Review

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Katherine G. Johnston , Abdelfatah Abomohra , Christopher E. French , Abdelrahman S. Zaky *

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Recent Advances in Seaweed Biorefineries and Assessment of Their Potential for Carbon Capture and Storage

Katherine G. Johnston [1], Abdelfatah Abomohra [2], Christopher E. French [1,3] and Abdelrahman S. Zaky [1,*]

[1] School of Biological Sciences, University of Edinburgh, King's Buildings, Edinburgh EH9 3FF, UK; k.johnst@yahoo.com

[2] Aquatic Ecophysiology and Phycology, Institute of Plant Science and Microbiology, University of Hamburg, Hamburg 22609, Germany; Abdelfatah.abomohra@uni-hamburg.de

[3] Zhejiang University-University of Edinburgh Joint Research Centre for Engineering Biology, International Campus, Zhejiang University, Haining, Zhejiang, 314400, China c.french@ed.ac.uk

* Correspondence: A.S.Zaky@live.com; Tel.: (00447474440333); orcid: 0000-0003-3141-3740

Abstract: Seaweeds are among the most important biomass feedstocks for the production of third generation biofuels. They are also efficient in carbon sequestration during growth, and produce a variety of high value chemicals. Given these characteristics together with the relatively high carbohydrate content, seaweeds have been discussed as an ideal means for CO2 capture and biofuel production. Though third generation biofuels have emerged as some of the best alternatives to fossil fuels, there is currently no large-scale production or mainstream use of such liquid fuels due to the many technical challenges and high production costs. The present study describes the concept of coastal marine biorefineries as the most cost-effective and sustainable approach for biofuel production from seaweeds as well as atmospheric carbon capture and storage (CCS). The suggested refinery system makes use of marine resources, namely seawater, seaweed, and marine microorganisms. Firstly, extensive screening of the current literature was performed to determine which technologies would enable the emergence of such a novel biorefinery system and its merits over conventional refineries. Secondly, the study investigates various scenarios assessing the potential of seaweeds as a means of carbon sequestration. We demonstrate that the removal of 100 Gigatons of excess CO_2 using seaweed farms can be achieved in around 4 months to less than 12 years depending on the area under cultivation and the seaweed species. The total bioethanol that could be generated from the harvested biomass is around 8 trillion litres. In addition, high-value chemicals (HVC) that could potentially be recovered from the process represent a considerable opportunity with multi-billion-dollar commercial value. Overall, coastal marine biorefineries have strong potential for a sustainable green economy and represent a rapid approach for climate change mitigation.

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Introduction

Since the industrial revolution, extensive utilization of fossil fuels has led to increasing greenhouse gas (GHG) emissions, mainly CO_2. This has resulted in an increase in global temperatures, leading to more and more extreme weather phenomena. In response, countries around the world signed the 2016 Paris Agreement as a commitment to combat the ongoing crisis [1]. However, according to the Intergovernmental Panel on Climate Change (IPCC) 2018 special report, today's actions aren't sufficient to stop the rise in temperature, and the report states that global warming will go beyond 1.5 °C by 2030 if the present pollution rates continue [2]. Exceeding the 1.5-2.0 °C temperature limit would lead to irreversible damage to the biosphere. The report also outlines two targets for the coming century: achieving carbon neutrality by 2050 and removal of 100 Gigatons of atmospheric CO_2 by 2100 [3]. These goals highlight the need to replace fossil fuels with more

sustainable energy sources and reduce atmospheric CO_2 levels to maintain the global temperature within the safe range.

Much research is being done to find sustainable alternatives to fossil fuels, with ground-breaking development in renewable electric power. However certain sectors, like transportation, are still reliant on liquid fuels. Biofuels have been discussed as a sustainable replacement to these liquid fuels. The term biofuel refers to extraction of energy from organic biomass in the form of liquid fuel. Biofuels are classified into three generations, depending on their sources [4]. First-generation biofuels are derived from food crop material. Though their production is efficient, the use of crop-based substrates poses a threat to food security. This led to the emergence of second-generation biofuels, derived from lignocellulosic biomass residues and other agricultural wastes. However, the production of second-generation biofuels is unsustainable on economic and environmental grounds due to the required pre-treatment step to degrade lignin [5], which increases the production cost and processing time. Third-generation biofuels can be produced from aquatic feedstocks including micro- and macroalgae as a solution to the aforementioned problems. In recent years, macroalgae (seaweeds) have been studied as a potential substrate for bioethanol [6,7] and biogas [8]. Seaweeds are also more efficient at carbon sequestration than land plants, making them an effective means of carbon capture and sequestration [9]. Furthermore, many seaweed-derived chemicals are of high commercial value.

Reducing the resource input and increasing the co-product potential of the production process are critical aspects for spurring the development of seaweed biorefineries. Thus, coastal-based integrated marine biorefinery (CIMB) systems were suggested for the efficient production of third generation biofuels [10–12]. These systems utilise marine resources (seawater, marine yeast and marine algae) to produce biofuels and high value chemicals (HVC) through integrated biological conversion technologies (i.e., fermentation and anaerobic digestion). The integration of these marine resources and conversion technologies could significantly enhance the production efficiency and totally eliminate the use of freshwater and arable land in biofuel industry. This could also enhance the CO_2 sequestration potential of the process, making biofuels a more sustainable and eco-friendly energy source [13,14]. CIMB systems have not yet been thoroughly researched and therefore, they need intensive investigation. Therefore, the present study aims firstly to discuss the concept of coastal marine biorefineries and their merits over the conventional biorefinery systems. The second part of the study aims to analyse the potential of seaweeds for CO_2 sequestration by calculating the time required to remove 100 GT of atmospheric CO_2 through seaweed farming. In addition, the study determines the total bioethanol and HVC that may be produced from the produced seaweed biomass.

Coastal Marine Biorefinery Systems

The concept of biorefineries for production of liquid fuels is not new, as the processing of different biomass feedstocks into bioethanol is a common practice in many countries such as Brazil and the USA. However, such industries consume huge amounts of freshwater. It was estimated that production of 1 litre of bioethanol requires 5-10 litres of water during the fermentation process alone, with the total water footprint ranging between 1,388 to 9,812 litres when taking into account conventional routes of biomass production [15–17]. Given the major concerns regarding freshwater shortages, this production method is unsustainable, and a coastal marine biorefinery could provide a solution. This is a conceptual refinery system that relies on the use of marine components (seawater, yeast, and seaweeds) to produce biofuels and other valuable products. In the case of bioethanol, seaweeds are used as a feedstock, seawater as a growth medium and marine yeast for marine fermentation (Figure 1). Though research has been done on each individual element, “coastal”, “marine”, “biorefinery”, a single system combining all the three elements has not yet been considered.

All major components of the coastal marine biorefineries offer environmental and economic advantages compared to their conventional counterparts. As will be detailed later in this review, macroalgal biomass is an ideal substrate for bioethanol production. As macroalgae generally grow in marine environments, they do not require freshwater or arable land and therefore do not compete with food production. The successful use of seaweed as a substrate in a biorefinery has been

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investigated in several studies [18–22]. Though often titled "marine biorefineries", these papers focused solely on the marine nature of the feedstock where, in reality, the majority of the processes still rely on the use of freshwater and conventional terrestrial yeast strains. The potential use of seawater and marine microorganisms for marine fermentation has nonetheless been demonstrated. Isolated marine yeast strains, such as *Saccharomyces cerevisiae* AZ65, have shown high capability of producing bioethanol from glucose and molasses in seawater media [15,23]. These strains also produce more bioethanol from glucose compared to terrestrial yeasts and are more tolerant to fermentation inhibitors [23,24]. Seawater's mineral content further eliminates the need for mineral nutrients and enables the production of sea-salts and salted animal feed as co-products. The process of marine fermentation can also yield high quality distilled water [25]. Furthermore, the high-salt environment may reduce the chances of microbial contamination within a bioreactor.

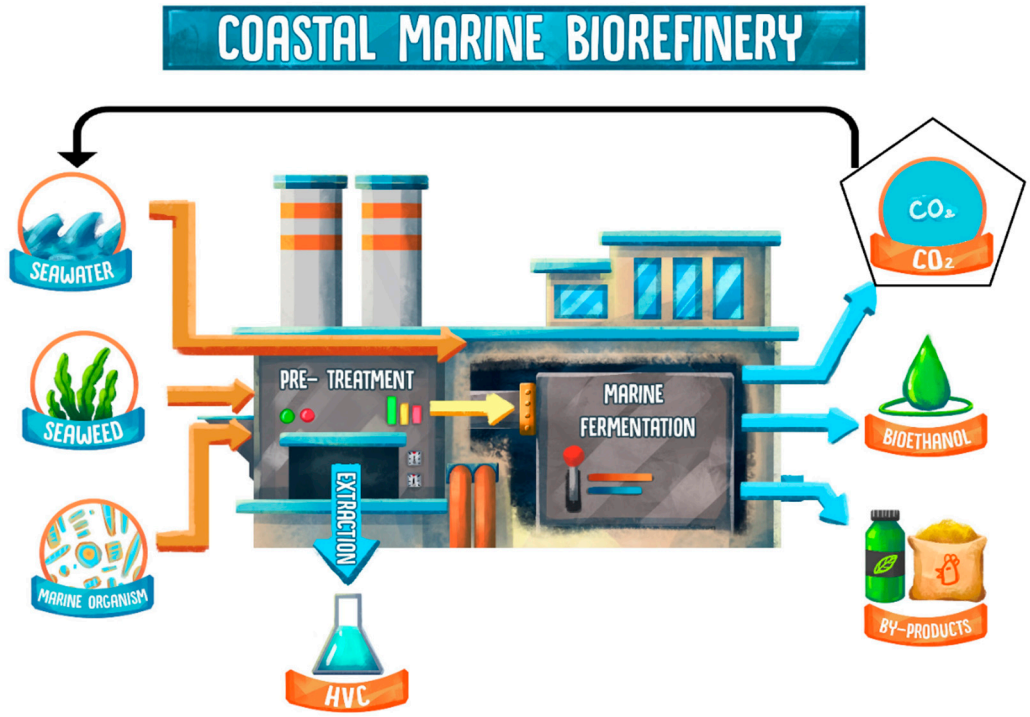

Figure 1. Visual diagram of a coastal seaweed marine biorefinery. Marine components include seawater, seaweed, and marine yeast (orange arrow). After processing, a number of outputs (blue arrows) are obtained. High value chemicals (HVC) are extracted during the pre-treatment step, while bioethanol and by-products (e.g. plant fertilizer & salted animal feed) are produced during or after the fermentation. Produced CO_2 is captured and stored in seawater or utilised to promote the growth of seaweed.

