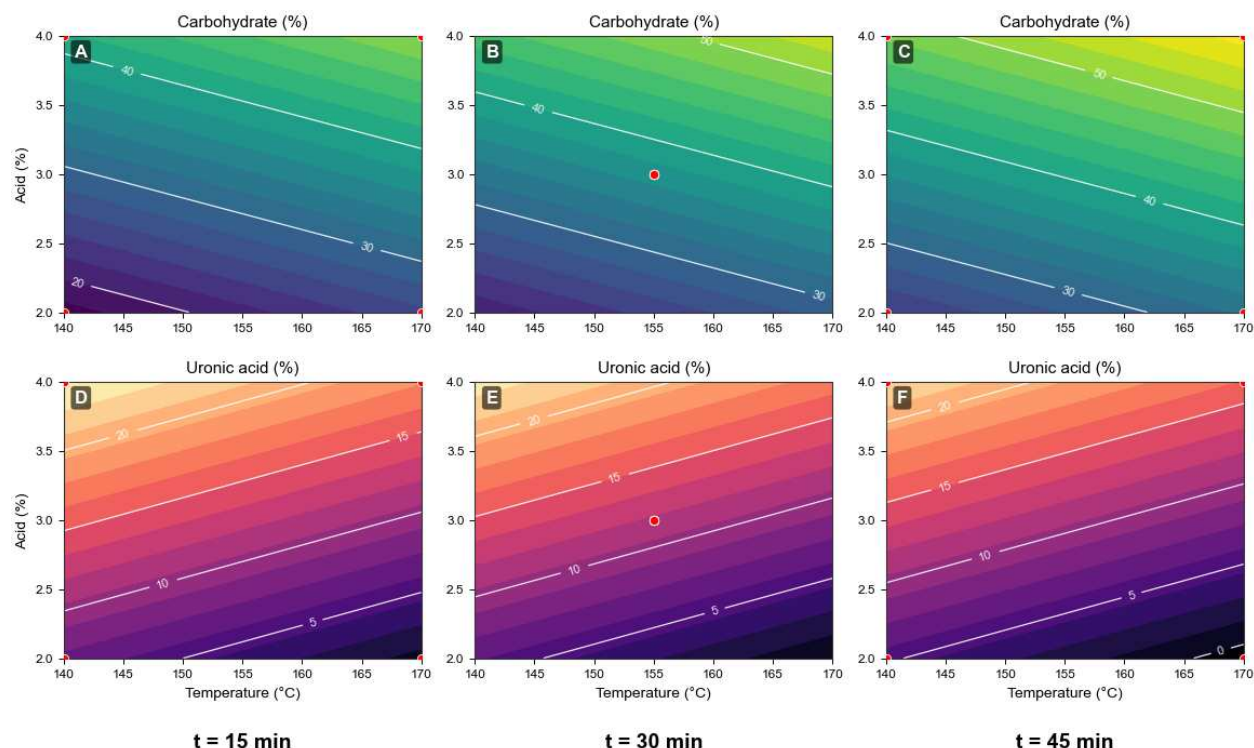


Figure 3: Response surface of monomeric sugar (A, B, C) and uronic acid (D, E, F) yields as calculated from pretreatment experiments at 10 mL scale in a microwave at different pretreatment times

The central composite design (CCD) experimentation at the 10 mL scale reveals several intrinsic phenomena related to extracting monomeric sugars and uronic acids from *Sargassum* via dilute acid pretreatment. These experiments were useful for comparative analysis of pretreatment approaches; however, due to the heterogeneity of *Sargassum* as a feed, scaling up the sample size of a reaction to 35 mL vessels offers more insight into reaction efficacy and further derisks the overall process as scales are increased. Based on the Response Surface Methodology results, three pretreatment conditions were selected for evaluation in a 35 mL reactor to minimize the impact of biomass heterogeneity. As shown in Table S-9, the monomeric sugar yield increased with temperature and residence time, reaching ~70% at 170 °C for 45 min. However, under these conditions, the uronic acid yield was the lowest (5.7%), resulting in a combined monomeric sugar and uronic acid yield of 34.9%. At a shorter pretreatment time (15 min) with the same acid loading and

temperature, the monomeric sugar yield decreased to 61.5%, while the uronic acid yield increased to 18.8%, leading to a slight improvement in the combined yield (38.3%). Thus, increasing the biomass loading appears to mitigate the effects of biomass heterogeneity in the 35 mL reactor, as evidenced by the low variation (<4.4%).

Data collected from microwave reactions informed the selection of an acceptable pretreatment condition for scale up to the 1 kg scale. Balancing the need for effective hydrolysis against the formation of potential fermentation inhibitors (HMF, furfural, etc.), a pretreatment condition of 160°C, 5/1 w/w (DCW/sulfuric acid), 23.5% dry matter content, and a residence time of 30 minutes was selected for 1 kg scale pretreatment. To mitigate pH buffering, the biomass was washed with water to remove ~50% of ash. Ultimately, yields in the 2-Gal Parr were consistent with prior 10-mL observations. The recalcitrant nature of biomass persisted and was potentially negatively affected by a modified heating method, direct steam injection. Direct steam injection is known to dilute the product significantly, approximately 75% dilution. As previously stated, the dry matter variation in the feed material must be accounted for in sugar and uronic acid recovery sensitivities. A 4% standard deviation in dry matter, corresponds to a ~16% variation in process carbohydrate recovery. Variation in dry matter can be improved by improved homogenization via mechanical refining and dewatering as seen with increased screw press severity and disk refining.

3.5: Bioconversion of monosaccharides

Microbial conversion has proven promising in conversion of hydrolyzed saccharides to lipids, which form the basis of chemical and fuel pathways. The organism selected for this work, *C. oleaginosum*, was selected for its broad substrate utilization profile as well as history of robust intracellular lipid production³⁸ and was shown to utilize most monosaccharides present in *Sargassum*. As *Sargassum* hydrolysate is a relatively dilute carbon stream (containing approximately 18 g/L total monomeric carbohydrates, Table 3), the addition of yeast peptone (YP) increased the monosaccharide uptake rates. The lipid accumulation and titer was higher without YP, approximately 3x more lipid was accumulated intracellularly, however, with the dilute carbon source, overall yields remained low (Table S-10).

Table 3: Saccharide concentration in hydrolysate-based fermentation media for *C. oleaginosum*, along with utilization efficiency (%) after 44 hrs, in the presence or absence of yeast extract and peptone (YP) additives. N = triplicate cultures

	Fucose	Galactose	Glucose	Mannose	Xylose	Guluronate	Glucuronate	Mannuronate
<i>Sargassum</i> hydrolysate media (g/L)	4.56 ±0.21	1.03 ±0.05	0.93 ±0.06	1.45 ±0.07	0.96 ±0.04	3.2 ±0.12	1.2 ±0.05	4.8 ±0.19
no YP (%)	3.2 ±5.56	100.0 ±0.00	100.0 ±0.00	99.0 ±0.08	100.0 ±0.00	0.0 ±0.00	87.1 ±0.72	100.0 ±0.00
+YP (%)	40.5 ±5.89	100.0 ±0.00	100.0 ±0.00	100.0 ±0.00	100.0 ±0.00	25.8 ±2.92	85.6 ±0.34	100.0 ±0.00

To understand its value as a stand-alone feed stream, we used the pressate as a sole saccharide source (Table S-10). The mannitol in the pressate was completely utilized within 48 hours without the need for supplemental nutrients and, as seen before when 0.1x YP was added, FAME content was dramatically lowered.

