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Review

Exploring the Prospects of Fermenting/Co-Fermenting Marine Biomass for Enhanced Bioethanol Production

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Abstract: With the rising demands for renewable fuels, there is growing interest in utilizing abundant and sustainable non-edible biomass as a feedstock for bioethanol production. Macroalgal biomass contains a high content of carbohydrates in the form of special polysaccharides like alginate, agar, and carrageenan that can be converted to fermentable sugars. In addition, using seagrass as a feedstock for bioethanol production can provide a sustainable and renewable energy source while addressing environmental concerns. It is a resource-rich plant that offers several advantages for bioethanol production, including its high cellulose content, rapid growth rates, and abundance in coastal regions. To reduce sugar content and support efficient microbial fermentation, co-fermentation of macroalgae with seagrass (marine biomass) can provide complementary sugars and nutrients to improve process yields and economics. This review comprehensively covers the current status and future potential of fermenting macroalgal biomass and seagrass, as well as possible combinations for maximizing bioethanol production from non-edible energy crops. An overview is provided on the biochemical composition of macroalgae and seagrass, pretreatment methods, hydrolysis, and fermentation processes. Key technical challenges and strategies to achieve balanced co-substrate fermentation are discussed. The feasibility of consolidated bioprocessing to directly convert mixed feedstocks to ethanol is also evaluated. Based on current research, macroalgae-seagrass co-fermentation shows good potential to improve the bioethanol yields, lower the cost, and enable more optimal utilization of diverse marine biomass resources compared to individual substrates.

Keywords: biorefinery; green energy; macroalgae; pretreatment; seagrass; seaweeds

1. Introduction

The growing concerns regarding climate change, energy security risks, fossil fuel prices, environmental degradation, and resource depletion have collectively fostered research on environmentally friendly and sustainable energy alternatives. Among other renewable energy sources, ethanol revenue from 2023 to 2029 is estimated to grow at 5.5%, reaching nearly US $152.24 Billion [1]. The traditional feedstocks for ethanol production include starchy/sugary biomass, including mainly corn, sugarcane, and sugar beet. These feedstocks are referred to as first-generation, which compete strongly with edible food and raise the *food-versus-fuel* paradigm. The second-generation ethanol feedstocks include lignocellulosic biomass/waste, potentially replacing first-generation feedstocks [2,3]. However, issues still need to be resolved, such as the high costs of the complicated methods required to convert lignocelluloses into fermentable sugars and the ineffective microbial technology

for fully fermenting these sugars into ethanol [4,5]. Alternatively, algae (including macroalgae and microalgae) have been extensively investigated as highly promising candidates for third-generation biofuels, boasting numerous advantages over their first- and second-generation counterparts [6–8]. However, their inherent limitations in natural productivity and efficiency have prompted researchers to explore metabolic engineering, aiming to position engineered algae as fourth-generation biofuels. This new generation seeks to harness the capabilities of oxygenic photosynthetic microorganisms for eco-friendly and economically viable biofuel production. Metabolic engineering has been used to enhance growth, stress tolerance, lipid accumulation, and biofuel synthesis [9,10]. In addition, the use of molecular techniques such as transformation, gene editing, and RNA interference for environmentally friendly and economically viable biofuel production, combined with enhanced CO_2 sequestration, has been gaining significant attention [11–13]. Genetically modified algae, like *Chlamydomonas reinhardtii* optimized for hydrogen production [9] and mutated *Parachlorella kessleri* for biodiesel production [14], present several benefits, including higher biomass yield, decreased land usage, reduced water demands, efficient carbon dioxide capture, and effective wastewater purification. However, several challenges need to be overcome, encompassing concerns related to biosafety, cost-effectiveness, scalability, regulatory compliance, and social acceptance. Continuous research and development efforts are imperative to fine-tune the metabolic engineering of algae, paving the way for the production of fourth-generation biofuels that contribute to a sustainable and environmentally responsible green future.

Macroalgae and seagrass (marine biomass) are two types of aquatic plants that grow in a wide variety of marine habitats. The term "macroalgae or seaweeds" encompasses multicellular algae that can be categorized into three primary groups based on their pigment composition: red, brown, and green. These distinctive pigment groups indicate the diverse and fascinating array of marine macroalgae species found in aquatic ecosystems worldwide. They can be found in various depths of seawater, ranging from the intertidal zone to depths as deep as approximately 200 m, where sufficient light sustains their growth [15,16]. Conversely, the term "seagrass" pertains to flowering aquatic plants. Seagrasses are specifically adapted to thrive in shallow and transparent seawater environments to access ample sunlight for their photosynthetic processes. This unique botanical group plays a crucial ecological role in coastal ecosystems and is distinguishable from macroalgae due to its vascular plant characteristics and its capacity to form underwater meadows in coastal waters.

Despite the optimistic outlook for the environmental benefits and commercial feasibility of cellulosic ethanol [17,18], its large-scale implementation has remained elusive. The processes associated with breaking down sugars from resilient renewable biomass and converting these mixed sugars into ethanol have proven to be intricate and formidable, presenting technical barriers and fundamental constraints that pose significant challenges. In addition, bioethanol production from marine plant biowaste remains relatively unexplored, with limited studies conducted on utilizing seagrass wrack as a potential feedstock for bioethanol production [19–21]. This underexplored area of research presents an exciting opportunity to tap into a largely untapped resource, potentially opening new avenues for sustainable and eco-friendly bioethanol production processes. Thus, co-fermentation of seagrass and seaweed may be successful, even though implementing individual cellulosic ethanol production is difficult. It could also provide a nutrient balance for enhanced microbial growth, enhancing conversion efficiency and bioethanol yield. Furthermore, incorporating macroalgae and seagrass into bioethanol production processes could potentially streamline the post-harvest sorting process, reducing time and energy expenditure. Therefore, this review evaluates various strategies of individual fermentation as well as simultaneous co-fermentation of marine biomass, including seaweeds and seagrass. Different methods of macroalgae farming, as well as harvest, are discussed. In addition, co-fermentation could have potential applications for bioethanol production from macroalgae and seagrass, which is further evaluated.