The importance of coastal locations must also be highlighted. As both the substrate and media are of marine source, establishment of marine biorefineries along coastal regions would decrease the transportation costs. Furthermore, coastal sites are easy water access points for arid and semi-arid areas. From an economic perspective, coastal locations enable rural regeneration, providing jobs to former coastal industrial sites that have historically been hard to maintain [26].

Though coastal marine seaweed biorefineries can operate on a stand-alone basis, there is the potential to pair such a system with other biorefineries and energy outlets to maximise the valuable outputs and minimise the cost. Such emerging combined systems are called coastal integrated marine biorefineries (CIMB). As can be seen in Figure 2, integrated biorefineries may combine both seaweed and microalgal refineries, with certain by-products of each serving as inputs for the other. CO_2 and spent seaweed hydrolysate may be used as organic and inorganic carbon substrates for the growth of microalgae [27–29]. Each fraction of microalgae has a certain potential industrial application. Lipids can be extracted and processed into biodiesel, proteins sold as livestock feed and carbohydrates fermented into bioethanol. Aside from the primary metabolites, microalgae contain a

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host of HVC, many of which have commercial uses. These include unsaturated long-chain fatty acids, shown to have a number of health benefits, as well as pigments, such as chlorophylls, phycocyanin and carotenoids [30,31]. As both biorefineries require electricity, this can be provided from sustainable sources, such as wind, solar, or wave energy. Conversely, coastal integrated marine biorefineries may also serve as a means of energy storage for renewable electricity. For example, peaks in solar electricity production generally surpass simultaneous energy demands. Excess electricity is often lost as there are currently no means of storage. Coastal integrated marine biorefineries enable the storage of excess renewable electricity by using it to produce biofuels. This integrated biorefinery system would lead to the production of less waste from each individual system and an increase in HVC and co-product yields.

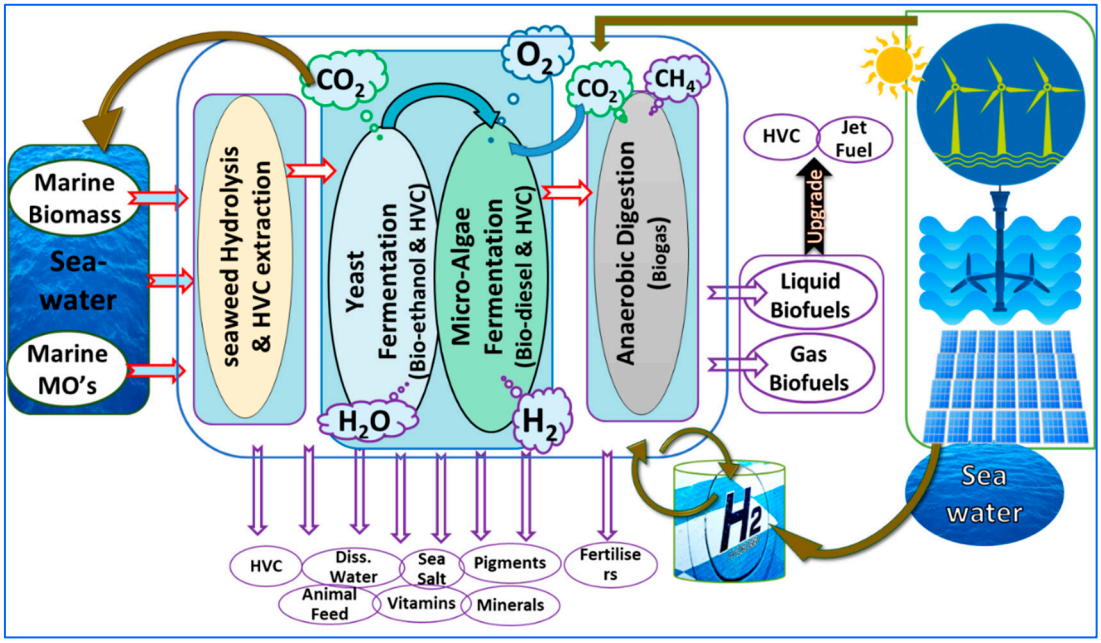

Figure 2. Coastal Integrated Marine Biorefinery (CIMB) system. Marine components (seawater, marine biomass and marine microorganisms) serve as inputs for the biorefineries. Different biological and biochemical conversion processes are performed (blue box). Biofuels and high value chemicals are obtained (purple arrows and ovals). Renewable energy sources (green box/arrow) are integrated with the biological system to improve efficiency.

Seaweed

Unlike terrestrial plants, algae do not require freshwater or agricultural land, two rapidly depleting world resources [32]. Furthermore, they can serve as a means for bioremediation as they have been shown to eliminate heavy metal and other contaminants in wastewater such as microplastic [34,35]. Macroalgae are also capable of removing high concentrations of nitrogen and phosphorous from coastal waters [36,37]. Therefore, seaweeds have been discussed as a potential feedstock for bioethanol and biogas production coupled with heavy metal removal [38]. Seaweeds can be classified into three groups: Red (Rhodophytes), Green (Chlorophytes), and Brown (Phaeophytes), which are differentiated mainly based on their pigmentation. Seaweeds have a wide variation in biochemical composition and, therefore, have many applications in HVC production.

Seaweeds Macro Chemical Composition

Like other biomass feedstocks, seaweeds are composed mainly of lipids, proteins, and carbohydrates, in addition to other specific components in relatively low proportions. Carbohydrates represent, on average, 50% of macroalgal dry weight [39]. In general, green seaweeds have the highest polysaccharide content, followed by red then brown seaweeds [40–42]. Each class is also characterised by the specific sugars they harbour. Proteins generally represent 10–30 % of dry matter

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in red and green seaweeds, and 3–15% of dry weight in brown seaweeds [43]. These values vary greatly between species and are particularly influenced by seasonal variation [44,45]. Regardless, seaweed can serve as a valuable protein source as most contain high levels of essential amino acids [46]. Lipid content of seaweeds is generally low, accounting for only 1-5 % of dry weight [43,47]. Though there exists a large variety of lipids, the most abundant are phospholipids and glycoglycerolipids [39,48,49] with a high proportion of polyunsaturated fatty acids (PUFAs), which have garnered much attention as they have shown a number of health benefits [50–52]. Other compounds also in seaweeds include pigments, such as carotenoids, sterols and vitamins, all of commercial value [39].

The remainder of seaweed dry mass is referred to as ash, representing around 22% of dry weight and comprised of macro-minerals (Na, K, Ca, Mg) and trace elements (Fe, Zn, Mn, and Cu) [53–57]. Sodium and calcium are the minerals found most abundantly whereas abundant trace minerals include zinc, manganese and arsenic [58,59]. As they are not biosynthesised, variations in mineral content are dependent on the seaweed's bio-absorption and bioaccumulation capacity and on its growth environment. Overall, macroalgae tend to have a higher spectrum and content of trace minerals compared with terrestrial plants, making them strong candidates for several industries, especially food, feed, and cosmetics [39].

The high variation in chemical composition of seaweeds depends on the seaweed species, the geographical location, and the seasonal fluctuations in biotic and abiotic nutrients available during seaweed growth [41,42]. However, reported variations are also due to differences in extraction procedures and the applied analytical methods [60,61]. For example, protein content is generally determined using direct chemical extraction methods or indirect application of nitrogen conversion factors. The former tends to underestimate total protein, whereas the latter overestimates it, as it assumes all nitrogen present in the biomass is protein. Errors in the initial values lead to further misrepresentation of the chemical composition. A 2015 meta-analysis determined that a conversion factor of 4.76, specifically tailored to seaweeds, provided a more accurate estimate of protein content compared to the universal conversion factor of 6.25 [62]. The use of better conversion factors or more efficient extraction and quantification methods would ensure the reporting of accurate values.

