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Review

Sustainable Seaweed Biotechnology Solutions for Carbon Capture, Composition, and Deconstruction

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Seaweeds or macroalgae are attractive candidates for carbon capture, while also supplying a sustainable photosynthetic bioenergy feedstock, thanks to their cultivation potential in offshore marine farms. Seaweed cultivation requires minimal external nutrient requirements and allows for year-round production of biomass. Despite this potential, there remain significant challenges associated with realizing large-scale, sustainable agronomics, as well as in the development of an efficient biomass deconstruction and conversion platform to fuels and products. Recent biotechnology progress in the identification of enzymatic deconstruction pathways, tailored to complex polymers in seaweeds, opens up opportunities for more complete utilization of seaweed biomass components. Effective, scalable, and economically viable conversion processes tailored to seaweed are discussed and gaps are identified for yield and efficiency improvements.

Highlights

A realistic framework around seaweed carbon capture potential and seaweed biomass conversion is needed to guide future bioenergy production.

Maximizing carbon storage in the biomass based on marine agronomy in the underutilized exclusive economic zone of the oceans represents significant untapped resources.

Using biotechnological applications for deconstruction of the famously complex polysaccharides provides a route towards the discovery of new enzymatic cascades present in microbial communities.

Seaweed-based industrial biotechnology allows for exploitation of the intrinsic biomass value based on its biochemical composition.

It is necessary to place the seaweed biorefinery discussion in the context of the large-scale, offshore farms that are envisioned for bioenergy production to create market opportunities commensurate with the volumes produced.

The Timely Promise of Seaweed Biotechnology for Carbon Capture

The landscape of future sustainable biobased fuels and products will likely rely on a portfolio of different feedstock sources to meet the growing demand for replacements for petroleum-derived fuels and products [1–3]. One of the promising emerging sources in this area is biomass derived from seaweed (or macroalgae). Macroalgae are capable of producing more biomass per acre in offshore marine farms compared with their terrestrial crop counterparts and can be sustainably harvested and produced without utilizing valuable arable land or unsustainable nutrient requirements [4–9]. In particular, the topical emergence of seaweed in algal blooms occurring around the world has the bioenergy community focused on developing solutions to harvest and then maximize the conversion and recovery of the biochemicals entrained in the biomass. Similar to microalgae, the biomass conversion process for seaweeds is modeled as a biorefinery and necessitates a discussion around biomass composition, intrinsic value of the components, and, ultimately, conversion to products as a success metric [1]. Despite this potential, there are significant challenges associated with realizing both the cultivation and harvesting logistics, as well as in developing an efficient biomass deconstruction and conversion platform to fuels and products. Effective, scalable, and economically viable conversion processes tailored to seaweeds are discussed in detail and gaps are identified that outline the needs for yield and efficiency improvements. In particular, the recent interest and multiyear, multimillion dollar investment of the Department of Energy's Advanced Research Projects Agency-Energy supports a shift in both government and industry interest (https://arpa-e.energy.gov/?q=arpa-e-programs/mariner).

The concept of developing a biorefinery approach to maximize the value derived from seaweed biomass is represented in the literature but is often not placed in the context of sustainable

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bioproducts and bioenergy productions. Often, literature reports relate to the ongoing challenges with respect to carbon capture potential in an agronomic setting that does not compete within a food versus fuel debate. With the discussion here, we aim to drive the narrative to a more realistic framework around seaweed carbon capture and bioenergy conversion. This review will be focused around the following topics: carbon storage in the biomass and carbon capture potential based on the underutilized exclusive economic zone (EEZ) of the oceans that represent ideal marine agriculture locations; application of biotechnological applications towards deconstruction of the complex biomass composition, including the discovery of new enzyme cascades present in microbial communities; consideration of seaweed intrinsic biomass value based on composition and the challenges associated with in-depth characterization of macroalgae; and placing the biorefinery discussion in the context of the large-scale, offshore farms that are envisioned for bioenergy production and thus create market opportunities commensurate with the volumes produced.

Seaweed as a Sustainable Bioenergy Feedstock

Global Seaweed Production Potential

In the context of supplying a feedstock for bioenergy applications, the primary consideration is the scale of production capacity. It is estimated that current global marine agronomy is able to produce around 30 MMT (million metric, dry, tonnes) of seaweed per year, of which production in the USA is estimated to be around 425 000 T [10,11]. Of this, 29.4 MMT was cultivated in a marine agriculture setting and produced and 1.1 MMT was harvested in the wild. The primary driver for this production is to support the growing global markets in seaweed-derived hydrocolloid polymers and other high-value products for food, feed, and agriculture application [12]. Almost all of this production is driven by a couple of species: *Kappaphycus alvarezii*, *Eucheuma* spp., *Laminaria* spp., *Gracilaria* spp., *Undaria pinnatifida*, *Porphyra* spp. (some species in this genus were renamed as *Pyropia* spp., a.k.a. nori), *Sargassum fusiforme*, and *Ulva* spp. in global production [10,12–14]. Most recently, there is an additional and growing interest in harvesting fast-growing seaweeds that are entrapped in natural algae blooms for conversion to bioenergy. The unpredictability of algal blooms may present challenges in a bioenergy supply chain, however, it is a source that is currently not utilized and may become a supplemental source of biomass [15].