2. Chemical Composition of Different Feedstocks Hydrolysates

The composition of hydrolysate from lignocelluloses, seagrass, and different macroalgal species depends on the type and source of the biomass, pretreatment method, and hydrolysis conditions [22]. Generally, most lignocellulosic hydrolysates, such as maize cobs sawdust, and sugar cane bagasse, contain mainly glucose and xylose, in addition to other sugars such as arabinose, galactose, and mannose (Figure 1). In addition, the substantial presence of lignin in most land plant biomass plays a pivotal role in conferring resistance of biomass to hydrolysis. Macroalgal hydrolysates contain mainly glucose and mannitol, as well as other sugars such as galactose, fucose, and rhamnose. They also have some beneficial compounds, such as alginic acid, laminarin, fucoidan, and iodine, that can enhance the growth and metabolism of microorganisms [23]. Whereas a hydrolyzate of maize stover has around 40% glucose and 30% xylose, the hydrolysates of macroalgae have completely different sugar compositions (Figure 1), even among different macroalgal groups [4,24,25]. For instance, red macroalgae have dominance in glucose (22%) and galactose (23%). Along with glucose and mannitol, brown macroalgae contain additional carbohydrates (about 14%) in the form of alginate. The combination of brown seaweed alginate, mannitol, and glucose has recently been shown to be a promising source for bioethanol generation [4]. Interestingly, seagrass contains mainly cellulose, hemicellulose, and pectin (Figure 1), which could provide a promising substrate for co-fermentation with macroalgae.
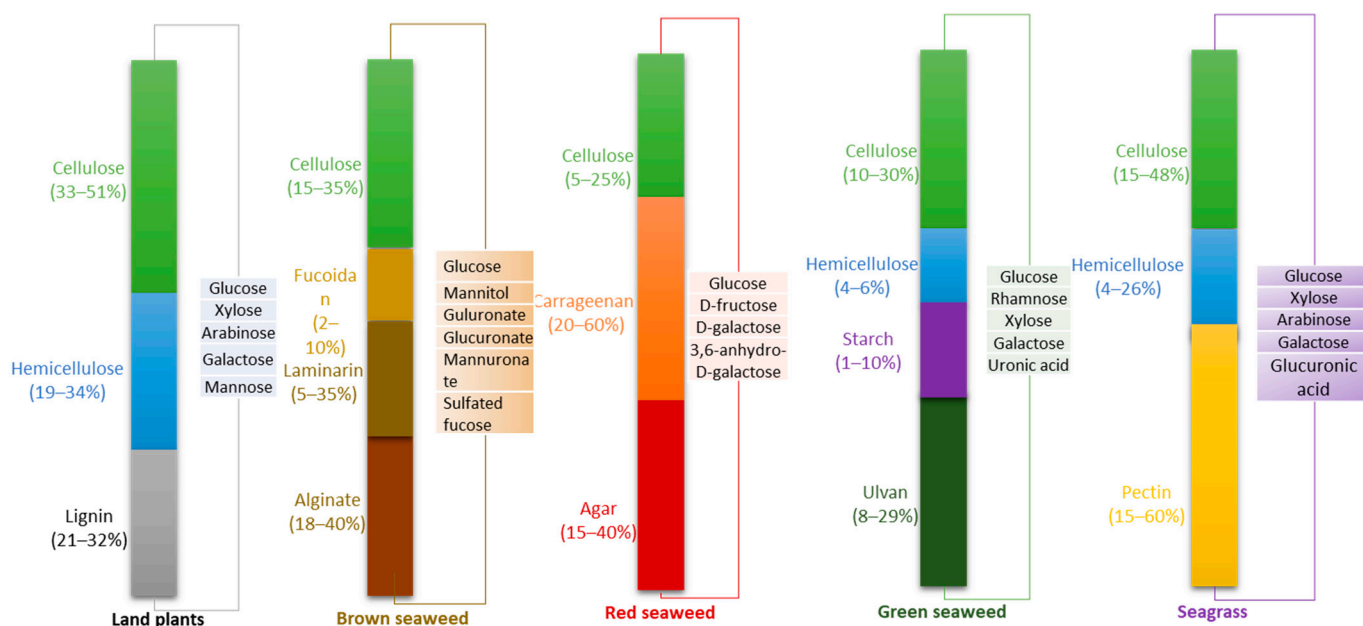

Figure 1. Main polymers and hydrolytic components of lignocellulosic biomasses (land plants) in comparison to different groups of macroalgae and seagrass. Data are extracted from Cosenza et al. [26], Ibraheem and Ndimba [27], and Pfeifer et al. [28].

In the soluble portion of non-starch polysaccharides in the majority of seagrass hydrolysates, galactose, glucose, and mannose are the predominant sugars (>10 g kg^{-1} of dry matter) [29]. Glucose makes up the majority of the neutral sugars (74% of the total output) in newly senescent tissues [30], while the major component of the seagrass cell wall is cellulose [31]. On the other hand, certain seagrasses include sulfated polysaccharides (SP), similar to red, brown, and green macroalgae [32–34]. The presence of uncommon pectic polysaccharides known as apiogalacturonans is another distinctive characteristic of the cell walls of seagrasses. The unusual monosaccharide apiose (*Apif*) is replaced with large numbers of low-methyl esterified galacturonic acid (*GalAp*) units [35,36]. The large variation in sugar content indicates the importance of developing fermentation processes that are suited for the target biomass resource.