High Value Chemicals (HVC)

Seaweeds contain several interesting compounds with a range of bioactivities, from potential anti-cancer to food preserving agents. Several studies have detailed the extensive properties of certain HVC with potential industrial applications [39,63,64]. However, most of these attributes have only been demonstrated as part of proof-of-concept studies. As there is no established market for many macroalgal compounds, this review will focus on HVC with existing applications. Polysaccharide composition of seaweeds varies greatly from that of land plants, and each class harbours their own unique set. Specific carbohydrate distribution is as follows: fucoidan, laminarin, alginate, mannitol are present in brown seaweeds, carrageenan and agar in red seaweeds, while ulvan is found in green seaweeds [65]. Fucoidan, carrageenan and ulvan are known as sulphated polysaccharides given the sulphate moieties that form part of their backbone. This chemical structure affects their water-solubility, resulting in unique gelling properties with applications in various industrial sectors (Table 1). Sulphated polysaccharides are also sources of rare sugars with high market value [64]. For example, ulvan's main components, rhamnose and iduronic acid, are used as precursors for the synthesis of artificial flavours and anticoagulant analogues of heparin, respectively [39]. Other seaweed polysaccharides are also highly traded. Though agar from Rhodophyta has the highest market value, considering its high price and large quantity, brown seaweeds are a host to a greater number of HVC, namely laminarin, alginate, and mannitol, all with food applications. Commercial alginates represent an increasingly growing market, set to reach 529.2 million USD by 2025 [66]. Starch and cellulose are also major macroalgal polysaccharides. These are particularly abundant in green seaweeds, with *Ulva ohnoi* shown to be a potential source for marine starch [20,63,67]. Although seaweed cellulose has potential as a HVC, starch and cellulose from seaweed are often reserved for biofuel production as they can be easily hydrolysed to fermentable sugars.

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In addition to containing most essential amino acids, seaweeds also contain unusual amino acids or similar compounds, such as D-homocysteic acid, kainic acid, and taurine. Taurine in particular has high economic value given its increasing importance as a dietary supplement [68]. Though many algal amino acids are generally found in relatively low concentrations, the quantities are higher than those of land plants, thus making seaweeds an interesting source of bioactive peptides. The most prominent algal proteins are lectin and phycobiliproteins [69]. Lectin has shown great promise as an anti-HIV and anti-cancer drug but has yet to be commercially extracted and produced [70]. Phycobiliproteins are photosynthetic pigments found in red algae, used in the biomedical field as fluorescent markers [71]. R-phycoerythrin is the most common phycobiliprotein on the market and has a selling value of 180-250 M USD valuation per kilogram (Table 1). This high price tag is mostly due to the difficulty of protein extraction. Much work is being done to optimise the process, which may possibly result in improved yields and/or cheaper extraction procedures [72–74].

Carotenoids are another class of HVC including two major classes depending on their structure, namely carotenes, made of carbon, and xanthophylls, oxygenated carotene derivatives [75]. Fucoxanthin, extracted from brown seaweeds, is the most dominant xanthophyll on the market, often used as a basal metabolism booster in slimming diets. Despite a high price tag, it is only made in small quantities as current extraction processes make large-scale production difficult [76]. Other smaller classes of seaweed compounds have also attracted commercial attention. Phlorotannin is a brown seaweed polyphenol that can be used as an antimicrobial agent in animal feeds and serve as a means to reduce livestock antibiotic resistance [77]. Squalene-2,3-epoxide, isolated from green seaweeds, can serve as a source of squalene derivatives, highly prized compounds in the cosmetics industry [50,78].

Though an exhaustive list of all high value chemicals is not possible, it is evident that seaweeds are a host to a plethora of bioactive compounds of commercial value. The cost-effective extraction of these HVC is essential for the economic viability of a coastal seaweed marine biorefinery. Therefore, integration of HVC extraction with other applications could enhance the process feasibility. In addition, sole production of biofuels from seaweeds is time-consuming and not economically feasible given the competitiveness of the petrochemical industry. Indeed, many second-generation biofuel businesses, focussed only on fuel production, have shut down. This is due to biofuels being a high volume, low margin product with high capital investment requirements and relatively expensive production costs. Acquiring profits from such a business model is therefore difficult. Complete exploitation of input substrate is needed to maximise earnings. Therefore, extraction of HVC alongside biofuel production from seaweeds is necessary to create self-standing profitable sustainable refinery systems. Because seaweeds are rich in carbohydrates with low lipid content, they have been suggested as a potential feedstock for bioethanol production. As will be seen in this article, there exist a host of technologies, both conventional and cutting-edge, that enable simultaneous macroalgal breakdown and HVC extraction.

Table 1. Representative market price and applications of high-value chemicals (HVC) from different classes of seaweeds.

HVC	Compound	Seaweed Class	Applications	Market Price (USD/kg)	Ref
Carrageenan	Sulfated polysaccharide	Red	Stabilizer / gelling agent / texture modifier/ thickener	10.5	[63,79]*
Furcellaran	Sulfated polysaccharide	Red	Gelling agent / food preservative / bacterial growth media	1	[80,81]*
Ulvan	Sulfated polysaccharide	Green	Animal feed / anticoagulant / immune modulator / drug delivery	4	[82]*
Fucoidan	Sulfated polysaccharide	Brown	Bioactive agent in food, cosmetics, pharmaceuticals	11	[63,83]*
Agar	Polysaccharide	Red	Stabilizer / thickener / culture media / moisturizing agent / packing material	18	[84,79]*
Alginate	Polysaccharide	Brown	Thickener, gelling agent, stabilizer, emulsifiers	12	[63,79]*
Laminarin	Polysaccharide	Brown	Ethanol production / Biomedical agent	0.42 – 0.94	[63]*

Mannitol	Sugar alcohol	Brown	Diabetic sweetener / dehydrating agent	7.3	[85,64]*
Starch	Polysaccharide	All	Biofuel / bioplastic / thickener / stabilizers	_	[63]
Cellulose	Polysaccharide	All	Bioethanol production / nano filter / drug carrier / paper making	_	[63]
R-phyco-erythrin	Phycobiliprotein	Red	Pigment dye / fluorescent label	180 M – 250 M	[71]*
Lectin	Protein	Red & Green	Anti-viral / cancer biomarkers	_	[86]
Taurine	Sulphonic β-amino acid	All	Nutritional and medical dietary supplement	3	[63,68,87]*
Squalene	Lipid	Green	Antioxidant & moisturising agent / drug carrier	18	[39,88]
Fucoxanthin	β-carotene	Brown	Medical and nutritional supplement	200	[89,90]*
Phloro-tannin	Polyphenol	Brown	Animal feed antimicrobial agent / anti-vasoconstriction medication	70	[77,91]**

* Reference for market price; ** Calculated average price.

Extraction methods for Seaweed HVC

Extraction of HVC from seaweeds can be divided into 2 stages. The first is a pre-treatment step, whereby the biomass is broken down to liberate compounds and later give access to hydrolytic enzymes, followed by the second stage of HVC recovery [92]. Though independent reactions, both steps are intrinsically linked and may occur simultaneously depending on the chosen procedure. Early steps of pre-treatment include washing, drying, and size reduction. Washing is necessary to remove any debris present on the biomass [93]. Though the process leads to the loss of some compounds, such as polysaccharides, this is outweighed by the benefits of removing all undesirable matter [94]. Washing can also be done using seawater to decrease the total freshwater footprint [22]. Drying is done to extend the storage life of biomass and decrease the transportation costs [94]. Within the context of a coastal marine biorefinery, this step is unnecessary, given the coastal location, thus saving energy and cost. Size reduction aims to decrease the particulate size, increasing the overall surface area on which subsequent treatments can act. Indeed, particulate size reduction has been shown to positively correlate with the biofuel yields [95,96]. Possible methods include milling, grinding, extrusion, and high-pressure homogenization, with ball-milling being the most common and most effective method [97,98].

Conventional pre-treatment and extraction technologies

Dilute acid pre-treatment (DA), hydrothermal pre-treatment, and enzymatic pre-treatment are the most commonly used conventional technologies for biomass pre-treatment. They were originally proposed for lignocellulosic materials but have recently applied successfully on seaweed. Dilute acid pre-treatment (DA) involves the use of strong acids at high temperatures to hydrolyse biomass and yield high concentrations of monosaccharides [99]. As it is well established and cheap, DA is the most widely used pre-treatment procedure in industry [100]. However, the technology has a number of disadvantages, namely long reaction times, the use of high concentrations of environmentally toxic acids, and potential corrosion to equipment [101]. The biggest issue within the context of an ethanol biorefinery is the formation of 5-hydroxymethylfurfural (5-HMF), a potent fermentation inhibitor [102]. A direct alternative to dilute acid pre-treatment is dilute alkali pre-treatment. However, research on its use for seaweed treatment is limited, as it requires larger solution quantities compared to DA [103].

Hydrothermal pre-treatment uses water at high pressures and temperatures (100 °C to 374°C) to fractionate biomass. This liberates a variety of products that can be fermented further into bioethanol [20]. Though shown to be effective for the breakdown of macroalgal and lignocellulosic biomass, the technology has high energy demands and is not suitable for thermo-sensitive compounds (Table 2) [104]. Enzymatic pre-treatment is a well-established method, often used alongside dilute acid pre-treatment [105]. The non-toxic nature of enzymes and their mild reaction conditions make them one of the few green conventional pre-treatment methods [106]. Depending on the chosen enzyme, the reaction can be more or less selective, with little negative impact on the compound's bioactivity [90]. However, the cost of enzymes is high, therefore their use is generally limited to sugar hydrolysis,

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which will be discussed later (Section 2.4.1). On-site enzyme production could reduce the costs [107]. In addition, hyperthermophilic enzymes have been suggested recently for efficient treatment [108]. As seaweeds have been shown to be a particularly good substrate for the production of hydrolytic enzymes, this represents a further potential market to exploit [109].