Sargassum hydrolysate was tested in a bioreactor to provide better process control (Figure 4). In this experiment, *C. oleaginosum* exhibited diauxic growth with a clear preference for glucose. Once glucose was depleted, mannitol, galactose, mannose, xylose, and glucuronic acid were all quickly utilized. While these secondary saccharides were present, utilization rates of fucose and mannuronic acid were rapid. However, a second phase inflection occurred, slowing down fucose and mannuronic acid when co-utilization of mannitol, galactose, mannose, xylose and glucuronic acid was no longer possible. This suggests that without these sugars, energy or redox requirements are not well met and the cells are not as capable of metabolizing these more difficult carbohydrates. Guluronic acid was minimally utilized even in the presence of first- and second-tier monosaccharides.

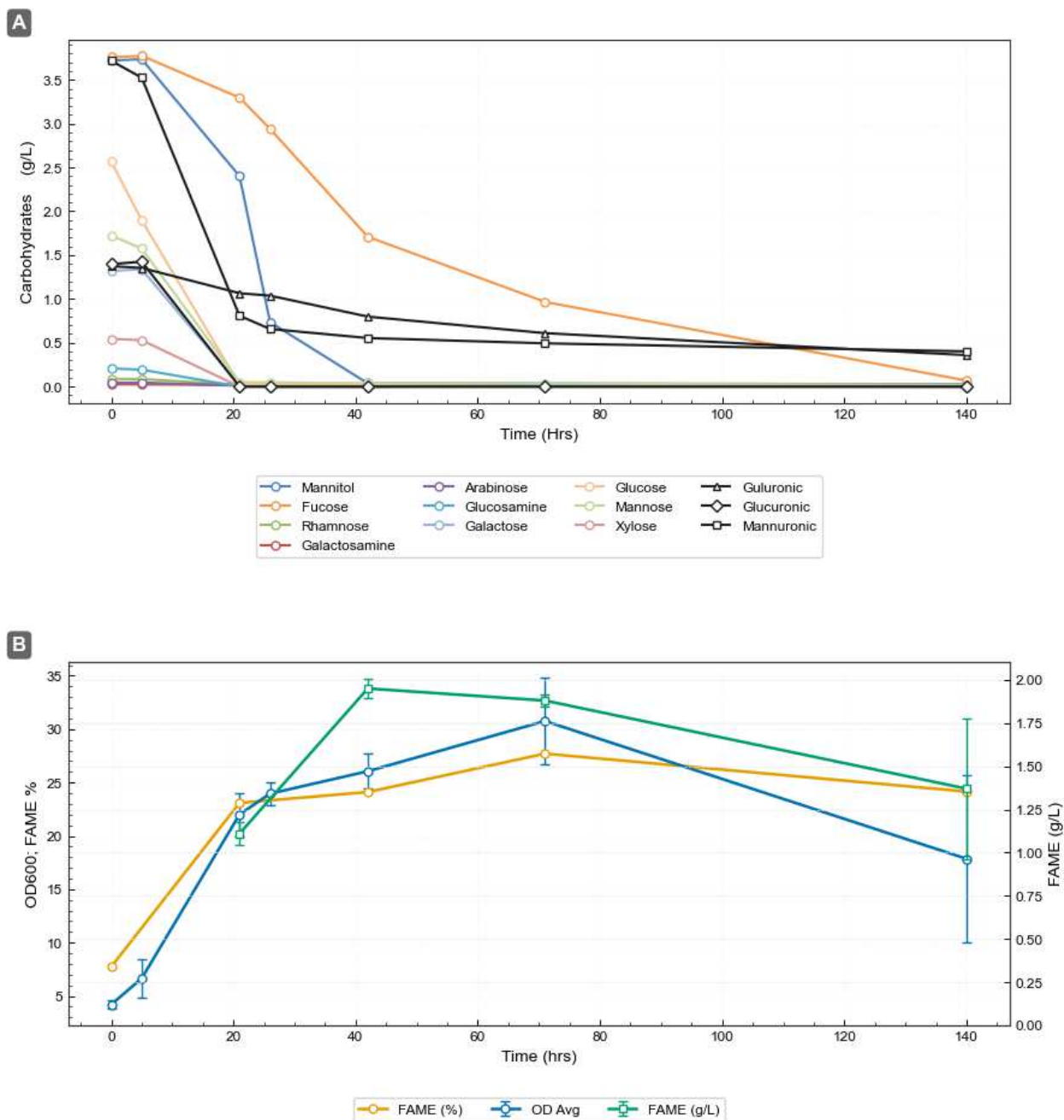


Figure 4: (A) *C. oleaginosum* monosaccharide utilization in mock hydrolysate representing *Sargassum* hydrolyzed carbohydrates, and (B) growth (OD600), lipid content (%) and concentration (g/L).

The dilute nature and low carbon content (and C:N ratio) of the hydrolysate causes low lipid content of the biomass ($30 \pm 2.06\%$ DCW), and titer and yield (2.0 ± 0.15 g/L, 0.095 ± 0.007 g/g yield). These bioconversion metrics (titer, rate, yield) on *Sargassum*-derived hydrolysate and pressate are challenging primarily due to low concentrations of fermentable sugars in the current hydrolysates. Concentration of these feed streams would increase overall

salinity of the feed stream and could impact *C. oleaginosum* physiology, shown to attenuate growth at salinity levels of 35 ppt and above (data not shown).

To address the challenges of a dilute feed stream and that lipid accumulation occurs intracellularly, we opted for biological concentration in the form of fed-batch style fermentation with a cell-recycle system (Figure S-3, Table S-11). Leveraging the intracellular accumulation of lipid product, a hollow fiber filtration system was employed to retain cells within the bioreactor while removing spent medium, similar to a repeated batch. The bioreactor was initially operated in standard batch mode; upon complete carbohydrate utilization, a peristaltic pump circulated the culture through the filter. A small pressure differential enabled the removal of spent medium while retaining the cells in the reactor. Once the reactor volume was sufficiently reduced, fresh medium was added and fermentation resumed. This cycle was repeated multiple times. Using this approach, a portion of the lipid containing biomass could be periodically harvested for lipid recovery while maintaining high productivity due to elevated cell densities.