After pretreatment and hydrolysis, biomass hydrolysates are composed of different hexoses and pentoses. Although the sugar composition of hydrolysates from different feedstocks varies to some extent, xylose and glucose are the two main sugars found in the majority of hydrolysates from terrestrial plant biomass. Differently glucose and galactose are the two main sugars in hydrolysates produced from marine biomass. Wild-type *Saccharomyces cerevisiae* cannot produce ethanol directly from hydrolysates of lignocellulosic biomass because xylose cannot be fermented by *S. cerevisiae*, while galactose can be fermented, even though less than glucose [37]. *S. cerevisiae* lacks the metabolic pathways to utilize xylose, which is the second most abundant sugar in lignocellulosic hydrolyzates after glucose [37]. To address this issue, the conversion of xylose-rich hydrolysate into xylulose is necessary, and this conversion can be achieved through the use of enzymes like xylitol dehydrogenase and xylose reductase, which are not naturally present in wild-type *S. cerevisiae* strains [38,39]. However, galactose can be converted by *S. cerevisiae* to glucose-6-phosphate by the Leloir pathway, which involves four enzymatic steps and consumes one molecule of ATP. The expression of the Leloir pathway genes is regulated by the galactose (GAL) system, which is repressed by glucose and induced by galactose. Therefore, when glucose and galactose are present together in the hydrolysate, *S. cerevisiae* prefers to consume glucose first and represses the uptake and metabolism of galactose [40]. To enhance the efficiency, it has been of the utmost importance to metabolically engineer *S. cerevisiae* to ferment xylose. However, ethanol yields from galactose by modified *S. cerevisiae* still need to be enhanced for marketable uses despite the fact that there are several methods for enhancing galactose consumption to varied degrees.

3. Macroalgae

Macroalgae are multicellular macroscopic organisms that can be classified into three divisions, namely Rhodophyta (red algae), Phaeophyta (brown algae), and Chlorophyta (green algae). These distinct divisions are based on their pigmentation and provide a classification framework for the diverse world of macroalgae. Different macroalgae can be found at various seawater depths, from the intertidal zone to the greatest depths. They have different shapes and sizes, ranging from filamentous and leafy to calcareous forms [41]. Depending on the species and taxonomical group, macroalgae are impacted by their habitat's characteristics, such as temperature, light, salinity, pollution, nutrients, and even water current [42]. The distribution of macroalgae within their habitat is shaped by their physiological and ecological adaptations to various environmental factors and grazing pressures. Certain macroalgae exhibit greater resilience to fluctuations in these conditions, enabling them to thrive across diverse habitats. For instance, members of the green alga genus *Ulva* can thrive in environments with elevated nutrient levels and pollution, whereas members of the brown alga genus *Laminaria* are well-suited for cold and deep-water ecosystems [43]. In general, macroalgae can be sourced from two primary origins: natural marine ecosystems and aquaculture systems.

3.1. Naturally-Growing Macroalgae

Macroalgae and phytoplankton are predominant contributors to marine primary production [44,45]. Despite once being considered predecessors of land plants, algae lack the characteristic plant structures such as stems, leaves, and roots. On the other hand, they have a level of complexity surpassing that of single-celled microalgae but do not exhibit the intricate anatomical features found in terrestrial plants necessary to live under aerial conditions. Seaweeds are multicellular eukaryotic aquatic organisms, boasting around 9000 distinct species, and they display specialized cell structures and functions, leaning more towards a plant-like nature [46,47]. They generally have simple reproductive structures without vascular tissue. In their natural habitats, macroalgae can be found attached to, for example, sand particles or rocky substrates and sometimes free-floating. They form a multilayered stable vegetation that captures the available solar photons.

Because macroalgae do not compete with other crops for land or freshwater, they are becoming extremely important in aquaculture [48]. They also produce large amounts of biomass in a short time and have relatively high carbohydrate contents. One of the promising marine algal species, sugar kelp, has a high potential to provide biofuels if grown and used sustainably [49]. The majority of macroalgal species are restricted to the marine environment. Due to their diversity, algae utilize various light spectra, and certain light-capturing pigments are thought to contribute to the diversity of algal species to water depth. Large brown algae produce kelp forests with extremely high productivity, upwards of 3000 g cm^{-2} per year. Macroalgae are the dominant autotrophic coastal marine biomass, providing food and shelter for associated fauna [50]. Because macroalgae are photoautotrophic, they may use either CO_2 or HCO_3 to create and store organic carbon [51,52]. Due to the relatively slow diffusion rate of CO_2 and low concentration at a pH of about 8 in saltwater, the majority of macroalgae directly absorb HCO_3^- for growth [53]. However, certain macroalgae may use CO_2 directly as a substrate or can speed up the interconversion of CO_2 and HCO_3 by employing enzymes like carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase [42]. In addition, macroalgae possess significant photosynthetic potential, allowing them to produce and store ample carbon resources, rendering them promising candidates for biofuel production and carbon sequestration. Therefore, coastal vegetated habitats have garnered global attention for their ability to facilitate significant carbon sequestration [54,55]. However, it is important to note that their photosynthetic efficiency can vary considerably among species, even within the same taxonomic group. The net primary production (NPP) per unit area in many macroalgae stands out as one of the highest among all global habitats, rivaling that of tropical rainforests [56]. In the context of naturally grown algae harvest, it is estimated that macroalgal forests constitute a significant biome covering approximately 6.06–7.22 million km^2 worldwide [15]. They are primarily dominated by red algae, contributing to an NPP of approximately 1.32 Pg C/year, with brown algae playing a predominant role in this production [15]. However, the growing interest in various applications of macroalgae has led to an increasing focus on macroalgae farming in recent years.