Once the biomass has been pre-treated, product recovery is conducted through extraction steps. Traditional extraction procedures include solid–liquid extraction and liquid–liquid extraction, but Soxhlet extraction is the most widely used method in industry due to its simplicity, safety and scalability [92,110]. All recovery methods make use of organic solvents with appropriate solvent choice depending on the properties of the HVC that are to be extracted. For example, hexane may be used to extract non-polar compounds and water may be used for polar ones [111]. Table 2 summarises different methods used in pretreatment showing the advantages and disadvantages of each method. It also indicates whether the method is considered sustainable or not based on their environmental impact, mainly their energy consumption and waste generation.

Table 2. Comparison of different pre-treatment methods showing the advantages and disadvantages of each method.

Type	Pre-treatment	Advantages	Disadvantages	Sust.	Ref
Physical	Milling & Extrusion	Maintains biochemical activity	High cost & energy	No	[97,104]*
	Microwave	High yields / Fast / Low solvent use	High energy / Not suitable for heat-sensitive metabolites	Yes	[92]·[112]·[113] [104]*
	Ultrasonication	Cheap / Fast / High yields / Low solvent / Suitable for labile compounds	High energy / Wave attenuation	Yes	[92]·[106,114,115] [116]*
	Pulsed Electric Field	Fast / selectivity /Low solvent & energy use	Cost / incomplete breakdown	Yes	[117–119][120]*
Physico-chemical	Hydrothermal	Less equipment / low inhibitors (at low temp.)	High energy / Low yields at high temperature	No	[121,122] [104]*
	Supercritical fluids	Fast, efficient & eco-friendly / Pure final product	High costs	Yes	[92,106,123][104]*
	Pressurized liquids	Fast reaction / Increased solubility and transfer rate /Low solvent	Unsuitable for unstable metabolites/ Not selective	Yes	[92,115][104]*
Chemical	Dilute acid/alkali	Cheap / Simple/ Efficient	Fermentation inhibitors / eco-toxicity	No	[103,104][104]*
	Ionic liquid	Efficient / Mild / low energy / fewer inhibitory compounds	High cost / Eco-toxicity / Inhibited by water	Yes	[124][104]*
	Deep eutectic solvents	Green / Cheap / Tailorable / Low to non- toxic	Few research	Yes	[125][104]*
Biological	Enzymatic	High yield / Selective / Mild	High cost / Long extraction time	Yes	[126–128] [104]*
	Fungal	Eco-friendly / low chemical needs / Feed co-product	Slow / High space requirements / constant growth monitoring	Yes	[129–131]
	Bacterial	Eco-friendly / Ideal environment for enzyme activity	Slow / Few optimised processes / used in combination with other conventional methods	Yes	[132–135]

* Reference for sustainability.

Emerging pre-treatment and extraction technologies

Several enhanced biomass pre-treatment methodologies and extraction technologies could be applied on seaweed. These include microwave-assisted extraction (MAE), ultrasound assisted extraction (UAE), pulsed electric field (PEF), supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), ionic liquids (IL), and deep eutectic solvents (DES).

Microwave-assisted extraction (MAE)

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MAE relies on the energy transfer from nonionizing electromagnetic waves to the moisture within the biomass cellular matrix. In the case of seaweeds, these waves cause heating of the internal water. The resulting steam generates an increase in intracellular pressure, which upon escape, causes cell rupture and leakage of the cytoplasmic contents [112]. HVC that are liberated during cellular breakdown are dissolved in the chosen solvent for targeted chemical recovery. MAE works best with polar solvents due to their high dielectric constant, leading to faster energy absorption and coming up to temperature. Of the commercial solvents, the best is water, followed by methanol, ethanol, acetone, ethyl acetate, and finally hexane [136]. MAE is often used for extraction of polysaccharides and polyphenols (Table 3) [137]. Though it is fast, effective and requires little solvent, MAE is not suitable for heat-sensitive metabolites as operating temperature conditions are quite high. A 2017 Boulho et al. [138] study extracted carageenans at temperatures between 90°C and 105°C, combined with high pressures. Although such temperatures are generally considered mild for extraction methods, they are still too high for heat-sensitive HVC. Even when MAE procedures are optimised, temperatures are still around 80°C [139,140]. However, given its numerous advantages over conventional methods, MAE is becoming an increasingly viable route for bioactive compound extraction.

Ultrasound assisted extraction (UAE)

UAE works using a mechanical component that vibrates at frequencies above 20 kHz, creating ultrasonic waves which propagate throughout the medium, transferring their energy to surrounding particles. This causes a number of physical effects, most prominently cavitation. Cavitation results from the alternate pressures caused by the longitudinal movement of ultrasonic waves. The pressure variation leads to formation of a bubble, whose oscillation or collapse results in the mechanical breakdown of the cell and leakage of the intracellular content [141]. UAE is fast, makes use of less solvent, and can be used for extraction of thermo-sensitive compounds, unlike MAE (Table 2). Furthermore, UAE has been used to extract a host of bioactive compounds from seaweeds, notably phycobiliproteins of very high value (Table 3). Current research is also aiming to optimise the running parameters of ultrasonic assisted extraction in order to improve yields [142,143].

Pulsed electric field (PEF)

PEF relies on the principle of electroporation to increase the mass transfer [136]. During PEF, the biomass is placed between 2 electrodes and is subjected to high voltage pulses. The PEF leads to formation of irreversible pores and diffusion of intracellular components out of the cell. Though PEF uses less solvent and is more energy efficient than conventional methods (Table 2), one drawback is that it does not lead to complete cellular breakdown. Coupling of PEF with other mechanical procedures, such as ball-milling, is therefore necessary to gain access to membrane-bound compounds [118]. A variation of PEF, known as high voltage electrical discharge (HVED), inflicts more damage on the biomass, leading to efficient cellular breakdown, but may not be suitable for the extraction of unstable compounds [144]. Although this method has been shown to be effective for extraction of minerals, polysaccharides and proteins from seaweeds, a major challenge limiting the wide-spread use of PEF is the high equipment costs [145]. However, given the push towards greener technologies, projects like FieldFOOD, set up by the EU, are aiming to make PEF more accessible [146].

Supercritical fluid extraction (SFE)

SFE makes use of fluids subjected to critical temperatures and pressures, giving them gas- and liquid-like physicochemical properties. Supercritical fluids (SF) are compressible like gases but also have solvent-like activity as they retain liquid density. These particular behaviours give them a number of advantages, such as better transport properties, and better diffusion through solid material, leading to faster extraction with high yields. Furthermore, as density impacts solubility, solubility in SF can be fine-tuned by varying pressure and temperature parameters [147]. A number

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of solvents can be used as SF, with carbon dioxide being the most widely used industrially. As it is a gas under ambient conditions, CO_2 can be easily removed and recycled, producing a solvent-free extract. However, CO_2 is non-polar and therefore not suitable for polar compound extraction [148]. Therefore, addition of co-solvents and solubility modifiers would be necessary to improve efficiency [149]. The unique properties of various SF enable highly specific extraction. However, given the high energy costs of SFE, the process is limited to HVC. These include polysaccharides, polyphenols, and carotenoids (Table 3) [150].

Pressurized liquid extraction (PLE)

PEL involves the use of solvents subjected to high temperature and pressure to maintain liquid states past their boiling point. These conditions cause a decrease in solvent viscosity and an increase in solubility, allowing for easier and better penetration into biomass matrix. This method results in fast extraction, low solvent use, decreased sample handling and increased yields [151]. PLE extraction selectivity is largely dependent on the employed solvent. For environmental reasons, Generally Recognized As Safe (GRAS) solvents, such as ethanol or ethyl acetate, are preferred, with water being the best. PLE using water is also known as subcritical water extraction or hot-water extraction. This procedure is similar to hydrothermal pre-treatment, though the conditions differ [152]. PLE has been used to extract polyphenols, carotenoids, and terpenoids from solid matrices (Table 3). Response surface methodology studies have shown that the efficacy of extraction is mainly influenced by temperature. As fewer parameters impact its activity, PLE is a potentially easier method than SFE [153–155].

Ionic liquids (ILs)

ILs are non-molecular ionic compounds with melting points below 100 °C [156]. ILs have high thermal and electrochemical stability, strong miscibility with aqueous substances, and negligible vapor pressure, making them strong solvents [157,158]. This diversity of ILs enables the tailoring of extraction procedures depending on the target HVC, leading to better yields [159]. As can be seen in table 3, IL can be used for the extraction of phycobiliproteins, polysaccharides and iodine compounds from macroalgae. Though ILs represent an attractive "green" emerging extraction process, they are a relatively new technology with its own set of drawbacks, most prominent being the cost [124]. The high cost of IL extraction is due to the difficult purification step that occurs during their production. Consequently, ILs can sell sometimes for up to 300 USD per Kg [160]. Current research is aiming to improve and optimise IL extraction to make the technology cost-competitive [161,162].