The cell recycle system offers several advantages over established fermentation modes. Unlike conventional batch processes, it avoids reduced productivity associated with low initial cell density. Although repeated batch systems retain some biomass, a substantial fraction is typically lost during medium exchange, leading to decreased productivity between cycles. Compared with fed-batch operation, which is constrained by reactor volume and requires termination upon reaching capacity, the cell-recycle approach enables extended operation without such limitations. In continuous fermentation, medium is replaced at a constant rate, which can limit substrate utilization under diauxic growth conditions due to insufficient residence time, thereby reducing yields. In contrast, the cell-recycle strategy allows complete substrate utilization prior to medium removal. On average, conversion rates of 0.02 ± 0.002 g/L/hr and yields of 0.11 ± 0.01 g/g were observed, matching batch bioconversion performance while enabling a more efficient overall batch time.

3.6: Techno-economic analysis

The experimental results described informed the mass and energy balance of a conceptual integrated process. The sequential operations were simulated in Aspen Plus, and the resulting process model was used to estimate the overall cost and energy implications of each modular process unit in an extensive TEA. The facility design centered around production of lipids from *Sargassum*, which was successfully demonstrated experimentally. These lipids have value as fuel precursors and may also warrant higher values due to a high fraction (45%) of polyunsaturated fatty acids, which are useful feedstocks for polymer synthesis.^{54–56} However, despite this value, the TEA indicated that

lipid revenues were not high enough to support the costs for biomass harvesting and conversion in isolation, and that the bulk of revenue (>90%) would have to come from a coproduct derived from the residual solids. Thus, the primary metric of the TEA is the value that this solid coproduct must be sold at to attain a 10% internal rate of return for the facility (defined as the minimum solid selling price, MSSP). Potential applications for the residual solids are discussed further below and include soil amendments, aquaculture and animal feeds, and thermoplastics.

The results of the base case TEA, summarized in Table 4, indicate a MSSP of \$942/tonne. The large scale of the proposed facility necessitates a significant total capital investment of \$866 million and high annual operating expenditures of \$186 million, most of which are variable costs. As detailed in Figure 5; raw materials account for a large portion of the MSSP (\$652/tonne, 69% of the total; also see Table S-12). The most significant contributors are the *Sargassum* feedstock (\$348/tonne, 37%), acid and base consumption in pretreatment (\$121/tonne, 13%), wastewater treatment costs (\$74/ton, 8%) and natural gas consumption (\$70/tonne, 7%). Fixed operating costs contributed an additional \$58/ton, 6% of the total MSSP (Table S-13)

Capital costs contributed a total cost of \$304/tonne (32% of the total) to the MSSP, with primary contributors including fermentation operations (47% of CAPEX) and solid dewatering and drying (26% of CAPEX); additional CAPEX details are provided in Table S-14. In contrast, revenue credits from coproducts were modest. The renewable lipid product produced provided a modest MSSP credit (–\$50/ton), though was much less than the combined cost of the fermentation and lipid extraction operations. This suggests that significant improvements to the fermentation operation are needed to justify it economically. Electricity co-produced by the facility also provided a credit (–\$22/ton), a byproduct of the high natural gas usage required for solid drying (51% of total heat), solvent recovery (27% of total heat), and pretreatment (19% of total heat).

The MSSP associated with the base case indicates challenging economics for the *Sargassum* biorefinery when considered alongside the market values of its potential applications, including soil amendments, aquaculture and animal feed operations, and thermoplastics (here, we intentionally limit coproduct applications to those with significantly high market volume in order to support large-scale *Sargassum* valorization). One challenge associated with *Sargassum* in these applications is the presence of heavy metals, which can have detrimental consequences for soil amendments^{47–49} and feed.⁵² Heavy metal content may be partially remediated by the washing and processing operations of the biorefinery; however, this has not yet been quantified. If these challenges can be addressed, the residual solids may be suitable for these applications,^{47,48,50,51} but the associated product values are generally lower than the required MSSP (<\$100/tonne for

soil amendments and <\$500/tonne for feed and aquaculture applications). One potential market which could fetch higher values (\$800-\$1000/tonne) is thermoplastics, as has shown promise microalgae.^{39,54} The chemical makeup of the residual *Sargassum* solids may limit its use in this technology pathway, though other bioplastic technologies may serve as higher-value, scalable applications suitable for supporting the biorefinery concept described here; in particular, the high proportion of alginate in *Sargassum* presents a significant opportunity.^{57,58}

Table 4. Key TEA results for the base case.

	Value	Units
Minimum Solid Selling Price	\$942	\$/tonne
Solid yield	630	kg/tonne biomass
Lipid yield	8.5	gal/tonne biomass
Total capital investment	\$866	MM \$
Variable operating costs	\$244	MM \$/yr
Fixed operating costs	\$21	MM \$/yr
Average fuel & coproduct revenue	\$365	MM \$/yr
Revenue breakdown:		
Fuels	5.0%	%
Solid Coproduct	92.9%	%
Electricity	2.1%	%

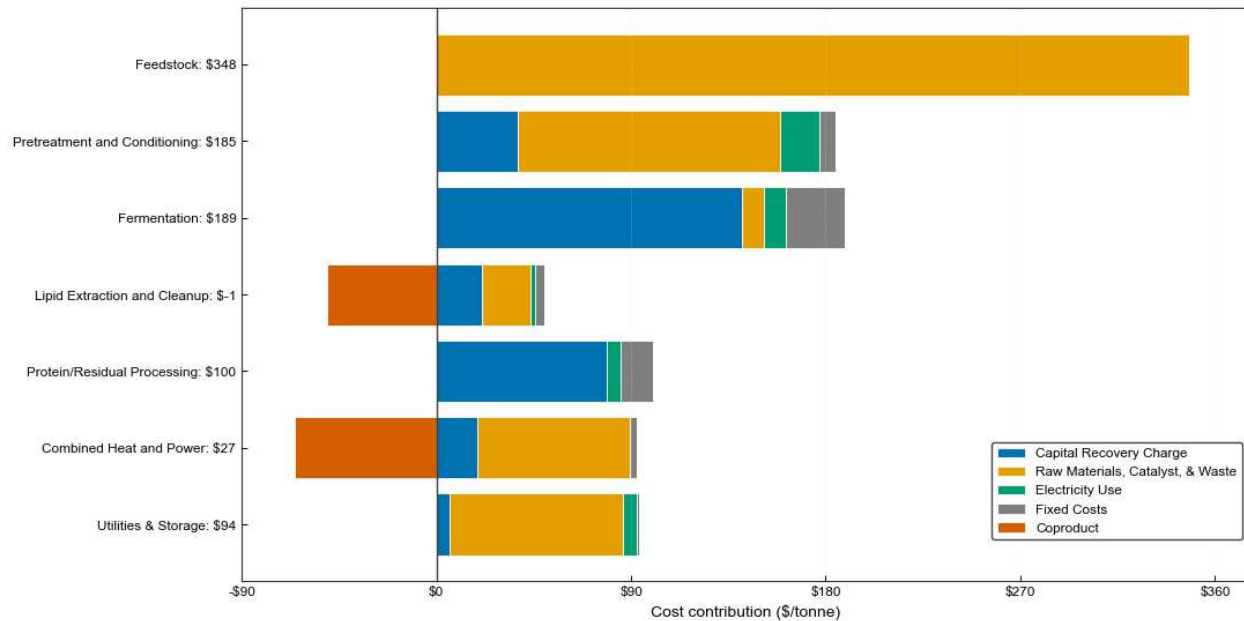


Figure 5. Cost breakdown of the MSSP by processing area and cost category.