3.2. Macroalgae Farming

The global seaweed output (both farmed and wild) has increased nearly three-fold in the last two decades, from 118,000 tons in 2000 to 358,200 tons in 2019 [57]. Due to their great nutritional content, macroalgae are globally recognized as a food source, particularly in Asian cuisine. Furthermore, their potential in the pharmaceutical sector is gaining momentum because of the richness of bioactive compounds [58,59]. It is reported that in 2019, a staggering 97% of the world's aquaculture production was derived from artificial seaweed farming [60].

Each hectare of macroalgae farming has the potential to yield approximately 59 dry metric tons of macroalgae per year, with estimated bioethanol production of about 322 L per metric ton of dry macroalgae [61]. According to the provided data, the maximum bioethanol production rate from macroalgae is estimated to be 19 m^3 per hectare per year. Remarkably, this yield significantly surpasses that of corn (five times higher) and sugarcane (two times higher), underscoring the potential of macroalgae as a more efficient bioethanol source [2,4,61,62]. The commercial market for products derived from macroalgae has expanded considerably in recent years, driving advancements in macroalgae cultivation. Notably, a significant focus has been on developing macroalgal species that exhibit disease resistance, thrive in higher-temperature environments, and display rapid growth rates. These efforts are indicative of the growing interest in enhancing and optimizing macroalgae cultivation methods to meet the demands of various applications and market needs [2,63]. However, promoting the industrial cultivation of macroalgae can be expedited by establishing a seed bank dedicated to preserving species that exhibit favorable growth and production attributes. This seed bank would serve as a valuable resource for macroalgae cultivators. The macroalgae cultivation process typically comprises two main stages:

the hatchery phase, where young macroalgae are initially nurtured, and the on-growing phase, where they are further developed and cultivated to reach maturity for various industrial applications. Macroalgae farming was previously discussed by Panahi et al. [2], as summarized in this section.

3.2.1. Hatchery Production

In this phase, germlings from spores are cultivated in a controlled system while attached to substrates throughout this phase. Once they reach a suitable size, these young plants are relocated to coastal farms [2]. Hatchery production can be accomplished through gametophyte or direct seeding techniques [64]. Due to the absence of the hatchery management phase, the direct seeding approach is relatively easier, faster, and more cost-effective but more labor-intensive than the gametophyte seeding method. However, it requires more fertile materials as gametophyte cultures are not bulked up; it has less control over the time of seeding and deployment. It is also unable to preserve chosen seaweed strains for growth and can use only wild types. Throughout every phase of hatchery production, contamination must be prevented, and the environment must be kept constant. The latter is mainly accomplished by keeping the hatchery temperature, e.g., at 10 °C, ensuring that there is appropriate illumination, maintaining clean filters and tanks, changing the water often, and continually monitoring culture. The hatchery equipment has to be cleaned and disinfected once juveniles are transported from the hatchery to the sea. To reduce the cost of water transportation by reducing energy consumption, the hatchery should be situated close to the sea on flat, low-lying terrain. A hatchery must also have enough area to accommodate the equipment and the space needed for basins, laboratories, and departments [2,50].

3.2.2. On-Site Seaweed Farming

In the on-site farming phase, juveniles can be placed on longlines or other systems at the sea after they have grown to a suitable size (6–7 months) until harvest (around June–July) [64]. Farms for macroalgal cultivation can be established in offshore locations, nearshore coastal areas, or land-based ponds [50].

4. Seagrass

Seagrass plays a crucial role in coastal marine ecosystems globally, serving as a foundational element. It provides sustenance for marine creatures such as dugongs and turtles and functions as an essential nursery for various fish and prawn species. It serves as a habitat for a wide range of small marine organisms. Thus, the presence and health of seagrass beds are vital for maintaining the overall ecological balance and biodiversity of coastal marine environments. These meadows are essential to shoreline communities, supporting diverse marine life that sustains local livelihoods. In recent decades, human-induced factors such as diminished water quality, rising temperatures, amplified sedimentation, and intensified grazing pressure have resulted in a worldwide decrease in seagrass populations and the expanse of seagrass beds [65,66]. On the other hand, the overgrowth of seagrass can have negative impacts on the coastal marine ecosystems and the surrounding environment. For instance, the non-native salt marsh plant *Spartina alterniflora* expanded its range deeper into the intertidal zone within China's Yellow River Delta, which posed a threat to the natural habitat of the native seagrass *Zostera japonica* [67]. Thick and overgrown seagrass beds can impede water circulation and flow, leading to stagnant areas with reduced oxygen levels, which can harm aquatic life. In addition, the excessive seagrass growth can alter the physical structure of coastal habitats. It may create overly dense and monotonous underwater landscapes, reducing the availability of open spaces needed by various species for feeding, reproduction, and movement. While seagrass beds are known for their biodiversity, an overgrowth can lead to a decline in species diversity, as some species may thrive in the dense seagrass while others that require more open habitat may suffer. Moreover, the overgrown seagrass can trap sediments and organic matter, leading to increased turbidity

(cloudiness) in the water, negatively affecting light penetration and hindering the growth of other marine plants, such as macroalgae or even corals. Furthermore, it may provide excessive shelter for herbivores like sea urchins and parrotfish, making it difficult for them to graze and control algal growth. This can lead to simultaneous algal overgrowth, negatively impacting the coastal area. In order to maintain a healthy balance, it is important to monitor and manage seagrass ecosystems carefully. Proper management practices may involve seagrass restoration, selective thinning, and maintaining water quality to prevent excessive growth while ensuring the continued ecological benefits of these vital coastal habitats. Simultaneous management of seaweeds has the potential to help maintain the equilibrium of seagrass ecosystems, particularly in temperate and tropical regions where seagrass decline has been linked to the proliferation of macroalgal bloom [68].