Deep eutectic solvents (DESs)

DESs are a new generation of solvents comprised of a eutectic mix of Lewis or Brønsted acids and bases with a resulting melting point lower than that of each individual component [163,164]. They evolved as an alternative to ILs as they are inexpensive, readily available, and biodegradable whilst still retaining most properties of ILs. Furthermore, DESs can be recycled without significant loss of activity [165]. They are, however, less chemically inert than ILs, thus exert more damage on equipment [163]. Research on DESs is very recent and studies on HVC extraction are very limited. A 2016 study demonstrated the possible extraction of κ-carrageenan using 10% hydrated choline-chloride-glycerol DES, resulting in a 64.70% κ-carrageenan yield increase compared to the conventional extraction methods [166]. Other studies highlighted the potential of DESs in combination with other technologies, such as combination of DESs and ultrasound, as an effective means of polysaccharide extraction from *S. horneri* [167]. Polysaccharides can also be extracted from brown seaweeds using DESs in combination with subcritical water hydrolysis [168]. Though very promising, further research is needed to better establish DESs as an extraction procedure.

As highlighted with DESs, alternative extraction procedures can be combined, both with conventional and "green" methods, to improve HVC release and yields [157,169,170]. However, application of multiple treatments entails more investment capital for multiple pieces of equipment.

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Furthermore, many emerging methods have not been optimised or only on a laboratory scale. More research is needed to facilitate the use of such technologies on an industrial scale.

Table 3. HVC extraction from seaweeds using green and emerging technologies.

Extraction Method	Compound	Seaweed species	Conditions	Concentration	Ref
Microwave-Assisted Extraction	Polyphenols	*A. nodosum*	1:10 seaweed:methanol, 110 °C for 15 min at 2.45 GHz	3.738 mg/g	[171]
	Ulvan	*U. pertusa*	3:40 (seaweed:ethanol), 43.63 min, 600 W, pH 6.57	12.573 mg/g	[139]
	Carotenoids	*C. glomerata*	4 g dw algae per 100 mL of solvent, 60 min, 40°C, 800 W	3 mg/ml	[172]
	Polysaccharides	*S. henslowianum*	40 min, 330 W, solid-to-liquid ratio 1:36 g/mL	126.3 mg/g	[173]
	Polyphenols	*S. henslowianum*	1 g extract, 102 min, 377 W	114 mg/g	[173]
Ultrasound-Assisted Extraction	R-phycoerythrin	*G. turuturu*	20% seaweed: 80% water, 300- 340 W, 6 h	3.25 mg/g	[174]
	Polyphenols	*F. vesiculosus*	35 kHz, 30 min, 50% ethanol	572.3 mg/g	[175]
	Phlorotannins	*F. vesiculosus*	35 kHz, 30 min, 50% ethanol	476.3 mg/g	[175]
	Flavonoids	*F. vesiculosus*	35 kHz, 30 min, 50% ethanol	281 mg/g	[175]
Pulsed Electric Field	Proteins	*U. rigida* & *U. ohno* mix	140 g, 50 pulses of 50 kV, 70.3 mm	1.92 mg/ml	[176]
	Starch	*U. ohnoi*	200 pulses, field strength of 1 kV cm^{-1}, pulse:50 µs, 3 Hz	1.54 g/g	[145]
Supercritical Fluid Extraction	Aliphatic hydrocarbons	*U. pinnatifida*	0.5 g sample, 50 min,1 mL min^{-1} CO_2, density 0.55 g mL^{-1}	13.6–21.7 µg/g	[177]
	Fucoxanthin	*U. pinnatifida*	SC-CO_2, 200 bar, 323 K	7.53 µg/g	[178]
	Polyphenol	*U. pinnatifida*	250 bar, 333 K	780 mg/g	[178]
	Lipids	*S. hemiphyllum*	SC-CO_2, 1 mL/min, 37.9 MPa/ 323.15 K,	55.8 mg/g	[179]
Pressurised Liquid Extraction	Polyphenols	*L. ochroleuca*	1 g, 20 mL ethanol:water (1:1), 160°C, 100 bars, 10 min	173.65 mg/g	[180]
	Fatty Acids	*F. vesiculous*	1 g, 10 min, 120°C, 100 bar, ethyl acetate 10 ml	693.20 mg/g	[151]

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	Phenols	*A. nodosum*	5g, 50°C, ethanol, 1500 psi, 5 min	50.2 mg /g	[181]
	Carotenoids	*A. nodosum*	5g, 50°C, ethanol, 1500 psi, 5 min	85 μg/g	[181]
	Phycobiliproteins	*Gracilaria* sp.	0.7 fw/solvent, 20 min, 5.9 pH, 1 M [Ch]Cl	0.40 mg/g	[182]
Ionic Liquid	Agarose	*G. dura*	0.5 g, 10 g [Emim][OAc], 2 h, 100°C, IL ([EPy]Br)	175 mg/g	[183]
	Iodine compounds	*Laminaria* sp.	200 mM, 30 min, 6.5 pH	3754 μg/g	[184]
Deep Eutectic Solvents	κ-carrageenan	*K. alvarezii*	500 mg, 10g 10% Hydrated choline-Chloride-Glycerol 1:2, 1 h	301 mg/g	[166]

Bioethanol production from seaweeds

After HVC extraction, the remaining biomass rich in carbohydrates can be converted into bioethanol through four steps: pre-treatment, hydrolysis, fermentation and distillation. As pre-treatment was covered in previous sections, the focus of the present section will be on the hydrolysis and fermentation steps, where various adaptations are needed to enable the production of bioethanol through marine fermentation.

Hydrolysis

Hydrolysis enables the transformation of complex polysaccharides into fermentable sugars. Enzymes are generally used to complete this saccharification step, with cellulases being used most frequently. This class of enzymes is divided into 3 types, endoglucanases, exoglucanases, and β-glucosidases, depending on the enzyme's specific hydrolytic activity [185,186]. Enzyme mixtures are often used on a commercial scale to maximise saccharification [187]. Enzymatic hydrolysis is highly efficient, specific, and mild, leading to less release of fermentation inhibitors [188,189]. However, the activity of conventional industrial enzymes is often inhibited at high salt concentrations. Nevertheless, for the purpose of the marine biorefinery, it is necessary to use enzymes that have good activity at around 6% salt concentration, to ensure the efficient hydrolysis of seaweed's polysaccharides using seawater. Marine organisms represent an ideal source for halotolerant enzymes. Firstly, the metabolisms of marine organisms have evolved to function in high salt conditions. Given the shared environment with macroalgae, it is also likely that they have developed enzymes capable of degrading seaweed biomass to be used as a carbon source. Furthermore, marine enzymes are generally more stable at high temperatures and varying pH conditions due to the complex and dynamic nature of marine environments [190].

Many hydrolytic enzymes capable of seaweed polysaccharide degradation have been produced from marine organisms, most commonly bacteria and fungi (Table 4). Multifunctional enzymes, such as Amy63 from *Vibrio alginolyticus* 63, are particularly interesting as they can degrade multiple types of carbohydrates. Given their broad hydrolytic activity, such enzymes would limit the number and quantity required for saccharification, thus reducing the production costs. Conventional enzymes have also been genetically engineered to make them thermoresistant, halotolerant, and other properties useful for bioethanol production [109]. Though many halotolerant hydrolytic enzymes have been produced in proof-of-concept studies, there is a general lack of research on their exact saccharification activities and whether they can be used in an industrial setting. For example, no enzymes capable of macroalgal starch degradation have been found. Therefore, much research is still necessary in order to optimise the hydrolysis conditions and minimise the production costs before enzymatic hydrolysis is economically viable.

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Table 4. Halotolerant enzymes used for hydrolysis of seaweeds.

Enzymes	Producing Organism	Targeted Polysaccharide	Substrate Seaweed Sp.	Reducing sugars yield	Ref.
Green Seaweeds					
Xylanase	*Bacillus* sp. strain BT21	Xylan	*U. lactuca*	45.84 µg/mg	[191]
Xylanase	*H. meridiana*	Xylan	*U. lactuca*	50.03 mg/g	[192]
Cellulase	*V. parahaemolyticus*	Cellulose	*U. lactuca*	107.6 mg/g	[193]
Cellulase	*V. parahaemolyticus*	Cellulose	*U. intestinalis*	135.9 mg/g	[193]
Ulvan lyase	*Alteromonas* sp.	Ulvan	na	na	[194]
Red Seaweeds					
Xylanase	*Bacillus* sp. strain BT21	Xylan	*A. plicata*	12.16 µg/mg	[191]
Agarase	*S. degradans* 2–40	Agar	*G. verrucosa*	na	[195]
Amy63	*V. alginolyticus* 63	Amylose Agarose Carrageenan	na	na	[196]
Aga4436	*Flammeovirga* sp. OC4	Agarose	na	na	[197]
Brown Seaweeds					
Laminarinase	*Bacillus* sp. (8D)	Laminarin	*Sargassum sp*	1.97 mg/mL	[198]
Bgl1B	*S. degradans* 2-40T	Laminarin	na	na	[199]
Xylanase	*Bacillus* sp. strain BT21	Xylan	*P. tetrastromatica*	59.56 µg/mg	[191]
OLA	*V. splendidus* 12B01 (12B01)	Alginate	na	1.6 mg/ml	[200]
Fucoidanase	*Formosa* algae strain KMM 3553	Fucoidan	*F. evanescens* *F. vesiculosus*	na	[201]

na Not available.