Sensitivity analyses were also conducted to further elucidate key cost drivers and assumptions for the biorefinery. First, two known cost drivers (scale and feedstock cost) were modified together to show cost implications over a wide range of variations (Figure 6A); following this, we consider a broader list of variables and their impact on the MSSP (Figure 6B).

The impact of scale on process economics is very significant, with large scales (on the order of 1 million wet tonnes/year [435 dry tonne/day] or greater) required for MSSP to approach the market values of the potential coproducts for the biorefinery. For scales below this value, the MSSP sharply increases to untenable values unless selling into exceptionally high-value markets. Feedstock cost also has a pronounced impact on economics; a feedstock price of \$35/ton (reflecting a nominal cost of *Sargassum* delivered to a floating biorefinery⁴¹) lowers the MSSP of the base case scale to \$642/tonne (though noting that this cost does not reflect the increased capital and operating expenditures that would be associated with a floating facility). Alternatively, a higher feedstock cost of \$419/tonne (reflecting the average cost for the first 50 million dry tonnes/year of farmed macroalgae as reported in the 2023 Billion Ton Study⁵⁹) makes economics significantly more challenging, with MSSPs greater than \$1000/tonne even for the highest plant scales considered.

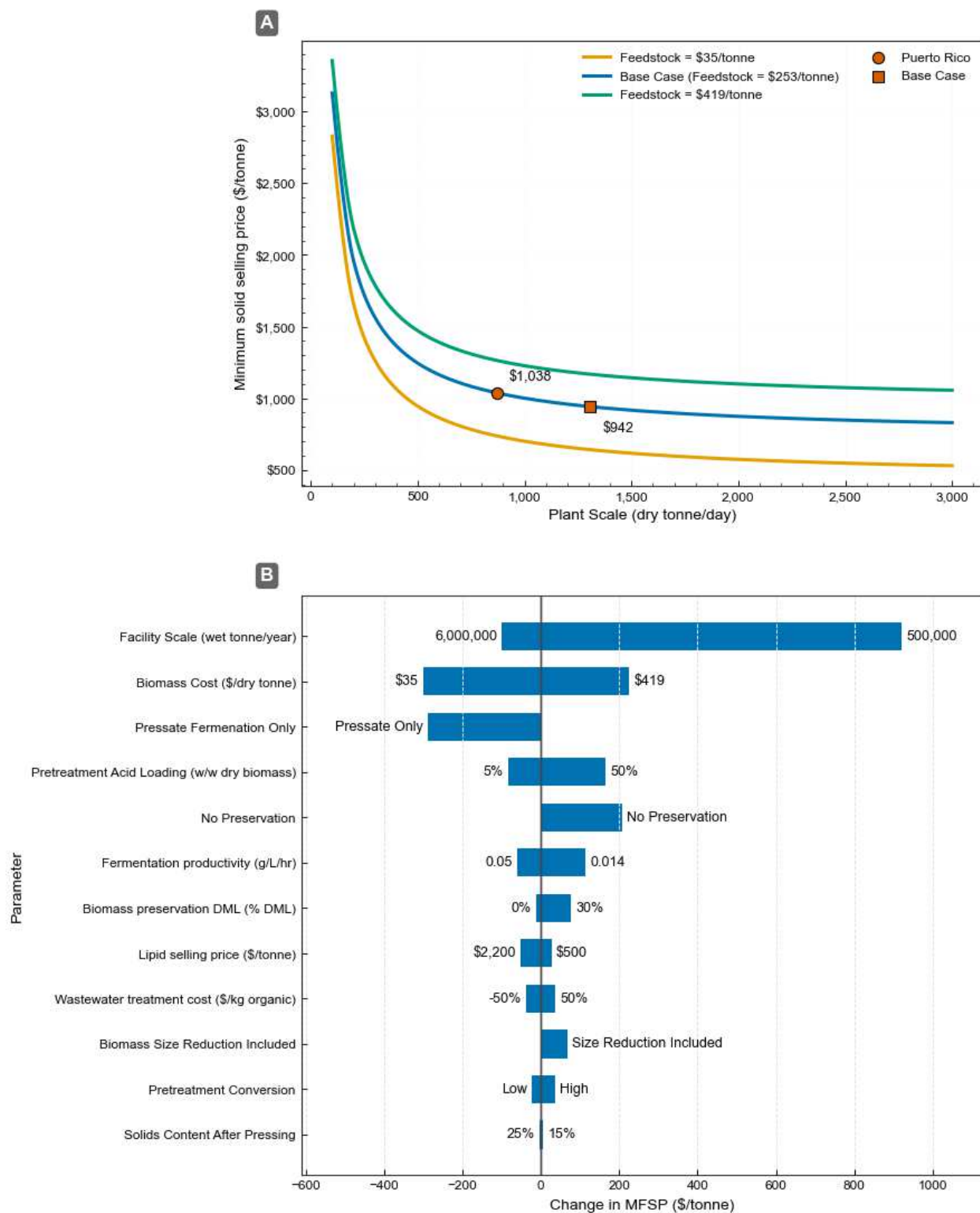


Figure 6. (A) Sensitivity analysis of plant scale and feedstock cost on minimum fuel selling price. Point values for the base case (3 MM wet tonne/year) and an estimate of biomass available around Puerto Rico (2 MM wet tonne year) are shown on the line for the base case. (B) Tornado plot depicting the sensitivity of MSSP to key process parameters.

Beyond scale and biomass cost, which are the most significant cost drivers as shown in Figure 6B, we conducted a single-point sensitivity analysis on other key variables. An alternative scenario considering fermentation of the pressate only (forgoing pretreatment and lipid extraction of *Sargassum* biomass) showed potential to reduce costs by nearly \$300/tonne, highlighting the high cost of pretreatment and fermentation. This is further emphasized by cost sensitivity to pretreatment acid loading and fermentation productivity. Improving these variables could reduce biomass costs by \$59-83/ton, while conservative assumptions could increase it by \$114-166/ton. Thus, significant improvements could be made in process economics by focusing on valorization of the pressate in isolation, which allows for isolation of nearly 50% of carbohydrates and uronics without costly pretreatment and extraction operations.