Tropical seagrass meadows have gained recent research focus due to declines in iconic marine species like the dugong. Initiatives like neighborhood watch programs and extensive research efforts are vital for managing the uncertain future of coastal marine life. The seagrass habitats in tropical systems can be categorized into river estuary, coastal, deep water, and reef. These categories vary based on water depth and seabed type, influencing factors such as light availability crucial for photosynthesis. Deepwater coastal ecosystems, found between 10 and 70 m deep, contain sand-covered seafloors, while reef environments are formed by coral-derived calcium carbonate rock. Shallow water habitats, exposed during low tide, pose challenges to seagrass growth due to air exposure and intense light. The ongoing research in these diverse habitats contributes crucial knowledge for the conservation of coastal marine ecosystems [69,70].

4.1. Species Diversity

Seagrasses represent a polyphyletic group of marine angiosperms comprising approximately 60 species distributed across five families: Zosteraceae, Hydrocharitaceae, Posidoniaceae, Cymodoceaceae, and Ruppiaceae [71]. These seagrasses are classified within the order Alismatales, following the Angiosperm Phylogeny Group IV proposed system. Important coastal habitats are formed by seagrasses [72]. These species have different morphological, physiological, and ecological characteristics that allow them to adapt to various environmental conditions. Seagrasses can be found in a variety of habitats, such as sandy/muddy or rocky shores, kelp forests, coral reefs, estuaries, lagoons, and deep waters. Many factors, such as climate, light, nutrients, salinity, sedimentation, herbivory, and human activities, influence seagrass diversity. Seagrass diversity is important for maintaining the resilience and functioning of seagrass ecosystems and the services they provide. Seagrasses were recorded and found in 159 countries across six continents [73]. According to Short et al. [74], seagrasses exhibit five primary centers of high global diversity, all of which are situated in the eastern hemisphere. Among these centers, four are in the Tropical Indo-Pacific region, while the fifth is in southwestern Australia, falling within the adjacent Temperate Southern Oceans bioregion. The study reported that the largest and foremost center of seagrass diversity, boasting the highest number of seagrass species of 19, is situated across insular Southeast Asia and extends through north tropical Australia, including the Great Barrier Reef. A second, comparatively smaller center of diversity is identified in southeastern India, encompassing 13 exclusively tropical species. The remaining three high-diversity global centers are located in eastern Africa, southern Japan, and southwestern Australia. In East Africa, there are 12 seagrass species, with only one, *Z. capensis*, being of temperate origin, resulting in a predominantly tropical species mix. Southern Japan also boasts 12 seagrass species, with *Z. japonica* being the sole temperate species contributing to the diversity of this otherwise tropical region. Within the temperate Southern Oceans bioregion, southwestern Australia is home to 13 seagrass species, four of which are tropical in origin and contribute to its high diversity. A deeper insight into these diversity patterns and the specific distribution ranges of individual seagrass species has been discussed by Green and Short [75].

4.2. Cell Wall Structure

Genome data has revealed that significant alterations in cell wall composition are essential for organisms to adapt successfully to diverse marine environments [76,77]. However, the composition and characteristics of seagrass cell walls remain relatively enigmatic. Beyond the ancestral traits inherited from land plants, seagrasses are expected to have undergone a habitat-driven adaptation process to their unique environment. This environment is marked by various abiotic factors, such as high salinity, as well as biotic factors, like diverse seagrass grazers and bacterial colonization, all of which contribute to the stressors faced by these plants. Seagrass cell walls are composed of polysaccharides that are familiar from angiosperm land plants, i.e., cellulose, despite the lack of current information [31]. However, sulfated polysaccharides (SP) are characteristic of the macroalgae. They are also present in the cell walls of certain seagrasses [32,33,78]. Another distinctive characteristic is the presence of peculiar pectic polysaccharides known as apiogalacturonans in seagrass cell walls. The peculiar monosaccharide apiose (Apif) is substituted with significant numbers of low-methyl esterified galacturonic acid (GalAp) units [35,36]. Seagrasses feature highly glycosylated arabinogalactan-proteins (AGPs) that are of particular interest due to their dual role in wall architecture and cellular regulatory processes [79,80]. These AGPs are characterized by their complex structure, featuring extensive polysaccharide components consisting of arabinogalactans (AGs). AGPs were recently isolated from seagrasses and structurally described for the first time [81]. In seagrasses, phenolic polymers (i.e., lignin) responsible for the mechanical strengthening of the wall have also been detected, even though in much lower amounts than angiosperm land plants [30,82–84]. As a result, the cell walls of seagrasses appear to be intriguing fusions of traits from marine macroalgae and angiosperm land plants with novel structural components. More details about the cell wall structure of seagrasses have been previously discussed [81]. However, it is imperative to deepen the comprehension of seagrass cell walls, especially from a technical perspective, given their potential utility in various applications, such as biofuel production, biodegradable materials, and as a source of valuable compounds for pharmaceutical and industrial purposes.

4.3. Seagrass Cultivation

Seagrass cultivation can be performed in different ways, depending on the species, the environmental conditions, and the project's objectives. Some common methods of seagrass cultivation include seed collection and sowing [85]. This method involves collecting seagrass seeds from natural populations or the lab, then sowing them directly into the seabed or into biodegradable mats or bags that can be transplanted later. This method can produce large numbers of plants with high genetic diversity, but it requires careful timing, handling, and monitoring of the seeds and seedlings. Vegetative propagation is another cultivation method [86]. It involves cutting seagrass shoots or rhizomes from donor plants and planting them into the seabed or into pots or trays that can be transplanted later. This method can produce fast-growing plants with high survival rates, but it requires sufficient donor material and may reduce the genetic diversity of the plants. In addition to the aforementioned methods, tissue culture is another promising cultivation technique [87]. This method involves growing seagrass cells or tissues in a sterile laboratory environment and then transferring them to a greenhouse or a nursery for further growth and acclimation. This method can produce disease-free plants with high genetic diversity, but it requires advanced equipment/skills and is costly and time-consuming.