When comparing the current production levels with the total entrained energy potential of seaweed, there is potential for seaweed to displace a significant burden on imported energy and thus contribute to the global carbon capture and utilization solution. In particular, the EEZ of the USA represents a significant portion that could be used for aquaculture and marine agronomic development (Figure 1). In the context of the large marine ecosystems approach to preserve and restore the natural ecosystems around the world, there are tremendous opportunities to develop a sustainable macroalgae-based aquaculture. The value that macroalgae provide to global geochemical cycling (among other benefits) includes a carbon and excess nutrient-capture approach. Especially in the Gulf of Mexico, macroalgae opportunities exist and address the needed nutrient cycling that is rapidly becoming a priority for ocean and coastal management [16]. Similarly, offshore large-scale integrated multitrophic aquaculture is a promising route to support aquaculture in combination with effective biofiltration of excess nutrients [17]. If only a fraction (~2.5%) of the EEZ (250 000 km^2) could be used for deploying a national marine agriculture program, estimated (future) yields of between 300 and 1120 million tons of seaweed can be achieved [5,13,18]. Assuming an effective conversion process is available to convert at least half of the energy entrained in the harvested macroalgal biomass (with an assumed caloric content of 14 MJ kg^{-1}), to for example biogas, between 10% and 20% of the 31 Quad BTU (equivalent to approximately 31 exajoules, EJ) imported fossil energy natural gas used in the USA in 2018 (https://www.eia.gov/energyexplained/us-energy-facts/) could be displaced. Similarly, if an

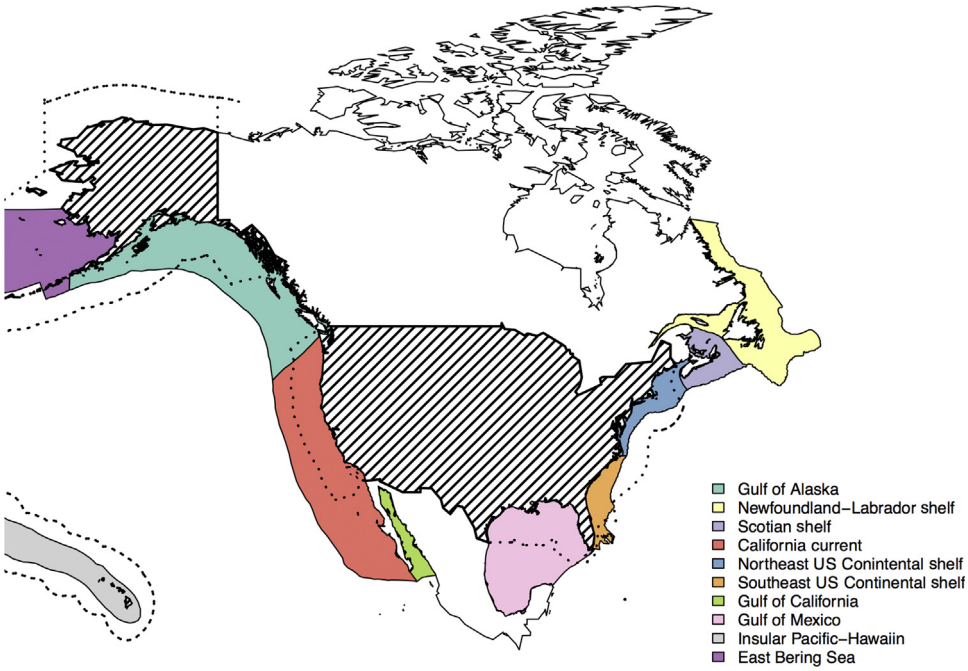

Figure 1. Visualization of the Exclusive Economic Zone Ocean Locations of the USA. This is where the USA has exclusive jurisdiction over the natural resources, of almost 10 million km^2 (3.4 million square miles), extending up to 200 nautical miles from territorial shores (broken lines off the coast), overlaid with the large marine ecosystems around the US coastline (colored regions). Created using Open Access shapefiles available from Flanders Marine Institute, GeoNetwork Open Source (geonetwork-opensource.org) and ThematicMapping (thematicMapping.org).

efficient, high yielding, biofuel conversion process is found, a similar fraction of the imported petroleum (36 Quad BTU, or 36 EJ) can be displaced. These values correspond to just over 200 million tons of CO_2 [19,20]. Since much of the EEZ is currently underutilized, a fraction of this area could be developed for marine bioenergy applications, while respecting competing uses for ocean resources [21].

The carbon capture potential of cultivated seaweed at least matches and often exceeds that of terrestrial farmed crops, with minimally intensive agricultural practices and nutrient requirements. Seaweed biomass productivities of between 1450 and 14 000 T volatile solids (VS, or ash-free dry weight) km^{-2} $year^{-1}$ have been reported, corresponding to between 6 and 57 dry T $acre^{-1}$ $year^{-1}$, depending on species and growth environment, that is, wild harvest or (intensively) cultivated (Table 1) [5,13,18,20,22,23]. This compares against 40 fresh T $acre^{-1}$ $year^{-1}$ for sugar cane as the representative highest yielding terrestrial crop (yielding 5 T sugar $acre^{-1}$ $year^{-1}$) (USDA, ERS, 2019 figures, www.ers.usda.gov/media/8310/table15.xls). Because of the order of magnitude reported range in seaweed productivity, it is hard to estimate the derivative data on carbon capture potential of seaweed. For carbon capture calculations, we have assumed a 30% carbon content on a dry weight basis, comparable with measured and representative carbon in wild-harvested *Ulva*, *Sargassum*, *and Gracilaria* 27–32%, or 37–45% on a VS basis (Box 1). For the purpose of this review, it is important to remain cognizant of both the seaweed production potential as well as the variation in carbon content, which, when both are optimized, can have a positive impact on overall carbon capture potential of the future marine agronomy.