Preserving biomass to ensure consistent, year-round operation is a key enabler of economic feasibility. As the sensitivity analysis shows, omitting preservation would require oversizing the facility for peak summer months while it sits idle in winter, leading to underutilized capital and a substantial MSSP increase of over \$200/ton. However, if preservation is successfully implemented, dry matter loss is not as strong as a cost driver, with a 30% loss resulting in a cost increase of only \$77/ton.

The economic impact of the other variables considered was lower, but still notable. Inclusion of a size reduction step (as represented by an increased feedstock cost of \$49/tonne, based on processing costs for cornstover⁶⁰) resulted in an MSSP increase of \$67/tonne. Wastewater treatment contributed significant costs; a variation of $\pm 50\%$ resulted in an MSSP change of $\pm \$38/\text{ton}$. The lipid selling price had a similar impact; a higher value of \$2200 per tonne of lipids would reduce the MSSP by \$51/tonne, while a more conservative value of \$500 per ton would increase it by \$28/ton. Pretreatment conversion efficiency was not a major cost driver within the bounds of the experimentally demonstrated deviation, changing biomass price by less than \$36/ton in either direction. Notably, increased pretreatment conversion has an inverse impact on MSSP, because revenue obtained from conversion to fuels is less than that obtained from unconverted solids. Finally, variation solids content after pressing had very limited impact on MSSP ($< \$7/\text{tonne}$).

4. Conclusions

The results presented here provide an integrated biorefinery framework specifically tailored to *Sargassum* seaweed. The process concept we experimentally demonstrated includes initial processing of harvested *Sargassum* biomass, preservation of biomass quality for long-duration storage, and bioconversion to lipids. The *Sargassum* biomass was initially condensed through pressing, which generated a higher density, lower ash content, and ultimately more stable material. The process for monomeric sugar conversion is initiated by acid pretreatment to release over 60% of the carbohydrates as monomers, with the suitability of microbial conversion of these monomers to high-lipid feedstocks. We then explored the suitability of historically-deployed microorganisms for fermenting the unique suite of macroalgae sugars to lipid precursors and were able to narrow down to candidate species with demonstrated effective utilization of algae-specific saccharides.

The data obtained were used to establish a rigorous process model of a commercial scale process, which informed a detailed TEA. The analysis identified a cost-competitive biorefinery concept which hinges on producing a high-value solid coproduct alongside renewable lipids and procuring a preservable *Sargassum* feedstock at sufficient scales.

While we demonstrated feasibility of a biorefinery approach for *Sargassum* conversion to fuels and products, the systematic assessment of preservation, pre-processing, pretreatment, and fermentation also identified challenges that should be addressed in future work: (1) the lack of full characterization of the biomass, specifically the unknown nature of the polyphenolic residues of the cell walls; (2) the high buffering capacity of the biomass that necessitates high acid loading to achieve hydrolysis; (3) the recalcitrant nature of glucans, which limits hydrolysis yields and microbiological conversion yields—introducing a critical tradeoff where increased pretreatment severity improves carbohydrate recovery but degrades uronic acids; (4) the bioconversion and challenging microbial metabolism of fucose and uronic acids; and (5) the application of the residual solids as demonstrated high quality bioproduct application. Despite these challenges, this study provides a systems-level framework for the valorization of pelagic *Sargassum*, shifting the paradigm from a costly waste management challenge to a potential bioeconomy feedstock.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MW, ABL, TD, SVW, JL, RN, EN, AM, and LL drafted the manuscript. ABL, TD, JL, RN, EK, XC, and JK performed the experiments. SVW conducted the physical sample and statistical analyses. MW conducted process modeling and techno-economic analysis. JK and LL led initial funding procurement, and LL served as the principal investigator of the project. CL and GG acquired the biomass feedstocks and provided technical expertise on *Sargassum*. AM contributed the biomass harvesting cost assumptions. All authors have read and approved the final manuscript.

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Supplemental Information

Biomass Compositional Analysis

Monomeric sugars (mannitol, fucose, rhamnose, glucosamine, arabinose, galactosamine, galactose, glucose, mannose, xylose, and ribose) released during hydrolysis were injected onto and analyzed on a Dionex ICS-5000+, HPAEC-PAD system equipped with a PA-1 column (Dionex #035391) and guard cartridge (Dionex #043096) set to 35 ° C. The column was washed at 1 mL/min for 10 min at 200mM NaOH then equilibrated for 30 min at 14mM NaOH, after which a 20 min isocratic run at 14mM NaOH separated the carbohydrates, which were detected by a pulsed amperometric detector (PAD). To separate four uronic acids (galacturonic, guluronic, glucuronic, and mannuronic), the following gradient was utilized: 10-minute pre-run column flush with 200mM NaOH, 20-minute pre-run equilibration with 1mM sodium acetate/100mM NaOH at 17% (remainder 18.2 megaohm water), and a 10-minute run with the 17% 1mM sodium acetate/100mM NaOH (remainder 18.2 megaohm water) ^{61,62} Whole biomass transesterification of lipids to fatty acid methyl esters (FAME) was performed to quantify the lipid content as FAME ³³.

Biomass Harvest Cost Assumptions

Fearless Fund⁶³ (Fearlessfund.org) designed a scalable process to harvest floating *Sargassum natans* and *Sargassum fluitans*, that formed the basis of the estimated collection cost of \$195/DMT. This collection cost is expected to decrease over time and is associated with an energy ratio, defined as the energy output to energy input, of 10:1. The harvest location for the TEA is the Great Atlantic *Sargassum* Belt (GASB), which extends across the subtropical Atlantic from West Africa through the Caribbean, including Puerto Rico and the US Virgin Islands. The GASB offers the potential for large scale macroalgae production without the need for macroalgae farming infrastructure. The biomass is distributed across the system without containment, so the team designed a process by which to maximize the floating macroalgae's habitat provision at sea and harvest near shore (within 25 miles, or 40.2 km) to minimize economic and ecosystem impacts from beaching biomass harmful to local economies.

The team developed and tested a predictive model using remote sensing, winds, currents, and growth as inputs, which was then used for a techno-economic analysis (TEA) which estimated the cost per dry metric tonne of *Sargassum* biomass. The model was based on data on biomass mat structure and methods to efficiently collect the biomass for energy purposes, and analyzed biomass quantities across the Caribbean. The Fearless Ocean Energy from Macroalgae (OEM) model was used to predict the path of floating biomass for efficient and lowest cost harvest. The model incorporates satellite imagery, wind, currents

and growth to locate and plot trajectory over time and space. The model predicts location over three (3) days horizon after which accuracy declines, and the location and harvest distance to shore is included in the overall cost calculation. Validation of the forecasting model was conducted using remote sensing and high-resolution georeferenced location data using sea surface drifters (SSD's) to track mats and train the model.

Techno-economic Analysis (TEA) basis for Sargassum harvesting

The season is defined as a 5-month period starting 3 months before the cycle peak, with seasonal harvest determined as a function of a selected home port. The location of maximum mass flux within a 10 nautical mile (18.5 km) range of the port is found based on the mass flux in the peak month of each year. The range is extended to find suitable locations for harvest if the flux is insufficient close to port. Operational testing characterized bycatch and defined potential mitigation strategies to traverse regulatory challenges associated with bycatch.