Overall, seagrass cultivation is a promising technique to enhance the conservation and restoration of seagrass ecosystems, which are threatened by global and local stressors such as climate change, pollution, coastal development, and overfishing. In addition, seagrass land-based co-cultivation with seaweeds can also provide benefits for human well-being, such as food security, shoreline protection, carbon sequestration, water filtration, and fisheries production [88]. The existing literature reveals a scarcity of studies focusing on cultivating seagrass for biomass production. In contrast to macroalgae, most research

concerning seagrass biomass relies on natural cultivation systems. However, macroalgae have been relatively more extensively investigated for their potential biomass production through cultivation. The emphasis has been placed on understanding seagrass ecosystems within their natural habitats rather than developing dedicated cultivation strategies for biomass production. In that context, the co-cultivation of seagrass and macroalgae could be of significant ecological, environmental, and economic importance for carbon sequestration, wastewater treatment, enhanced biodiversity, food security, and biofuel production.

5. Biochemical Composition of Macroalgae and Seagrass

The biochemical composition of such weeds has been a subject of significant interest due to their potential as valuable resources for various industrial applications such as food, pharmaceuticals, cosmetics, and biofuels. Macroalgae have a complex lifecycle displaying annual and perennial life histories, alternation of generation, as well as asexual and sexual reproduction. They are a rich source of bioactive compounds, including polysaccharides, proteins, lipids, vitamins, minerals, and secondary metabolites [45]. The composition of these bioactive compounds varies among different species, as well as based on growth factors such as environmental conditions, growth stage, and geographical location [89,90].

Carbohydrates are the most abundant component in most species, ranging from 23% dw to 55% dw. The major structural polysaccharides in different macroalgae include alginates in brown algae, agar and carrageenan in red algae, and ulvans in green macroalgae [91], which account for up to 40–70% dw. Other polysaccharides include laminarin (storage glucan) in brown algae, xylans, and mannans in small amounts in red and green macroalgae [92]. In general, macroalgae contain very low levels of cellulose or hemicellulose (less than 5%) compared to terrestrial plants [52]. They are mainly composed of structural polysaccharides (such as agar, carrageenan, and alginates) and storage polysaccharides (such as starch, amylopectin-like, and laminarin). Carbohydrates are important macromolecules for providing energy and structural support for macroalgae, as well as for potential applications in the biorefining and food industry [93].

In terms of protein content, macroalgae show variability, with levels ranging from 10% to 47% of their dry weight. Remarkably, red and green macroalgae surpass brown algae in their protein content [94,95]. The protein makeup demonstrates a well-balanced amino acid profile, while their digestibility exceeds that of terrestrial vegetation, making them a valuable dietary source [96]. The lipid content of macroalgae ranges between 1% dw to 5% dw, with brown macroalgae generally containing higher lipid proportions than red and green macroalgae [95,97]. These lipids are particularly notable for including polyunsaturated fatty acids (PUFAs), such as omega-3 and omega-6, which can constitute 15–40% of the total fatty acid composition [98].

The principal structural polysaccharides of seagrass encompass cellulose, constituting 15–48% dw, and hemicellulose, constituting 4–26% dw [99]. Additional carbohydrates include starch (3–20% dw) and the sugar alcohol mannitol acting as an osmoprotectant at levels 1–15% dw [100]. The maximum amount of carbohydrates was recorded in the leaf and rhizome of *Cymodocea serrulata* and *Cymodocea rotundata* [101]. To determine if seagrass biomass is suitable for the production of biofuels, it is required to understand the cellulose content and the makeup of hemicelluloses [81]. Previous studies determined the composition of cellulose in the dried plant material from various seagrasses, including specimens of the genus *Enhalus*, *Thalassia*, *Posidonia*, and *Cymodocea*. Comparatively, less cellulose was found in *Posidonia australis* [29] than *Enhalus acoroides* (77%) [31]. The amount of insoluble material remaining after hydrolysis was used to assess the composition of cellulose. Different hydrolysis techniques were used, and the insoluble residue was occasionally thought to include cellulose [81]. The photometric approach of Dubois et al. [102] can be used to determine the carbohydrate component of the insoluble residue.

Regarding proteins, seagrasses exhibit a range of crude protein content spanning 6–16% dw, comparable to their terrestrial counterparts. These proteins showcase a balanced amino acid profile, with a notable dominance of aspartic acid, glutamic acid, glycine, and

leucine [103]. Lipids in seagrasses compose 1–4% dw, approximately half the proportion observed in macroalgae. Among these lipids, PUFAs take up a significant portion, accounting for 30% to 40% of total fatty acids [104]. Hence, seagrass has the potential to serve as a feed and protein source, and this potential can be further optimized through the utilization of a biorefinery approach. In summary, both macroalgae and seagrasses contain a substantial amount of carbohydrates that can serve as valuable feedstock for bioethanol production. The subsequent section provides a detailed overview of the carbohydrates found in macroalgae and seagrasses.

5.1. Carbohydrates in Marine Biomass

In macroalgae, there are many carbohydrate-based molecules. Green, red, and brown seaweeds have carbohydrate levels of 25–50% dw, 30–60% dw, and 30–50% dw, respectively [52]. Understanding the carbohydrate compositions in macroalgae as well as seagrass is crucial for the efficient utilization of these marine biomasses as a carbon source for bioethanol production. Table 1 shows the storage carbohydrates and cell wall polymers present in various seaweed taxa and seagrass.