Recently, new opportunities in marine bioenergy technology development were included in a comprehensive report, for example, 'Powering the Blue Economy' [11], positioning seaweed as

Table 1. Summary of Reported Seaweed Biomass Productivity Potential and Associated CO_2 Capture

Species	Biomass T VS km^{-2} $year^{-1}$ (T VS $acre^{-1}$ $year^{-1}$)[a]	Biomass (g VS m^{-2} day^{-1})	Carbon T km^{-2} $year^{-1}$ (30% C)	CO_2 capture T km^{-2} $year^{-1}$ (T $acre^{-1}$ $year^{-1}$)	Refs
ND	1450 (6)	4.0	435	1595 (6)	[13]
Macrocystis pyrifera	1800 (7)	4.9	540	1980 (8)	[24]
M. pyrifera	2000 (8)	5.5	600	2200 (9)	[5,13]
Ulva lactuca	4500 (18)	12.3	1350	4950 (20)	[18]
Laminaria longicruris	7407 (30)	20.3	2222	8149 (33)	[23]
Gracilaria chilensis	14 000 (57)	38.4	4200	15 400 (62)	[20]

[a]Data collected across literature reports and normalized based on areal productivity, all assuming a 30% carbon content on the basis of volatile solids (VS), or ash-free dry weight; ND refers to an undefined seaweed species and an average (conservative) value of biomass productivity [13].

a supply of liquid biofuels and energy for offshore installations, as an integral part of a future vision of marine renewable energy.

Harvesting and Processing Logistics

While seaweed farming has existed for years, primarily for food and feed applications, a vision for marine agronomy at the bioenergy scale needs to take into account the costs of all aspects of processing along the seaweed value chain [25]. The logistics of harvesting a crop that is produced in near-shore waters is a complex process and there are significant challenges associated with recovering the biomass feedstocks for on-shore conversion. While an in-depth review of the harvesting and processing logistics of different seaweeds is outside of the scope of this review, we briefly introduce some important considerations. In some examples, the crop may be completely removed to be replanted the next season, in other cases, 10–40% of the biomass is left behind to start the next cultivation cycle [26]. Both seeding and harvesting operations rely primarily on human labor and, generally, overall process requirements for large-scale macroalgae operations at sea are challenging and only industrialized for some species of seaweeds [14,21,27]. Because of the highly manual nature of the process, the scale of implementation is currently limited and needs to be automated to reach autonomous harvesting operations for full-scale bioenergy feedstock production [19,21,28].

Seaweed Composition

Biomass Composition

Similar to other cultivated bioenergy crops, the high polysaccharide content of seaweeds positions them well to meet the demands of a changing market for producing both fuels and a suite of products [29–31]. The biomass composition changes dramatically for different algae species and across seasons for some genera of seaweeds [32]. An in-depth compositional profiling of the seaweed biomass can thus inform a different product portfolio by species selection (Box 1). Other influences on the biomass composition can be assigned to environmental manipulation. This unique contribution of seaweeds can be aligned with the appropriate conversion or fractionation technology, allowing for the utilization of algal biomass components to ultimately help to drive down the cost of bioproducts compatible with a large-scale conversion process.

The genera *Sargassum*, *Laminaria*, *Porphyra/Pyropia*, *Gracilaria*, and *Ulva* are representatives of the primary taxa of seaweeds. The primary discussion around the composition of seaweeds will focus on representatives from this set, distinguished by large phylogenetic phyla of green (e.g., *Ulva*), red (e.g., *Gracilaria*, *Porphyra/Pyropia*), and brown (e.g., *Sargassum*, *Laminaria*) seaweeds. This simple classification allows for some generalizations to be drawn with respect to the presence of

Box 1. Compositional Profile of Major Seaweed Representatives

Distinct biomass compositional profiles are associated with each of three representative seaweed species, *Ulva fasciata*, *Gracilaria parvispora*, and *Sargassum echinocarpum* (Table I). These three genera are of interest in current research on conversion to bioenergy products and their respective biomass composition is described in detail here. Consistently, about a third of the biomass is entrained in ash, with the majority of the rest of the measured biomass composition found in the carbohydrate fraction, which reaches around 40% of the ash-free portion of the biomass, and is likely higher due to the incomplete measurement of all monosaccharides that make up the complex polymeric structures. On an ash-free or volatile solids (VS) basis, the biomass carbon content reaches up to 45% for *Sargassum*, consistent with the high observed carbohydrate content.