As the maximum flux forecast is given with 11 km resolution, booms are used to gather half of the biomass passing through the maximum flux location. The same operation is carried out for the location with the second highest mass flux within the given range of the home port. The harvest vessel waits at the home port until one of the boom sets has accumulated adequate biomass, and travels to harvest that location area. Harvest with a conveyor-type system occurs at 120 MT/hr, and biomass is baled to a density of 230 kg/m³. The unconfirmed state of the art near shore harvest rate is 60 MT/hr. Scaling the equipment up for offshore harvest is expected to increase the harvest rate up to 180 MT/hr.

Supplemental Tables and Figures

Table S-1: Details of pretreatment conditions for central composite design (CCD) statistical experiment in 10 mL microwave reactor tubes.

	Time	Temperature (°C)	DCW/Acid ratio
1	45	170	10.00
2	15	170	5.00
3	15	170	10.00
4	30	155	6.67
5	30	155	6.67
6	45	140	10.00
7	30	155	6.67
8	30	155	6.67
9	45	170	5.00
10	30	155	6.67
11	4.77	155	6.67
12	30	180	6.67
13	30	155	15.17
14	30	155	6.67
15	55.2	155	6.67
16	30	155	4.27
17	15	140	5.00
18	45	140	5.00
19	15	140	10.00
20	30	130	6.67

Table S-2. Details of pretreatment conditions for 35 mL microwave pretreatment conditions

Temp	Scale	Time (min)	DCW/acid ratio
155	35mL	30	6.67
170	35mL	15	5
170	35mL	45	5

Table S-3. Financial assumptions and design basis used for the TEA model.

Financial Assumption	Value
Plant life	30 years
Plant throughput	3 million wet tonnes of <i>Sargassum</i> per year
Cost year dollar	2020
Capacity Factor	90%
Discount rate	10%
General plant depreciation	MACR
General plant recovery period	7 years
Steam plant depreciation	MACR
Steam plant recovery period	20 years
Federal tax rate	21%
Financing	40% equity
Loan terms	10-year loan at 8% APR
Construction period	3 years
<i>First 12 months' expenditures</i>	8%
<i>Next 12 months' expenditures</i>	60%
<i>Last 12 months' expenditures</i>	32%
Working capital	5% of fixed capital investment
Start-up time	6 months
<i>Revenues during start-up</i>	50%
<i>Variable costs during start-up</i>	75%

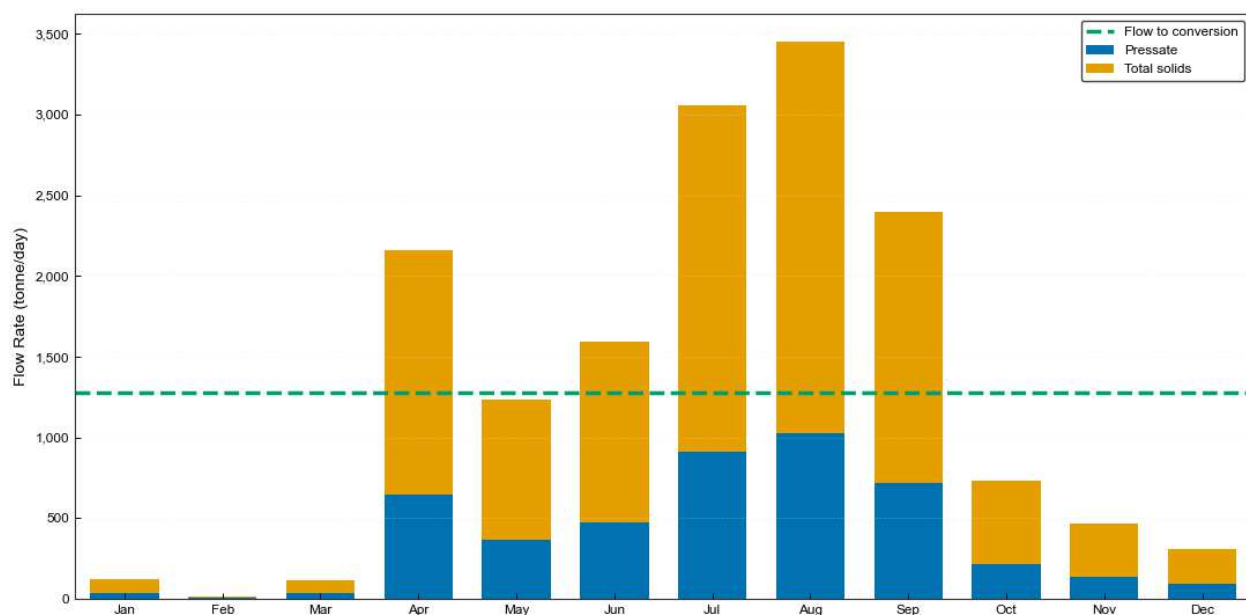


Figure S-1. Average profile of annual Sargassum available in Puerto Rico.⁴⁴ Monthly profile is applied to an assumed biomass availability of 3 MM wet tonne/year (1,306 dry tonne/day average). Raw flow rate is depicted by the bars; blue bars represent pressate, which is always used immediately, while yellow bars represent the pressed solids, which are partially stored in peak seasons. The consistent flowrate to conversion, after a 5% dry matter loss of stored material, is shown as a dashed green line.

Table S-4. Process parameters used in the base case process model. Experimentally demonstrated values are also shown for comparison.

Input	Experimental	Base Case	Units
<i>Sargassum</i> Harvesting			
Feed scale		3,000,000	wet tonnes/year
		429,000	dry tonnes/year
		1,306	dry tonnes/day
Feedstock cost		253	\$/dry tonne
At dock		195	\$/dry tonne
Ground transportation		58	\$/dry tonne
Harvested solids content	14.3%	14.3%	
Composition			

Carbohydrates	19.9%	19.9%	% dry weight
Uronics	29.4%	29.4%	% dry weight
Lipids	1.3%	1.3%	% dry weight
Protein	4.0%	4.0%	% dry weight
Ash	13.7%	13.7%	% dry weight
Other	31.7%	31.7%	% dry weight
Densification			
Solids content of densified biomass			
	23.6% ($\pm 4.6\%$)	20.0%	%
Carbohydrates to liquid	48.7%	48.7%	%
Uronics to liquid	48.1%	48.1%	%
Preservation			
w/w dry			
Acid loading ¹	0.03	0.03	biomass
Dry matter loss	1.1%	5%	% dry weight
Acid Pretreatment			
w/w dry			
Acid loading	0.2 – 1.0	0.2	biomass
Solids loading	24%-27%	20%	wt%
Temperature	155-175	155	°C
Residence time	15-60 minutes	30	min
Carbohydrate hydrolysis	41.5% (33.9-52.5%)	41.5%	%
	24.1% (19.7-30.5%)	24.1%	%
Uronic hydrolysis	14.6% (12.2-18.2%)	14.6%	%
Protein hydrolysis			
Hydrolysate Fermentation			
ATCC			
Organism	ATCC 20509	20509	
Lipid productivity	0.010-0.026	0.026	g/L/hr
Lipid yield	0.05-0.12	0.070	g/g sugar
Time	48	48	hours
Yeast composition			
Lipids		10%	% dry weight
PUFA (3+ double bonds)	45.3%	45.3%	% of lipids
Other (<3 double bonds)	54.7%	54.7%	% of lipids
Protein		50%	% dry weight