Fermentation using seawater-based media and yeast

Traditional biofuel biorefineries make use of fermenting microorganisms to turn biomass sugars into bioethanol. *S. cerevisiae*, in particular, has been optimised to produce high ethanol yields at industrial level. However, these industrial strains are of terrestrial origin and usually their activity is greatly inhibited by the presence of salts [202]. Marine fermentation, on the other hand, utilises marine-based resources (seawater, marine yeast, and seaweed). Hence, isolation and identification of new marine strains is necessary. Initial attempts at making efficient halotolerant strains used genetic engineering. Limtong et al. [203] generated a high ethanol fermenting halotolerant microorganism through hybridisation of *S. cerevisiae* and *Zygosaccharomyces rouxii*. The resulting RM11 mutant strain was able to produce 6.85% ethanol after 60 h of saltwater fermentation, which represented a 5.38% increase compared to the *S. cerevisiae* M30 and a 7.7% increase compared to the *Z. rouxii* parental strains However, the use of genetically engineered organisms is restricted in many countries, making them unsuitable for the global development of coastal marine biorefineries.

To avoid such restrictions, the search for halotolerant ethanogenic microorganisms has turned to marine environments. Urano et al. [204] isolated marine yeasts capable of ethanol fermentation using seawater-based fermentation media. Zaky et al. [23] have recently isolated many marine yeasts with potential for high ethanol production using seawater-based fermentation media. Using a two-stage fermentation procedure, an ethanol concentration of 50.32 g L^{-1} was produced by *S. cerevisiae* AZ65 from seawater-sugarcane molasses which is high enough to make commercial production viable [23]. Furthermore, these marine strains have been shown to be more tolerant to the

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inhibitors generated during the pre-treatment and hydrolysis of biomass [13,24]. Thus, marine yeasts are suitable candidates for marine fermentation, making seawater a viable replacement for freshwater. They are also a potential candidate for bioethanol production from seaweed hydrolysates which is an area requiring intensive research [11]. Table 5 represents examples of marine yeast used in ethanol production from different fermentation media.

Table 5. Marine microorganisms used for bioethanol production.

Marine yeasts	Source	Fermentation Media (Salt con.)	Pre-treatment	Sugar	Max. Prod uc. (g/L/h)	Max. EtOH (g/L)	Ref
S. cerevisiae YPS128	Plymouth, UK	*C. crispus* in Fresh water (na)	5 % H_2SO_4, 121°C, 15 min	2.02 g/L	0.108	13	[205]
Defluviitalea. haphyphila Alg1	Yellow Sea, China,	*S. japonica* in Salt water (3%)	Dried, powderised	5%	0.14	10	[206,207]
Candida sp.	West Coast India	*K. alvarezii* in Salt water (11.25%)	Acid hydrolysis, 100 °C for 1 h	5.5%	0.17	12.3	[208]
S. cerevisiae JN387604	Mangrove, Southeast India	Sawdust in 50% Seawater (1.75%)	0.8% phosphoric acid	6.84 mg/L	0.2	25.1	[209]
P. salicaria	Mangrove, SE India	Sawdust in Freshwater (na)	Dilute phosphoric acid	2%	0.37	28.5	[210,211]
C. albicans	Mangrove, SE India	Malt broth in 50% Seawater (1.75%)	na	3 g/L	0.49	47.3	[210]
S. cerevisiae C-19	Tokyo Bay	*U. pinnatifida* & Paper in Fresh water (na)	3% H2SO4, 121°C, 1 h & cellulase GC220 & α-amylase	230 g/L	0.73	87.7	[212,213]
S. cerevisiae AZ65	Caernarfon, Wales, UK	YPD -Seawater medium (3.5 %)	na	200 g/L	4.15	86.72	[23]
		Sugarcane molasses-Seawater (3.5 %)		91.27 g/L	2.46	50.32	

Co-products of marine fermentation

Aside from bioethanol, the marine fermentation process generates several additional products. In the exhaust gas of the fermentation process, CO_2 is the dominant gas. Such CO_2 can be considered a co-product because it is being utilised in many industries including the production of sparkling beverages, as an inert gas in welding and fire extinguishers, as a pressurizing gas in air guns and oil recovery, as a chemical feedstock and as a supercritical fluid solvent in decaffeination of coffee and supercritical drying. More interestingly, CO_2 can be stored in tanks and then used as a carbon substrate for microalgal and seaweed propagation [214,215]. It can also be further recycled for use as a solvent for supercritical extraction. Furthermore, CO_2, generated at the coast, can be liquefied and easily transferred or shipped to suitable geological spots, such as subsea in the North Sea, for permanent storage, which represents a permanent means of carbon capture and sequestration.

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The final recovery step of bioethanol involves the removal of water through distillation. Thus, high quality distilled water is another product of bioethanol production by marine fermentation. For every litre of bioethanol produced, around seven litres of freshwater can be obtained [25]. As a coastal marine biorefineries make use of seawater and marine biomass, high quantities of sea salts can also be recovered during the distillation process [18,216].

The leftover organic solids of fermentation, rich in minerals, are an interesting source of plant fertilizers and salted animal feeds [217–219]. Seaweeds also are rich sources of amino acids and bioactive peptides essential for nutrition [220]. The mineral composition of seaweed hydrolysate makes it an excellent plant fertilizer [221]. Other products may be generated from seaweed hydrolysate but require further processing. Seaweed hydrolysate can serve as an alternative carbon source for lactic acid and succinic acid production [215,222]. It is a possible carbohydrate source for microalgal growth [223]. Hydrolysate can also be pyrolyzed into biochar or digested anaerobically into biogas [19,224].

The subsequent use of leftover products after bioethanol production reduces the waste generated by the coastal marine biorefinery, thus making it more sustainable and closer to net zero. Furthermore, these co-products serve as a source of additional revenue for the biorefinery, thus maximising its efficiency and reducing the expenses in other areas of the business [21].

Evaluation of CO_2 removal and CCS by seaweeds

Over the course of the 21st century, an estimated 100-1000 Gigatons (Gt) of CO_2 need to be removed from the atmosphere to mitigate the impacts of the global warming expected by the end of this century [225]. In recent years, planting trees has gained traction, with certain campaigns amassing millions of dollars in funds [226]. Such efforts highlight the widespread interest in finding tangible solutions to the current crisis. However, afforestation is mostly suited to tropical regions, where fast plant growth is possible [227]. Furthermore, tree monoculture and planting in arid regions has been shown to have a negative environmental impact, increasing water scarcity and creation of "Green Deserts" [228,229]. Other carbon capture and sequestration (CCS) technologies are therefore necessary to combat climate change.

Seaweeds have been discussed as an alternative carbon sink to terrestrial biomass. As detailed in section 3, seaweeds do not use freshwater or arable land. Furthermore, as the ocean covers more than 70 % of the Earth's surface, the available area for seaweed growth is much larger than what can be grown on land. Most importantly, biomass productivity of seaweeds is much higher than that of terrestrial plants. Whereas carbon productivity of second-generation lignocellulosic crops is less than 1 kg Carbon (C) m^{-2} $year^{-1}$, seaweed productivity ranges between 1 and 3.4 kg C m^{-2} $year^{-1}$, depending on the species [230].

Seaweed carbon sequestration is part of blue carbon sequestration, referring to the removal of atmospheric CO_2 by marine ecosystems through the accumulation and sequestration of carbon by marine organisms. Blue carbon sequestration accounts for around 55 to 71 % of all biological carbon sequestration on the planet [231,232]. Wild seaweeds (naturally grown seaweed) have already been shown to be an effective means of carbon removal, permanently sequestering on average 0.634 Gt CO_2 per year, mainly through deep sea biomass exportation or coastal sediment burial [233]. In this study, we aim to determine how much time is required to sequester 100 Gt of CO_2 by growing seaweed biomass in large seaweed farms based on the available marine area. To achieve that, three scenarios (A, B, and C), have been explored based on the total cultivation area. Scenario A accounts for 5.7 M km^2 which is the inshore coastal area that is suitable for seaweed cultivation. Scenario B accounts for 100 M km^2 which is the total ocean area that could be used for seaweed farming. Scenario C accounts for 47 M km^2 which is the ecologically available ocean area for seaweed farming. In order to determine the total seaweed biomass production from each scenario, the average productivity of wild (naturally grown) seaweed and the productivity of the highly productive cultivated seaweed species, *M. pyrifera* and *Ulva sp.*, were used in the calculation. The biomass production in each scenario determines the number of years required to sequester 100 Gt of CO_2. In addition, the

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quantities and potential revenue of bioethanol and HVC from a coastal marine biorefinery system utilising such amounts of seaweed biomass were estimated.

Estimation of biomass production and CO_2 sequestration by seaweed mass production

As seaweeds are highly efficient at sequestering carbon, the first aim of this section was to estimate how much seaweed is required to remove 100 Gt of CO_2. The hypothesis is that enough seaweed can be grown to reach the target before the 2100 deadline. The total biomass and time required depends on the total carbon that is to be removed, the net primary productivity (NPP) of seaweed species, and the total surface areas available for growth. Average wild seaweeds NPP was chosen as a benchmark average and two seaweeds, *Macrocystis pyrifera* and *Ulva* sp., were chosen to represent highly productive seaweeds [234]. Explored surface areas include inshore coastal sites, total ocean surface available for *Ulva* sp. seaweed farms, and ecologically available area for global seaweed farming. The latter two include both inshore and offshore locations.