When looking at the molecular make-up of the monosaccharides that comprise the carbohydrate fraction (Table II), there are distinct sugars that can be found in the hydrolyzed liquors from each of the species. In the case of *Ulva*, the primary monosaccharides are glucose, rhamnose, and uronic acids, consistent with published reports on the presence of cellulose in green algae [76], while for *Gracilaria* it is galactose (most likely derived from the agar polymers) and glucose, and for *Sargassum* primary components are the uronic acids and glucose. Such different compositional structures will undoubtedly require adaptation of a selective fermentation approach [64].

The lipid content, reported as the sum of the fatty acids after *in situ* transesterification [77] is shown in Table I, while the fatty acids that make up the lipids before and after washing the biomass upon wild harvest are shown in Table III. In Table III, all data are expressed on a total fatty acid methyl ester (FAME) basis, reported on an ash-free dry basis (i.e., VS) after lyophilization of the biomass. All fatty acids are presented by their [carbon number]:[number of unsaturated bonds along the carbon chain]; C11CP and C13CP refer to the cyclopentyl dihydrochaulmoogric and dihydrohydnocarpic acids, respectively, as previously identified [78,79] and quantified by gas chromatography after *in situ* transesterification [77,80].

A fatty acid diversity and specificity was observed here that is consistent with the higher unsaturated fatty acids that make up algae lipids [29]. In particular, omega-3 polyunsaturated fatty acids (e.g., eicosapentaenoic acid, C20:5*n*3) were detected, albeit at very low concentrations of the biomass, in *Gracilaria* and *Sargassum*, potentially opening opportunities for high-value product extraction. Because the analytical methodology is based on a direct, or *in situ*, transesterification of the whole biomass, data on the origin of the fatty acids (i.e., which intact lipid the fatty acids were associated with) is not available. Cyclopentyl dihydrochaulmoogric and dihydrohydnocarpic acids, unusual metabolite fatty acids, have been detected in *Gracilaria*; these function as intercellular metabolites and 'local hormones', in particular in the red algae family [78,79]. The proposed identity of the fatty acid derivatives is based on electron impact fragmentation data by gas chromatography mass spectrometry for the two different products; their respective quantification is based on a similar size fatty acid standard, indicating that both together account for almost 15% of the total fatty acids and should not be dismissed as lipid contributors. Because of their so far unknown biological activity, their impact on a biological conversion process could be significant.

Table I. Overview of Macro-Elemental and Biochemical Composition of Three Seaweed Species, *Ulva fasciata*, *Gracilaria parvispora*, and *Sargassum echinocarpum*

	Ash (% DW)[a]	C (% VS)	H (% VS)	N (% VS)	Lipids (as FAME)[b] (% VS)	Protein (% VS)	Carbohydrates (% VS)
Ulva fasciata	28.57	38.2	6.7	2.9	1.55	13.92	42.8
Gracilaria parvispora	33.91	41.6	6.5	3.6	3.35	17.32	38.67
Sargassum echinocarpum	27.38	45.1	6.3	1.8	2.78	8.82	38.26

[a]All data are expressed on either a dry basis (%DW) or an ash-free dry basis (i.e., volatile solids, %VS) after lyophilization of the biomass upon wild harvest off the coast of Kailua Kona, Hawaii in June 2019.
[b]FAME, fatty acid methyl ester after direct transesterification of whole biomass, protein, and carbohydrates, measured as described in [43,73–75].

Table II. Overview of Biomass Monosaccharide Composition for Three Seaweed Species, *Ulva fasciata*, *Gracilaria parvispora*, and *Sargassum echinocarpum*

	Fucose[a]	Rhamnose	Galactose	Glucose	Mannose	Xylose	Mannitol	Uronic acids
Ulva	0	11.3	0.46	16.02	0	4.63	0	10.39
Gracilaria	0	0	23.79	12.66	0.66	0.88	0	0.69
Sargassum	3.96	0	1.79	10.27	1.09	0.57	5.04	15.54

[a]All data are expressed on an ash-free dry basis (i.e., volatile solids) after lyophilization of the biomass and sulfuric acid hydrolysis, followed by anion exchange chromatography quantification [43,44]. Arabinose and ribose were not detected in any of the seaweed samples.

Table III. Overview Biomass Lipid (Fatty Acid) Composition for Three Seaweed Species, *Ulva fasciata*, *Gracilaria parvispora*, and *Sargassum echinocarpum*

	C14:0	C16:0	C16:1n7	C18:0	C18:1n9	C18:1n7	C18:2n6	C18:3n3	C18:4n3	C20:3n6	C20:4n6	C20:5n3	C22:0	C22:1n9	C11CP	C13CP
Ulva	0.8	62.2	1.6	1.0	2.1	10.5	3.7	4.6	1.4	0.0	0.0	0.0	4.2	0.4	0.0	0.0
Gracilaria	0.7	38.4	0.4	0.7	2.4	1.1	0.3	0.0	0.0	1.5	18.0	12.9	0.0	0.1	11.3	2.6
Sargassum	4.5	36.0	2.9	0.9	13.3	0.3	4.0	6.6	6.4	0.8	14.3	4.4	0.7	0.2	0.0	0.0