Carbohydrate	30%	% dry weight
Ash	5%	% dry weight
Other	5%	% dry weight
Yeast Pretreatment		
		w/w dry
Acid loading	0.05	biomass
Solids loading	20%	wt%
Temperature	155	°C
Residence time	30	min
Carbohydrate hydrolysis	80%	%
Protein hydrolysis	50%	%
Lipid Extraction		
	3-stage	
Configuration	agitation	
Lipid extraction yield	96.0%	%

¹ Acid used for preservation is included in the total acid loading for pretreatment and is not considered a separate input.

Table S-5. Carbohydrate speciation for (1) freshly harvested and frozen Sargassum, (2) biomass after milling for size-reduction and washing, and (3) solids retained after pressing harvested biomass (both 15 and 40 psi pressure pressing is shown). All values are reported on a dry weight percent basis. <loq = monomers were present but below limit of quantitation; N/D = monomer was not detected.

	Mannitol	Fucose	Rhamnose	Galactosamine	Arabinose	Glucosamine	Galactose	Glucose	Mannose	Xylose	Ribose
Fresh <i>Sargassum</i> (frozen)	5.38	2.52	<loq	<loq	<loq	<loq	0.75	6.31	0.65	0.47	N/D
Wet-Milled and washed	<loq	4.51	<loq	<loq	<loq	<loq	1.1	12.2	1.08	1	N/D
Pressed Solids (15 psi)	2.51	2.89	<loq	<loq	<loq	0.14	0.9	7.13	0.76	0.56	N/D
Pressed Solids (40 psi)	2.48	3.04	<loq	<loq	<loq	0.14	0.95	7.54	0.82	0.56	N/D

Table S-6. Uronic speciation for (1) freshly harvested and frozen Sargassum, (2) biomass after milling for size-reduction and washing, and (3) solids retained after pressing harvested biomass (both 15 and 40 psi pressure pressing is shown). All values are reported on a dry weight percent basis. <loq = monomers were present but below limit of quantitation; N/D = monomer was not detected.

	Galacturonic	Guluronic ^a	Glucuronic	Mannuronic ^a
Fresh <i>Sargassum</i> (frozen)	<loq	5.43	2.02	8.3
Wet-Milled and washed	<loq	9.65	3.76	15.99
Pressed Solids (15 psi)	<loq	5.73	2.88	9.13
Pressed Solids (40 psi)	<loq	6.29	3.06	9.77

^a Guluronic and mannuronic were quantified using the galacturonic calibration curve and then applying a correction factor for the difference in response

Table S-7: Compositional Analysis of pressate liquid, after using screw press to increase density of Sargassum biomass (data shown as the mean $\pm$ stdev of duplicate analyses when available)

Sample ID	Monomeric Carbohydrates mg/ml	Total Carbohydrates mg/ml	Monomeric Uronics mg/ml	Total Uronics mg/ml
15 psi Pressate	9.33	9.82 $\pm$ 0.16	0.09	0.18 $\pm$ 0.00061
40 psi Pressate	9.64	9.77 $\pm$ 0.02	0.10	0.20 $\pm$ 0.00302

Table S-8: Dry matter loss of 3% sulfuric acid-treated Sargassum after 3 months in anaerobic syringe apparatus at 30°C. Roughly 1% of dry matter was lost during the incubation period, and % carbohydrates and % uronics were unchanged, indicating successful preservation of the biomass and its composition.

	T ₀	3 months
% carbohydrates	19.3	20.4 $\pm$ 0.5
% uronics	33.1	33.0 $\pm$ 0.5
% dry matter loss	0.0	1.1 $\pm$ 0.1

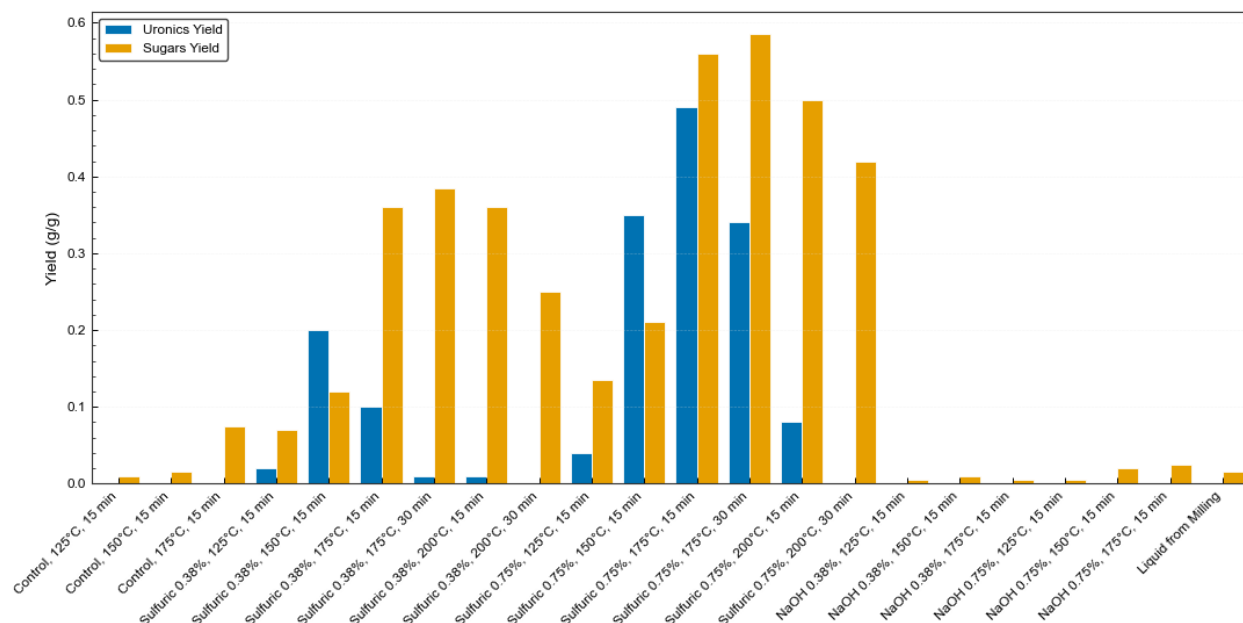


Figure S-2: Hydrolysis yield of Sargassum biomass (g/g uronic acids or sugars) when treated with acid or base following conditions listed in the x-axis labels.