Inputs, carbon (C), surface area (SA), and seaweed NPP were gathered from the literature (Table 6). Outputs, time (T), yearly carbon removal (YCR), biomass dry weight (Bdw), and biomass fresh weight (Bfw), were calculated as shown in Table 6 using Eqs. 1-4:

$$T = \frac{C}{YCR} \quad (1)$$

$$\mathrm{YCR} = \mathrm{NPP} \times \mathrm{SA} \quad (2)$$

$$\mathrm{Bdw} = \mathrm{YCR} \times 4 \quad (3)$$

$$Bfw = Bdw \times 4 \quad (4)$$

Scenario A represents the total time and biomass needed to remove 100 Gt of CO_2 when growing seaweeds on inshore coastal surface areas. Using an area of 5.7 million km^2, a total removal of excess CO_2 can be achieved in less than 11.28 years based on average wild seaweed NPP. When selecting highly productive species, the time goes down to 5.65 years using *Ulva* sp. and 3.64 years using *M. pyrifera* (Table 7).

In scenario B, the surface area for seaweed farming can be expanded to include the total inshore and offshore ocean surface area that can be theoretically used for seaweed farming. Based on *Ulva* sp., theoretical growth area of around 100 million km^2 can be farmed with 0.838 kg C m^{-2} yr^{-1} NPP to remove 100 Gt of CO_2 in just 116.8 days. Total yearly CO_2 removal using *Ulva* sp. farms in this scenario is 17 times higher than that of growth limited to inshore coastal sites (Table 8).

Though *Ulva* sp. can be theoretically grown over 100 M km^2 of ocean when only considering certain factors such as temperature, light, depth, and pH, the model does not consider all ecological limits on seaweeds growth. A better model taking into account these constraints estimates that the ocean surface area ecologically available for seaweed farms are in fact 48 million km^2. Therefore, in scenario C (Table 9), a total target CO_2 removal, considering NPP of wild seaweed, *M. pyrifera*, and *Ulva* sp. would take 1.34, 0.43, and 0.67 years, respectively. Across all seaweed classes, the yearly rate of CO_2 removal was 8.42 times greater for seaweed farms compared to inshore coastal areas (scenario A).

Estimation of theoretical bioethanol and HVC production and revenue by seaweed mass production

The second aim of this section was to determine the volume of bioethanol produced and the amount of HVC from the estimated biomass of *M. pyrifera*, as it was reported as one of the most productive seaweed species [235,236]. From these estimations, the value of all products was calculated based on the current bulk market prices. The hypothesis is that a sufficient quantity of HVC and bioethanol can be produced by a coastal seaweed marine biorefinery for it to be a sustainable business of CO_2 removal. The estimated market values for the HVC proposed in this study are available in Table 6.

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Biomass dry weight (Bdw), high-value chemical conversion factor (CF) and average market price (AMP) values were used as inputs. Final HVC mass (CM) and product value (PV) were calculated using Eqs. 5-6;

$$\mathrm{CM} = \mathrm{Bdw} * \mathrm{CF} \quad (5)$$

$$PV = CM * AMP \quad (6)$$

Having estimated the yearly growth needed for complete removal of 100 Gt of CO_2, further calculations were made in order to determine the HVC and bioethanol that could be produced from the biomass and their overall revenue. As exact compound calculation would be more specific on a single seaweed species basis, values associated to *M. pyrifera* were used. Total bioethanol and HVC mass and valuations were calculated based on 2 scenarios, Scenario A of inshore coastal growth (5.7 M km^2) and Scenario C, with seaweed farms in ecologically available ocean areas (48 M km^2). As can be seen in table 10, bioethanol, phlorotannin, alginate, mannitol, and protein can be produced from *M. pyrifera*. Based on coastal site growth, from 29.64 Gt of seaweed biomass energy equivalent, 6.31 Gt bioethanol can be produced, roughly equivalent to 8 trillion litres. With an average market price of 0.40 USD/litre, total seaweed bioethanol production worth over 3 trillion USD. The total revenue of the process including phlorotannin, alginate, mannitol, and protein, could be estimated at almost 143 trillion USD (Table 10). In scenario C, seaweed farms can generate 249.6 Gt of biomass, leading to production of 136 trillion litres of bioethanol with an estimated value of almost 27 trillion USD. Additionally, 1191 trillion USD of HVC and co-products can also be generated (Table 10). Once again, across all high value compounds, production quantities are 8.41 greater for aquacultures compared to coastal sites (Table 10).

Table 6. Inventory of data used in the present study to determine the time needed for removing 100GT of atmospheric CO_2 using three scenarios of seaweed mass cultivation.

Data	Value	Ref
Seaweed Biomass Estimation		
2100 CO_2 removal goal	100 Gt	[3]
CO_2 to Carbon conversion factor	3.67	[3]
Inshore Coastal Surface Area (Scenario A)	5.7 million km^2	[233]
Total theoretical ocean surface area for *Ulva* seaweed farms (Scenario B)	100 million km^2	[237]
Ecologically available ocean area for seaweed farms (Scenario C)	48 million km^2	[238]
Wild seaweed average net primary productivity	420 g C m^{-2} $year^{-1}$	[233]
M. pyrifera net primary productivity	1300 g C m^{-2} $year^{-1}$	[234]
Ulva sp. net primary productivity	838 g C g m^{-2} $year^{-1}$	[239]
Carbon to biomass dry weight conversion factor	4	[233]
Biomass dry weight to fresh weight conversion factor	4	[240]
HVC Extraction		
Biomass (dw) to bioethanol conversion factor	0.213 kg/kg	[241]
Ethanol density	783 kg/m^3	
Bioethanol market price	0.4 USD/L	[242]
Biomass (dw) to phlorotannin conversion factor	0.002005 mg/kg	[243]
Phlorotannin market price	70 USD/kg	[91]
Biomass (dw) to protein conversion factor	0.6169 mg/kg	[244]

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Carbohydrate ratio of *M. pyrifera* (dw)	0.648 kg/kg	[244]
Carbohydrate extraction efficiency	89.67 %	[244]
Alginate fraction of *M. pyrifera* carbohydrates	62.54 %	[244]
Alginate market price	12 USD/kg	[79]
Mannitol fraction of *M. pyrifera* carbohydrates	8.05 %	[244]
Mannitol market price	7.3 USD/kg	[64]
Single cell protein price	10.4 USD/kg	[245]

Table 7. Scenario A, estimated time required to remove 100 Gt of CO_2 using seaweed farms of *M. pyrifera, Ulva* sp., and average wild seaweed species over the inshore coastal sites (5.7 M km²).

Seaweed	NPP (kg C m^{-2} yr^{-1})	CO_2 removed (Gt/year)	Biomass fresh (Gt)	Biomass dry (Gt)	Time (year)
M. pyrifera	1.3	27.17	118.56	29.6	3.64
Ulva sp.	0.838	17.52	76.43	19.1	5.65
wild seaweed (average)*	0.42	8.78	38.30	9.58	11.28

*The calculations are based on the average NPP of the wild seaweed (naturally grown seaweed) in the marine environment.

Future perspectives

According to the Intergovernmental Panel on Climate Change (IPCC) and European Commission, a number of targets must be met in order for the planet to not reach a stage of irreversible climate change. Firstly, human activity must be carbon neutral by 2050 [246]. Second, a minimum of 100 $GtCO_2$ must be removed from the atmosphere using carbon dioxide removal (CDR) strategies by 2100 [3]. Both objectives aim to maintain global temperature increase to below 2°C. Our results show total sequestration of 100 $GtCO_2$ could take less than 12 years, based on wild seaweed cultivated in inshore coastal sites alone. This is already considerably shorter than the time scale left until the 2100 deadline. However, this period can be significantly reduced if high productive seaweed species are selected for farming. Therefore, seaweed cultivation is an efficient means of atmospheric carbon dioxide removal.

Scenario B of seaweed farms was limited to *Ulva sp*. as the used model was specifically designed for that genus of *Chlorophyta*. Though other types of macroalgae can generally grow within the same niche as *Ulva*, growth over 100 million km², around 10% of total ocean area, is restricted to *Ulva* sp. [247]. However, Table 7 highlights both the scale at which seaweed may be grown and, consequently, the efficient carbon capture and sequestration power of seaweed. Indeed, seaweed farms have the potential to offset total carbon emissions from entire industrial sectors. Seaweed farming on 3.8% of the West Coast Exclusive Economic Zones could offset carbon emission for the entire Californian land farming sector. Moreover, only an estimated 474 km² of seaweed farms are required to completely offset the entire global seafood aquaculture industry [238].

However, the *Ulva sp*. growth model does not take into account the ecological constraints of all seaweed species. A broader more accurate model estimates that 48 million km² of ocean surface could be used for seaweed farming [238]. Under these conditions, 100 $GtCO_2$ removal could be achieved in under a year when farming *M. pyrifera* and *Ulva sp.* (Table 8). Furthermore, as the yearly productivity of both species exceeds the minimum CDR target, there is the potential to go beyond the IPCC's

requirements. Further CO_2 removal would contribute to "negative carbon" emissions. This could not only completely limit global warming but also reverse the 1.3°C temperature rise that has already occurred [248]. Indeed, removal of the IPCC's upper limit, 1000 $GtCO_2$, would undo 20 years of global GHG emissions [249,250].