distinct polymeric structures [33]. For example, seaweed cell walls are known to contain (practically) no lignin and only low amounts of cellulose and lipids, which renders the seaweed-based biomass potentially higher in polysaccharides accessible for conversion processes, compared with hard-to-digest terrestrial ligno-cellulosic feedstocks [34,35]. Brown seaweeds can be rich in polyphenols, which are not only difficult to degrade but could also inhibit anaerobic digestion [36,37]. Brown seaweeds (e.g., *Laminaria*) are used as feedstocks to produce polysaccharides (e.g., alginate), which can find applications as thickeners, gelling agents, and stabilizers for frozen food and cosmetics [33,35,38,39]. Red seaweeds, such as *Gracilaria* and *Kappaphycus*, contain large quantities of agar and carrageenan, respectively. These industrial polysaccharides are often used in the food, pharma, textile, paint, and biotechnology industries, including antifouling, antibiotic, and antimalarial applications [40]. Similarly, green seaweeds such as those of the Ulvales family, are known to produce a complex, acidic, polysaccharide ulvan, also finding applications in the medical and cosmetics industries [38,41]. These polysaccharides also could also be exploited as prebiotic functional ingredients for both human and animal health applications [6,12].

It is critical that a robust analytical pipeline for macroalgae is focused around polysaccharide components, with the aim of defining the basis of biomass conversion and ultimately guiding the detection of products. While many surveys have been conducted on the major macromolecular composition of seaweeds in order to identify strains for different applications, the quantification methods have varied tremendously across the published literature. Precisely these biases in methods used for compositional characterization of the materials are at the basis for a problematic comparison of literature reports on seaweed biomass composition [42]. The value of understanding the macromolecular composition of seaweeds on a consistent basis will help to develop a basis for valuation of biomass and help tailor specific conversion pathways to maximize the deconstruction and bioenergy production.

While there are significant challenges associated with collecting quantitative characterization of seaweed biomass composition [42], a relative comparison between the three major taxa is shown in Tables I–III in Box 1. The basis of compositional analysis of seaweed polysaccharides, as recently recommended [43], includes a sulfuric acid hydrolysis process to monomeric sugar constituents that are subsequently quantified based on a liquid chromatography separation protocol [43,44]. Alternative methods are available for carbohydrates, however, not all have merit in the analytical characterization of algal biomass [42]. Using the hydrolysis and chromatography approach, it is clear that different species of seaweed can be classified based on the released monomeric sugar composition, such as rhamnose, fucose, guluronic acid, and mannuronic acid, along with many different derivatives (e.g., sulfated monomers), and thus a biochemical conversion process would need to be tailored specifically to metabolize these monomers.

Seasonality of Ultimate and Proximate Composition

The compositional characteristics can be represented in the elemental composition of seaweeds, which are usually measured as proximate [total solids (TS) and VS] and ultimate (carbon, hydrogen, and nitrogen) compositional analysis. The biodegradable element of the seaweed is also referred to as VS and salt is a major constituent of ash in seaweed. The total and VS content of all seaweeds range with species and with season [32,45,46]. With any changes in the biochemical composition of the seaweeds over different seasons, the respective process yields for any relevant bioenergy pathway are likely to be affected [32,45,47,48].

The elemental composition, and in particular the ratio between carbon and nitrogen (C:N), is an important characteristic of seaweed and often provides information that can be used to estimate the effectiveness of a conversion process [45,46]. Many brown seaweeds have C:N ratios in

excess of 15–20, while some green seaweeds (e.g., Ulva), had a low ratio, of 10 or lower [49]. This allows an elemental formula to be developed to describe the TS content of the substrate. For example, *Ulva* spp. generated the elemental formula $C_9H_{16}O_7N$ [50]. These ratios can have significant implications on downstream conversion processes, for example, for anaerobic digestion, the optimum C:N ratio is in the range of 20:1 or 30:1, with the much lower ratios of C:N causing levels of ammonium in the digester that could inhibit effective deconstruction and fermentation [51].

Limited information is available around the seasonality of compositional changes of seaweed composition, with the exception of a couple of detailed studies [32,47,52,53], but observations date back to the 1950s, where ash and carbohydrates were the predominant components tracked [54]. In particular, when looking at the laminarin and mannitol concentrations in Laminariaceae, these carbohydrates were found to be highest in October and lowest in winter months, with the ash proportion showing a reverse trend [54]. Similarly, the highest alginate content in kelp species (*Macrocystis pyrifera*) was in the summer months [52]. By contrast, protein content was found to be highest in the winter months and lowest during the summer [53,55].

However, some work on the elemental composition (ultimate and proximate) of *Laminaria digitata*, harvested over the course of a year, supports the notion that the highest carbohydrate content biomass coincided with the highest biomethane potential in a continuous anaerobic digester [45]. This indicates that the underlying biochemistry and storage products in the feedstock can dramatically influence the predicted conversion process characteristics.

Structural Biochemical Polymers

Seaweed polysaccharides are famously complex and have unique properties that make these polymers highly attractive in biotechnological applications (Box 2) [41]. The other side of the complexity is that these polymers are different when compared with terrestrial feedstocks (e.g., ligno-cellulosic biomass) for bioenergy production and thus not directly compatible with existing technologies and process operations, suggesting that much more research is needed to both elucidate the structure and associated needed degradation pathways.

Because the described complexity of the structural polymers that make up the polysaccharide or carbohydrate fraction of seaweed, there are still challenges in converting these polymers to useful products. While there have been a number of reports on the utilization and fermentation of the monosaccharides [56–58] that make up the polymers, there is little information on the deconstruction of the polymers beyond pretreatments.