5.1.1. Green Macroalgae

Starch (α-1,4-glucan) is a polysaccharide found in green algae as a storage molecule in a relatively sparse amount of 1–4%, while the content of lipids is up to 6% [105]. Green macroalgae also contain cellulose and sucrose [106,107] and may also contain other carbohydrates such as ulvan. The latter is a water-soluble polysaccharide sulfate found in relatively high amounts in *Ulva* (8−29% dw). It is widespread in the intercellular space, and fibrillary two-cell thick layer thalli belong to order Uvales [107,108].

Table 1. Major carbohydrate storage and cell wall polymers are present in various macroalgal taxa and seagrass [69,101,107–110].

Polysaccharides	Macroalgae			Seagrass
	Chlorophyta	Rhodophyta	Phaeophyta	
Crystalline polysaccharides	• Cellulose	• Cellulose • (1,4)-β-D-mannans • (1,4)-β-D-xylans • (1,3)-β-D-xylan	• Cellulose	• Cellulose
Hemicellulose	• Xyloglucan • Mannans • Glucuronan • (1,3)-β-glucan	• Glucomannan • (1,3), (1,4)-β-D-xylan	• Sulfated xylofucglucan • (1,3)-β- glucan	• Xylans, xyloglucans
Matrix Carboxylic polysaccharides	• Ulvans	*Not available*	• Alginates	*Not available*
Matrix-sulfated polysaccharides	• Ulvans	• Agar • Carrageenans • Porphyran	• Homofucans	*Not available*
Storage carbohydrates	• Inulin (fructan) • Starch	• Floridean glycogen (Semi-amylopectin)	• Laminarin	• Starch • Sucrose • Glucose • Fructose

5.1.2. Red Macroalgae

In almost all red algae, the heteroside floridoside [α-D-galactopyranosyl-(1–2)-glycerol], the sugar component that builds the florid starch (semi-amylopectin), is their primary photosynthetic storage molecules [110–112]. Apart from floridoside, some species also contain other carbohydrates such as mannitol (*Caloglossa, Ceramiales*), sorbitol, digeneaside (most

of the *Ceramiales* species), and digeneaside with d-isofloridoside (most of *Porphyridiales* species). It is possible to further split isofloridoside, an isomeric floridoside form, into D- and L-isofloridoside. Both floridoside and isofloridoside are important for osmotic acclimatization and storing carbon [113,114]. However, the most significant polysaccharides in red macroalgae from a commercial standpoint are galactans, which include carrageenan (up to 75% dw) or agar (up to 52% dw) [58,115]. As another major constituent, agar is made up of alternating β-D-galactose and α-L-galactose with scarce sulfations [115].

5.1.3. Brown Macroalgae

Laminarin (β-1, 3-glucans) is the unique polysaccharide in brown algae [52,116], with mannitol (M-chains) or glucose (G-chains) attached to the reduction end. This polysaccharide accounts for up to 30–35% dw in brown algal biomass [117]. However, the initial accumulation product of photosynthesis is the monomeric carbohydrate mannitol, which can make up to 30% dw at initial growth phases. The osmoregulatory property of mannitol is obtained from the six-carbon sugar D-mannose [118]. The amount of mannitol and laminarin varies according to the growth process, the reproduction, and the algal tissue component [119]. Alginic acid (also known as alginate) is another dominant polysaccharide that makes up the majority of the cell wall and contributes up to 40% dw of brown algal biomass [120]. Alginate is composed of three different uronic acids, namely mannuronic acid blocks, guluronic acid blocks, and alternative blocks of mannuronic and guluronic units [115].

5.1.4. Seagrass

The main structural polysaccharides found in seagrasses consist of cellulose and hemicellulose, playing significant roles in shaping their composition and functionality. These two macromolecules represent 15–48% dw and 4–26% dw of the biomass, respectively [121]. It is noteworthy to mention that cellulose content in seagrasses aligns with that of terrestrial plants, signifying its role in providing structural integrity. Within the realm of hemicelluloses, the diversity is evident, with constituents such as xyloglucans, xylans, mixed-linkage glucans, and mannans identified [122]. Seagrasses exhibit a strategic carbohydrate storage mechanism in the form of starch, a pivotal energy reservoir. Starch content varies within the range of 3–20% dw [123]. The dominant storage soluble carbohydrate in most seagrasses is the soluble disaccharide, sucrose [124]. Among different previously studied species of seagrasses (*Halodule wrightii, Enhalus acoroides, Syringodium filiforme, Halophila decipiens, Thalassia testudinum,* and *Zostera marina*), sucrose constituted over 90% of total soluble carbohydrate content. In addition to sucrose, other soluble monosaccharides, such as glucose and fructose, as well as polysaccharides, have been identified in small amounts. While comparatively less abundant, seagrasses also exhibit additional soluble carbohydrates such as apiose, arabinose, fucose, galactose, mannose, rhamnose, and xylose [124].

Notably, mannitol, a six-carbon sugar alcohol, emerges as a major storage carbohydrate that serves the pivotal function of osmoprotection. Constituting a proportion of 1–15% dw [100], mannitol exemplifies the dynamic strategies seagrasses employ for maintaining the optimal cellular balance. Furthermore, seagrasses contain an array of other carbohydrates in minor quantities. These include pectin, glucuronic acid residues, and fructans, which collectively contribute to the intricate carbohydrate landscape within these plants [125].