Table S-9: Summary of pretreatment of Sargassum conditions (time, temperature and acid ratio, along with hydrolysis yields)

PT Method	Temperature	Time (min)	DCW/acid ratio	Carbohydrate yield	Uronic acid yield
Microwave	155	30	6.67	36.5% ± 4.1%	13.5% ± 2.3%
Microwave	170	15	5	61.5% ± 4.2%	18.8% ± 2.1%
Microwave	170	45	5	69.6% ± 3.4%	5.7% ± 4.4%
Parr	160	30	5	41.5%	24.1%

Table S-10: Lipid accumulation, measured as FAME on *Sargassum* hydrolysate and pressate in the presence or absence of yeast peptone (YP) nutrient addition, shown as titer (g/L), %DW of the harvested biomass, and yield (g/g total saccharides).

		FAME	Lipid Yield
	g/L	%DW	g/g
Hydrolysate no YP	0.70	16.86	0.041
Hydrolysate +YP	0.48	5.66	0.028
Pressate no YP	1.2±0.05	20.5±0.01	0.094
Pressate +YP	0.5±0.04	7.6±0.14	0.039

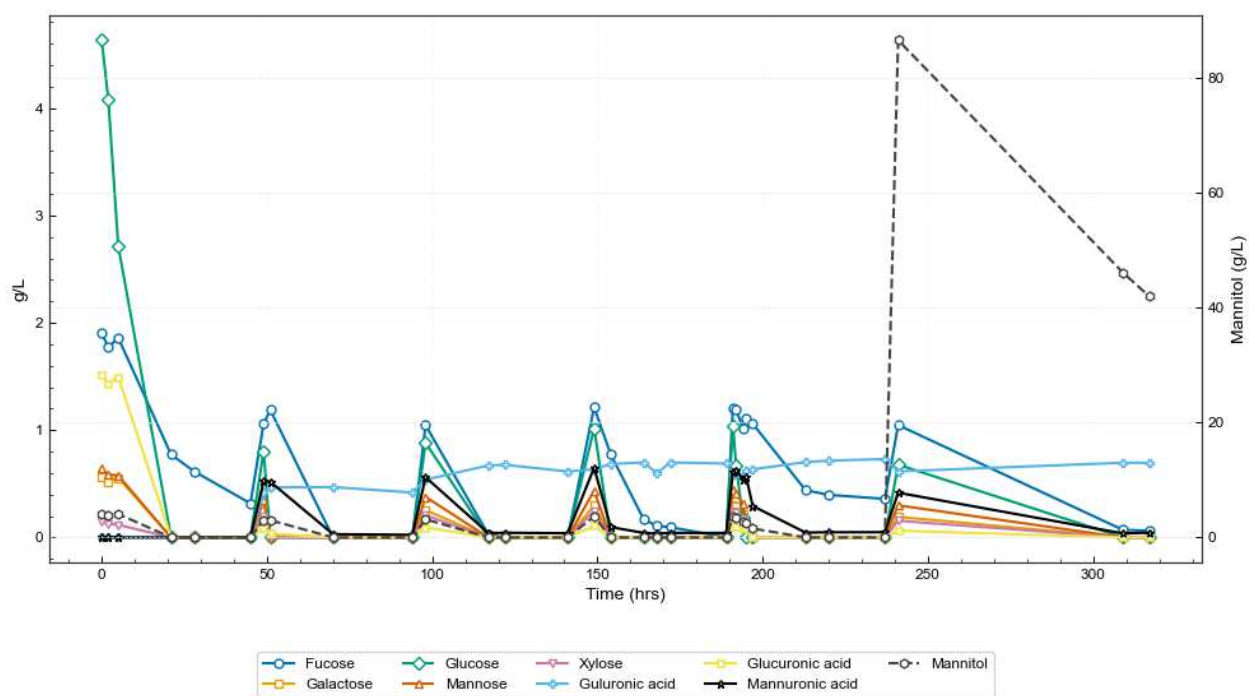


Figure S-3: Illustration of saccharide utilization of *Sargassum* hydrolysate over 6 consecutive feeding cycles of a fed-batch reactor with a continuous cell recycle set up

Table S-11: Overview of lipid yield, rate(productivity), titer for each of the consecutive feeding cycles of the continuous cell recycle system.

	Batch	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5
% FAME	16	18	17	17	19	54

Titer (g/L)	1.2	1.7	1.7	2.2	2.3	12.9
Yield (g/g)	0.09	0.09	0.00	0.06	0.01	0.22
Rate (g/L/hr)	0.026	0.018	0.012	0.011	0.010	0.041
Period (min)	45	45	43	40	46	76
Period Rate (g/L/hr)	0.026	0.011	0.000	0.000	0.002	0.133

Table S-12. Summary of raw material costs and relative contributions to MSSP for the base case TEA.

	Cost	Unit	Contribution to MSSP (\$/tonne)
Raw Materials	(2020\$)		
<i>Sargassum</i> Feedstock	\$253	\$/tonne	348.0
Sulfuric Acid	\$128	\$/tonne	27.7
NaOH (pretreatment)	\$569	\$/tonne	93.6
Ammonia	\$453	\$/tonne	1.6
Diammonium Phosphate	\$392	\$/tonne	8.5
Hexane	\$1,379	\$/tonne	9.8
Ethanol	\$2.53	\$/gal	8.5
NaOH (saponification)	\$569	\$/tonne	1.3
Phosphoric acid	\$910	\$/tonne	2.5
Natural gas	\$4.10	\$/MMBTU	70.3
Process Water	\$0.37	\$/tonne	6.1
Waste Treatment			
Wastewater	\$0.48	\$/kg organic removed	74.2
Products	Value (2020\$)		
Lipids	\$1,100	\$/tonne	-50.5
Electricity to grid	\$0.0572	\$/kWh	-21.5

Table S-13. Summary of annual fixed costs for the base case TEA. Maintenance costs are calculated as 3% of inside battery limits (ISBL) costs. Property insurance and taxes are calculated as 1% of the fixed capital investment.

Fixed Operating Costs	\$/year
Labor and Supervision	
Total Salaries	\$1,621,147
Labor Burden (90%)	\$1,459,033
Other Overhead	
Maintenance	\$12,242,512
Property Insurance and Taxes	\$5,774,259
Total Fixed Costs	\$21,096,951

Table S-14. Summary of capital expenditures for the base case TEA.

Process Area	Installed Cost
Pretreatment and Conditioning	\$55,132,000
Fermentation	\$206,778,000
Lipid Extraction and Cleanup	\$31,125,000
Protein/Residual Processing	\$115,049,000
Combined Heat and Power	\$27,554,000
Utilities & Storage	\$8,507,000
Total Installed Equipment Cost	\$444,145,000
Added Direct + Indirect Costs (% of TCI)	\$422,134,000 48.73%
Total Capital Investment (TCI)	\$866,279,000