Microbial Degradation of Structural Polymers

One of the critical challenges to macroalgae or seaweed conversion and degradation is tailoring the microbial deconstruction activities to the seaweed chemical compositional profile. There are numerous reports on the bioconversion (e.g., fermentation of released monosaccharides from seaweeds [33,48,57–62]. Products such as ethanol, butanol, and acetic, propionic, butyric, lactic, adipic, and succinct acids can be biologically produced from existing microbial platforms and become intermediates for next-generation fuels and bioproducts [61]. Typically, the organisms used for fermentation are not tailored to the unique molecular make-up of the hydrolyzed sugar liquor and are often unable to metabolize, for example, uronic acids and other saccharides [63]. Easily fermented sugars, such as glucose, galactose, mannose, and others, can comprise a smaller contribution of the biomass, with much more prominent contributions from deoxy- and anhydro-saccharides (fucose, rhamnose, anhydrogalactose, etc.), hydrogenated sugars (e.g., sugar alcohols such as mannitol), and uronic acids (Box 1). To achieve the necessary high yield and titer of fermentation products, there is a need for microbial organisms that are

able to effectively assimilate and metabolize these unique saccharides. Recent work has made progress in overcoming metabolic bottlenecks. In particular, the successful transfer of a uronic acid transporter, along with the required catabolic genetic machinery to a yeast host for the production of ethanol from brown seaweed monosaccharides, achieved up to 83% of the theoretical ethanol yield from consumed mannitol and uronic acids [64]. Simultaneously, the redox balance, and central metabolism energy resource allocation, needs to be carefully engineered to ascertain no yield losses occur [61,65].

While most of the bioenergy fermentation of seaweed polysaccharides have focused on metabolizing the unusual monosaccharides found in brown seaweed, it is understood that if the polysaccharide hydrolysis preceding the fermentation is not complete, a large fraction of the biomass remains untapped. In this context, metrics such as hydrolysis and fermentation carbon yield are important and are not always reported on the basis of the polysaccharide content in the original biomass [48,61].

There are large numbers of bacterial populations found on seaweeds that are being elucidated [66] and are an untapped and highly promising resource to find specific metabolic and fermentative activities. Many marine microbial communities are found naturally occurring on seaweeds or forming the basis of the microbiome in herbivorous fish and thus are able to utilize the seaweed polysaccharides as energy sources [67,68]. Recent work has begun to take a metagenomic approach to look specifically for bacterioplankton composition and microbial structure function degradation

Box 2. Complex Biopolymers of Prominent Seaweeds

Seaweed composition is dominated by polysaccharides that have highly complex compositional profiles. In the case of brown seaweeds, biomass structural heterogeneity plays a role in the respective polysaccharide contribution (Figure I). For example, the co-occurrence of multiple polysaccharides gives these seaweeds structural integrity but also challenges conversion approaches. We document here a summary of the primary polysaccharides and their respective molecular structures, illustrated in Figure I. The polysaccharides are present in the three main seaweed families and we focus on unique polysaccharides beyond cellulose and starch, which are thought to be minor contributors to seaweed polysaccharides and have been described previously [57].

Agar, found in red seaweeds, such as *Gracilaria*, is a heteropolymer mixed of two polysaccharides: agarose and agaropectin, with agarose making up the majority of the mixture. Agarose is a linear polymer, made up of repeating units of agarobiose, a disaccharide made up of D-galactose and 3,6-anhydro-L-galactopyranose (Figure IIA). Agaropectin is a heterogeneous mixture of smaller molecules that occur in lesser amounts and is made up of alternating units of D-galactose and L-galactose, heavily modified with acidic side-groups, such as sulfate and pyruvate [81].

Carrageenan is also an anionic polysaccharide, also typically found in the red seaweeds, where the monosaccharide units are sulfated. The polymer consists of straight chain backbones of alternating 3-linked β-D-galactopyranose, 4-linked α-galactopyranose residue. Some of the α-galactose residues may be in an anhydrous form and be substituted by sulfate ester, methyl groups, and pyruvic acid acetals. The 3,6 anhydro-α-D-galactose is responsible for gelation of the carrageenan polymers. A representative structure is shown in Figure IIF.

Laminarin is a storage polysaccharide, primarily accumulated by brown seaweeds, such as *Sargassum*, and consists of a β-1,3-glucan with β-1,6 branches, with terminal mannitol residues on the chains. It is a linear polysaccharide, with a β-1,3:β-1,6 ratio of 3:1 and its hydrolysis is catalyzed by enzymes such as laminarinase. A representative trimer is shown in Figure IIB.

Fucoidan consists of a backbone of sulfated fucans, or α-L-fucopyranose residues, and other minor sugars such as xylose, galactose, mannose, and glucuronic acid, typically isolated from brown seaweeds (e.g., *Fucus vesiculosus* or *Sargassum*). The fucoidan polymer contains a linear backbone of 1,3 linked, sulfated α-L-fucopyranose (Figure IIC) with branches of several residues as α-D-glucopyranosyluronic acid residues or α-L-fucopyranosyl residues, which forms quasi-regular carbohydrate chains with hexasaccharide [41].