Only available surface area for seaweed growth was explored in this analysis. However, improvements in seaweed farming conditions could enhance seaweed productivity and would thus shorten the time needed for CO_2 removal and/or a decrease in necessary surface area. There is however a lack of research on seaweed farming conditions and their direct impact on seaweed NPP. Certain studies have focussed on the various factors that influence biomass production but not NPP [251,252]. Aside from temperature, the most limiting parameter is the rate of photosynthesis, itself limited by multiple physiological processes [253]. A study by Golberg & Liberzon showed that the use of an external mixing system, one that would cycle seaweed culture plots, enabling optimized light exposure, could increase total energy gain by two orders of magnitude [254]. However, practical technologies based on this principle have yet to be developed.

Though seaweed farming could be a means for carbon capture, a number of studies have highlighted the economic and environmental costs of such a strategy [238,255]. There can be some debate on the feasibility of our three scenarios. For example, despite scenario C being ecologically possible, biomass transportation from distant offshore seaweed farms to marine biorefineries becomes a major challenge, leading to increased production costs. Indeed, until more advances are made in transportation technologies, seaweed farming will be restricted to areas close to the coast [237]. Another major issue is the environmental consequences of seaweed farming. This includes concerns regarding the release of artificial and organic materials into the environment, as well as the noise disturbance to marine wildlife [256]. However, seaweed farming has also been shown to have a number of ecological benefits. Macroalgae mitigate ocean acidification whilst replenishing oxygen supplies by removing CO_2 and producing O_2 through photosynthesis [255]. This is particularly important in hypoxic environments, resulting from the eutrophication of water bodies. Seaweed can further help to bioremediate nutrients and metals from agricultural and urban runoffs [257]. Aside from biochemical impacts, seaweed farms can also serve as a means of wave attenuation, providing protection from extreme weather phenomena [255].

To further explore the economic potential of seaweed, the mass and value of the bioethanol and HVC that could be produced from the biomass were calculated. In 2018, worldwide oil consumption was estimated at around 4622 million tonnes (Mtoe) [258,259]. According to our results, bioethanol production from coastal sites could generate around 6310 Mtoe of bioethanol. This value more than exceeds planetary oil requirements. In fact, total seafarm bioethanol production, estimated at 53200 Mtoe, greatly exceeds the 2018 global energy demand of 13864.9 Mtoe [258]. It is worth noting that, in this study, bioethanol estimates were based on production using fresh water and genetically modified *E. coli* [241]. Further research is needed for the bioethanol production on such a scale within a coastal marine biorefinery, using seawater and marine yeast. Nonetheless, the volumes of bioethanol that could be produced using carbon capture seaweed could meet worldwide energy demands and replace the petrol industry, a main driver of CO_2 emissions. Climate protection policies could also lead to the expansion of the bioethanol market. The Renewable Fuels Standard (RFS) mandates the blending of 36 billion gallons of renewable fuels by 2022, of which only 42 % can be corn-based ethanol [260]. The remaining gap can be filled by seaweed bioethanol, a market only set to grow in coming years. Global bioethanol production is projected to rise by 14%, with the biofuels market set to reach USD 246.52 billion by 2024, at a compound growth rate of 4.92% [242,261].

Turning to HVC, like all seaweed species, the chemical composition of *M. pyrifera* varies greatly depending on environmental conditions [262–264]. Specific values and parameters chosen for our calculation came down to the quality of the study [244]. As seen in Table 10, considerable amounts of phlorotannin can be extracted. Many phlorotannins have commercial value as they have been shown to have anti-oxidant, anti-diabetic, radioprotective, hepatoprotective, and anti-inflammatory activity [265]. Indeed, phlorotannin is the most valuable compound (~ 70 USD/kg) that can be extracted from *M.pyrifera* [79]. However, given the low production yields, they tend to generate the least revenue.

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Optimisation of extraction procedures could increase the total volume and revenue of the product. However, given the generally low phlorotannin content of seaweed, there is a ceiling limit [266].

Table 8. Scenario B, estimated time needed to remove 100 Gt of CO_2 using seaweed farms of *Ulva* sp. over the total theoretical ocean surface area available for inshore and offshore seaweed farming (100 M km^2).

Seaweed	NPP (Kg C m^{-2} yr^{-1})	CO_2 removed (Gt yr^{-1})	Biomass fresh (Gt)	Biomass dry (Gt)	Time frame (year)
Ulva sp.	0.838	307.29	1340.80	335	0.32

The most interesting seaweed HVC are alginate and mannitol as both sugars have multi-billion-dollar valuations. Alginate is the most abundant of the extractable HVC and is also the most lucrative while mannitol is the second most profitable. This is in contrast to bioethanol, which, despite having the highest production volumes, is the lowest grossing product. Given their abundance, polysaccharides are the most cost-effective HVC for future investments. Furthermore, the market for algal sugars is set to expand in the coming years. Alginate is finding increasing pharmaceutical and biomedical applications while mannitol, a low calorie sweetener, is facing increasing demand in a health concerned population [267,268].

Table 9. Scenario C, estimated time needed to remove 100 Gt of CO_2 using seaweed farms of common seaweeds, *M. pyrifera* and *Ulva sp*. NPP over ocean surface area ecologically available for offshore and inshore seaweed farming (48 M km^2).

Seaweed	NPP (kg C m-2 yr-1)	CO_2 removed (Gt/year)	Biomass wet (Gt)	Biomass dry (Gt)	Time frame (year)
M. pyrifera	1.3	228.82	998.4	249.6	0.43
Ulva sp.	0.838	147.50	643.584	161	0.67
Wild seaweed (average)	0.42	73.93	322.56	80.6	1.34

Though large quantities of proteins can be extracted from *M. pyrifera*, as most have not been characterised and therefore, they currently have no commercial applications; however, they may have tremendous potential especially as animal feed. Brown seaweeds also contain fucoidan, a sugar with interesting properties and commercial value. However, due to a lack of efficient extraction procedures, no values could be estimated for fucoidan from *M. pyrifera* [269,270].

In this study, the compound estimations were based on the individual extraction values of each chemical. This means that the extraction process was optimised for a single compound. For the simultaneous extraction of all HVC and the production of bioethanol, the design of a downstream process is necessary. However, in such downstream production processes, product yields and valuations may decrease as the extraction procedures are not tailored to the individual chemicals. Furthermore, the product valuations in this study are based on current market prices. With an influx of HVC on the market, the increased supply may exceed the demand, leading to an overall drop in price. However, a supply increase and price fall makes a product more accessible, thus opening its use to further markets. The average price of each compound is also based on its bulk sale value. Laboratory grade chemicals sell for a higher price but, in turn, entail higher purification costs. Nonetheless, seaweed biorefineries represent a potential multi-billion-dollar business that could potentially aid in the removal of excess CO_2 and help combat climate change.

Table 10. Potential annual bioethanol and HVC production and revenue from *M. pyrifera* biomass grown over 5.7 M km^2 (Scenario A) and 48 M km^2 (Scenario C).

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Product	Price (USD/Kg)	Scenario A (5.7 M km²)		Scenario C (48 M km²)	
		Weight (Million kg)	Value (Million USD)	Weight (Million kg)	Value (Million USD)
Bioethanol	0.5068	6,310,000	3,197,908	53,200,000	26,961,760
Phlorotannin	70	0.0594	4	0.5	35
Alginate	12	10,800,000	129,600,000	90,000,000	1,080,000,000
Mannitol	7.2	1,390,000	10,008,000	11,700,000	84,240,000
Protein	10.4	183	1,903	1,540	16,016
Total value			142,807,815.36		1,191,217,811

Conclusions

A coastal marine biorefinery is a novel conceptual refinery system that relies on marine components to produce sustainable biofuels. Sufficient research exists within the field for the emergence of such seaweed refinery systems. Seaweeds are an ideal substrate for bioethanol production as they do not require arable land or freshwater and require less intensive treatment procedures. Furthermore, all seaweed classes contain HVC with commercial applications. As conventional methods for seaweed pre-treatment and HVC extraction are often not ecologically benign, greener alternatives have been developed. Such technologies often combine biomass treatment and product recovery into a single process, thus using less power and organic solvents than conventional methods. However, each technology comes with its own drawbacks. Due to their novelty, further research is still required to optimise the individual extraction procedures. Within the context of a seaweed marine biorefinery, halotolerant enzymes and marine microorganisms are needed for saccharification and fermentation during bioethanol production. A variety of enzymes capable of seaweed polysaccharide breakdown have been isolated and identified from marine sources or genetically engineered. However, a main limitation for their use is their production costs. A number of organisms capable of saltwater fermentation have also been identified from marine environments. Additionally, bioethanol production co-products can serve as further sources of revenue or may be used as inputs for other industries, enabling the expansion of marine biorefineries into integrated marine biorefineries. Such systems allow maximum biomass utilisation whilst minimising waste.

As seaweeds are highly efficient at carbon capture, an analysis was conducted to investigate its CO_2 sequestration capacity. Based on the literature values of seaweed net primary productivity over three different surface areas, the required time and biomass for the removal of 100 gigatons of CO_2 was determined. It is possible to remove all excess CO_2 within 12 years. From the biomass estimated over the 3 scenarios, sufficient volumes of bioethanol can be produced so as meet global energy demands and replace the petrochemical industry. Moreover, the extractable HVC have a multi-billion-dollar valuation, making seaweed biorefineries an attractive business.

Though as of now only conceptual, coastal marine biorefineries have the potential to make their way onto the biofuel scene thanks to the already existing technologies. With further research, the individual aspects of such biorefineries could be optimised and better established. A move to coastal marine biorefineries may pave the way to carbon neutral energy production and hopefully a cleaner, more sustainable, and marine based future.

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