Ulvan, a branched polysaccharide, typically isolated from the green seaweed *Ulva*, is composed of repeating disaccharide units, in which D-glucuronic acid (GlcA) is β-1,4-linked or L-iduronic acid (IdoA) is α-1,4-linked to L-rhamnose 3-sulfate (Rha3S) (Figure IID), which is α-1,4-linked within the main chain. Some of the uronic acids are replaced by β-1,4-linked D-xylose (Xyl), which can be sulfated at position 2 (Xyl2S). Furthermore, Rha3S can be modified by β-1,2-linked GlcA side chains and the GlcA-Rha3S or IdoA- Rha3S pattern can be interrupted by consecutive GlcA residues [82].

Alginate is an anionic polysaccharide comprised of mannuronic acid and guluronic acid; it has gelation properties in water and is typically found in highest concentrations in brown seaweeds, including *Sargassum*. The main building blocks are (1,4)-linked α-L-guluronic acid (G residues) and (1,4)-linked β-D-mannuronic acid (M residues) and structures that contain alternating M and G residues. A representative structure is shown in Figure IIE.

Figure I. Macroscopic View of Biomass Heterogeneity (Air Bladders, Blades, and Stipes) of *Sargassum muticum*, an Invasive Species. Seaweed collected from local San Diego shores, used for microbial degradation studies (image courtesy of authors).

adaption that is adapted to live off the dissolved organic carbon (DOC) exudates from seaweeds [67,68]. The DOC studied comprised a complex mixture of major chemical compound classes of carbohydrates, protein, and lipids and thus microbial populations found associated with these products are likely harboring the necessary deconstruction and metabolic conversion machinery and can provide insights into seaweed polymer deconstruction activities [67,69].

Microbial diversity can present clues to novel activities specifically tailored to help digest the major biomass polymers. As an example, ulvan degradation pathways and enzymatic activity cascades have been identified in a bacterium *Formosa agariphila*, a marine flavobacterium, and build on the elucidation of novel β-glucuronyl hydrolase from the GH105 family [70]. Similarly, a microbial degradation cascade has been successfully engineered in *Escherichia coli*, based on a set of alginate lyase enzymes that were secreted into the immediate vicinity of the bacterial population [56].

The most promising microbes tailored to complex polysaccharide conversion to, for example, organic acids, can be found in representatives from the phyla Bacteroidetes and Firmicutes, with representatives of Clostridiaceae, Ruminococcaceae, and Lachnospiraceae within the Firmicutes phylum. A number of representatives of these genera are known carbohydrate-degrading bacteria, with a very high number of identified carbohydrate-active-enzymes (CAZymes) [71]. In particular, an agar degradation locus was recently discovered and characterized in a representative marine Bacteroidetes [72]. It remains to be demonstrated that such an informed search for novel and enriched microbial activities in seaweed-associated and/or fish-gut-associated microbiome communities can yield genetic information on novel encoded enzymes that can be applied to the biocatalytic degradation of the complex seaweed biomass.

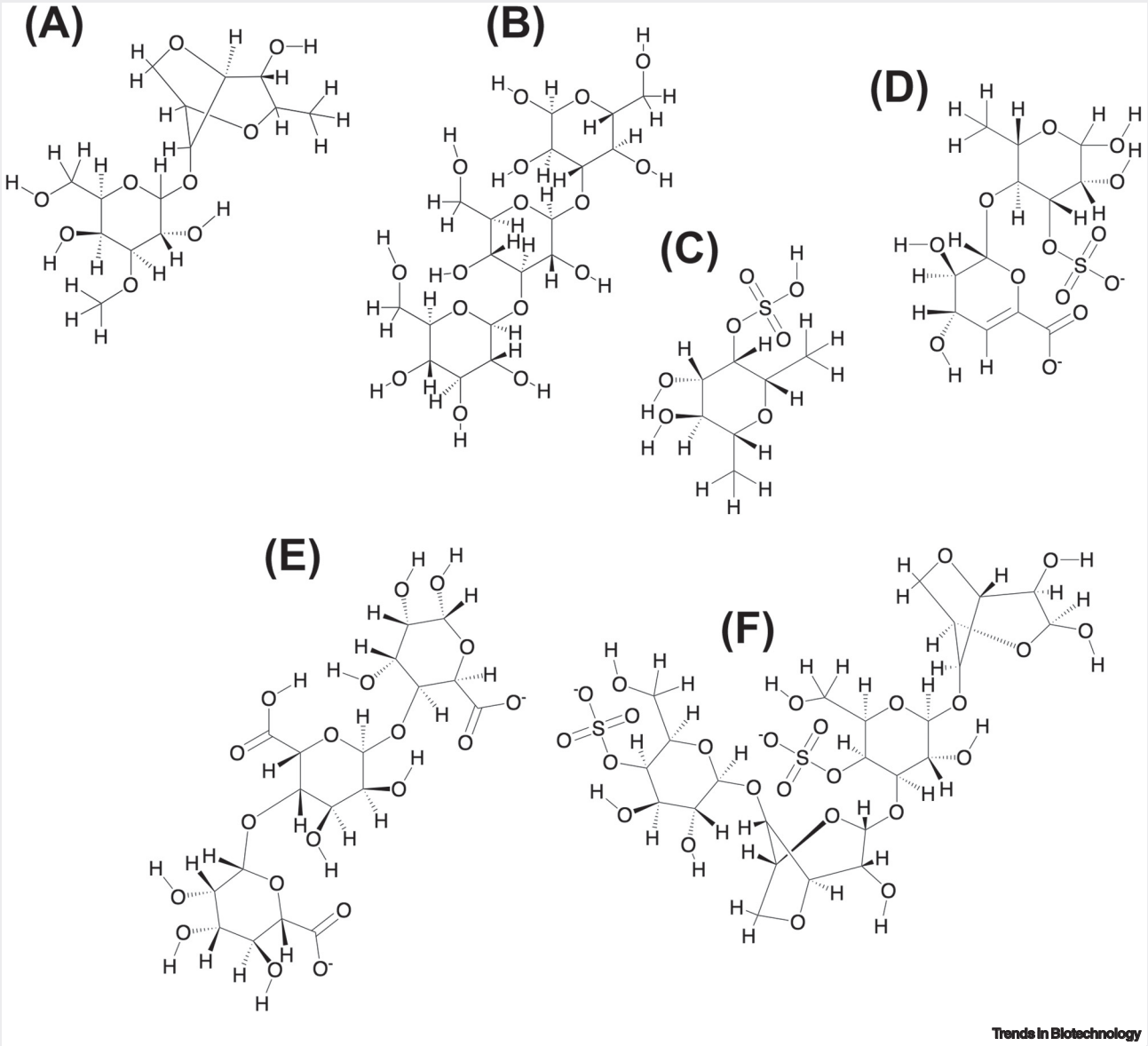

Figure II. Overview of Representative Chemical Structures of Major Representative Seaweed Polysaccharides. (A) Agar (shown as an aragobiose dimer of galactose and 3,6-anhydro-L-galactopyranose); (B) laminarin (shown as a trimer of β-1,3 linked glucose); (C) fucoidan (shown as a sulfated fucan unit); (D) ulvan [shown as α-L-rhamnopyranose 3-sulfate (Rha3S), linked to β-D-glucuronate (GlcA)]; (E) alginate (shown as a trimer of mannuronic and guluronic acid, MGM); (F) carrageenan (shown as a tetramer of alternating 3-linked β-D-galactopyranose and 4-linked sulfated α-galactopyranose residues).

Another remaining challenge is associated with improving accessibility of the microbial communities to the polymers that are needed as a carbon source for downstream conversion and ultimately to maximize the channeling of the biomass carbon in the products. Improving accessibility of the biomass for deconstruction can happen through physical biomass deconstruction and in nature (*in vivo*) can be accomplished by an animal chewing or physically shearing the seaweed to increase the available surface area. In a biological deconstruction process (e.g., in a fish gut), enzymes are secreted into the stomach to further deconstruct the seaweed, increasing

accessibility for the microbes to the biomass. These same strategies can be applied *in vitro* to potentially greater effect. Various milling techniques can reduce particle size and increase surface area. As has been done with terrestrial biomass, cocktails based on microbial enzymes can be optimized and mass produced for seaweed-based biomass.

Concluding Remarks and Future Perspectives

The interface between biotechnology and agriculture is set to position seaweeds or macroalgae in a critical nexus to meet the increasing demands of biomass for fuels and products. The strategic direction for seaweed as a highly efficient photosynthetic biofuel and bioproduct feedstock can be achieved through the integration of biotechnology, process engineering, and analysis. Primary strategies for bioenergy production from seaweed will likely need to rely on a multiproduct biorefinery approach to sustain economically feasible development. One of the products in the biorefinery would be fuels derived from fermentation products, such as alcohols, produced alongside higher-value bioproducts. The basic promise of seaweed-based bioenergy applications is valid; there does not need to be competition with existing food and feed supply or associated land-use change challenges, since seaweeds have the potential to be grown offshore in a globally underutilized EEZ. There is the added advantage of using waste or run-off nutrients as a cultivation fertilizer, allowing for the recovery of nutrients at each step of an integrated process to minimize the pressure on limited available resources (see Outstanding Questions). However, there are significant barriers currently impeding commercialization and economic production of seaweeds for relatively low-value energy and fuel markets. The barriers to commercialization span from accessing seaweed biology and structural composition and deconstruction chemistry (and biochemistry), to challenges associated with the integration of technologies at demonstration scale. Overcoming these barriers will require a concerted effort towards the discovery of novel biological and chemical conversion technologies. One area that shows promise is the realm of bioprospecting for novel microbial activities directed at converting the biomass to novel products. For example, the advent of microbiome sequencing of natural herbivorous fish gut communities provides access to a treasure trove of deconstruction and energy conversion metabolism that can guide novel biotechnology research. Even though many technologies have been demonstrated at the laboratory scale, this most often has focused on specific unit operations or aspects of the technology such that the challenge remains to fully integrate unit operations for bioenergy applications and demonstrate these through extended multiseason operations.

Outstanding Questions

Can future sustainable seaweed farming become a significant source of biomass for bioenergy production exceeding terrestrial areal carbon capture potential?

Which biomass deconstruction activities are most critical in defining and implementing a successful and economically feasible conversion pathway?

Can the deconstruction activities of unique seaweed polymers be tailored for maximum activity through microbial bioprospecting?

Can high-value products from seaweed contribute to the biorefineries of the future in a fully integrated, and economically viable, bioconversion process?

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