Altogether, total carbohydrate content within seagrasses spans from 20% dw to 60% dw, with cellulose and hemicellulose standing as the predominant macromolecules [124]. These structural polysaccharides play a pivotal role in shaping the mechanical properties and overall architecture of seagrass tissues. It is noteworthy to state that seagrasses exhibit remarkable adaptability in carbohydrate composition, with significant variations observed across species and growth conditions. Factors such as light availability, salinity, and temperature intricately influence the carbohydrate makeup, leading to unique physiological responses that define seagrass species. This variability, underscored by recent research [124],

showcases the intricate interplay between seagrasses and their environment, enriching the understanding of these invaluable coastal ecosystems.

6. Marine Biomass Conversion into Bioethanol

It is crucial to gain a comprehensive understanding of the types and quantities of chemical resources present in marine biomass. For example, the ratio of six- and five-carbon sugars is vital for selecting the appropriate fermentation microorganism and the method of bioethanol production. All varieties of macroalgae [126,127] and seagrass [19,21] can potentially undergo fermentation to produce bioethanol by converting their carbohydrates into simple sugars, followed by the use of suitable fermentable microorganisms. The successful fermentation of bioethanol relies on converting carbohydrates (such as starch, cellulose, laminarin, and/or floridean starch) into simple fermentable sugars, with careful selection of the right microorganism to perform the fermentation process. Consequently, various physical, chemical, and enzymatic methods are considered during the pretreatment and subsequent saccharification steps in the processing of marine biomass. Generally, seagrass bioethanol production follows similar procedures to the conversion of seaweeds.

Handling seaweed after harvest is a critical step, as improper handling can lead to biomass deterioration during storage and transportation. Additionally, the composition of the biomass may alter as a result of contamination by impurities such as sand, aquatic plants, and animals. After being harvested, seaweed is frequently cleaned with water to get rid of rocks, sand, and other unwanted contaminants [128,129]. Previous studies have examined the impact of washing and found that when *L. digitata* is rapidly rinsed under tap water, it results in a significant loss of carbohydrates, with laminarin experiencing reductions of up to 49% [130]. Despite these losses, it is not ideal to have debris, sand, and salts in process streams, particularly for the synthesis of bioethanol on a large scale. Utilizing saltwater for washing on site is a preferable option to conserve the limited freshwater resources. This not only makes it a more cost-effective choice but also aligns with environmentally friendly practices.

Drying macroalgal biomass is a pivotal and energy-intensive step in the process of bioethanol production, primarily because freshly harvested seaweed typically contains a high moisture content of 85–90% [131,132]. Various drying techniques showed high efficiency for seaweeds, including oven drying [133], sun drying [134], and freeze drying [135]. In a previous study evaluating the impact of different drying methods on the composition of *L. digitata*, it was observed that the quantities of laminarin, the primary carbohydrate in brown seaweeds, were 10.9, 10.8, and 14.7% dw for oven-dried, frozen-oven dried, and freeze-dried seaweed, respectively [130,136]. These findings indicate that freeze-drying is a more viable option. However, to be considered a sustainable choice, its economic benefits would need to be thoroughly evaluated and compared. It is worth noting that the high energy consumption associated with freeze-drying may make it impractical for seaweed farmers, particularly those located in underdeveloped coastal areas. An alternate drying technique that has been employed in several research is sun drying, which is now the most practical method for seaweed producers [134,137]. Its impact on the seaweed's carbohydrate content has not yet been studied, in addition to the documented loss of seaweed pigmentation or decolorization. Because sun drying lacks the precise control offered by instrument-based drying methods, there may be many concerns regarding the potential compositional alterations. Affordability remains a crucial determinant in selecting the drying method. Presently, the use of non-dried seaweed is not under examination because drying is essential for mitigating microbial degradation and reducing transportation costs. Thus, the viability of commercial bioethanol production relies on the seaweed being stored and transported in a dry state to processing plants. If the growing/harvest site can supply a nearby bioethanol facility with enough material at a feed rate that matches it, the drying process can be skipped. Another essential handling technique is size reduction or milling, which enhances the biomass surface area, particularly for the activity of catalysts in the phases of hydrolysis and fermentation [134]. Size reduction improves the effectiveness

of transportation and storage by lowering the bulk volume of macroalgal biomass. For dried macroalgae, milling has been shown to have a small hydrolytic effect on seaweed biomass [138].

6.1. Pretreatment

Both macroalgae and seagrass have various carbohydrates such as starch, cellulose, laminarin, alginate, mannitol, ulvan, carageenan, and agar, while they contain no lignin [61,108,139], which gives a benefit to microbial ethanol production over terrestrial biomass. This stage involves pretreating a wide variety of carbohydrates in marine biomass using a variety of techniques. After the pretreatment stage, the polysaccharide feedstock is more susceptible to rapid hydrolysis, which increases the yields of monomeric sugars. Pretreatments are necessary to improve the production of sugars directly or during the subsequent hydrolysis step, prevent the generation of compounds that inhibit the hydrolysis/fermentation processes, reduce the energy requirement, and lower the costs in order to increase the efficiency of the whole process. Macroalgae can be pretreated using physical (chopping, grinding, irradiation), physicochemical (hot water, steam explosion), and chemical (acid, base) methods, individually or combined. Checking for debris and foreign objects is crucial before treatments, as seaweeds may contain residues of adherent epifauna, sand, stones, or other materials after being harvested [2,105]. In addition, mechanical pretreatments are frequently used with other techniques to improve the surface area-to-volume ratios, considerably increasing the sugar yield during the subsequent hydrolysis step [140].

6.1.1. Physical Pretreatment

Exposure of biomass to high-energy irradiations, such as gamma rays, generates ions and/or radicals that initiate various chemical processes, often leading to breaking chemical bonds and a reduction in molecular weight, which directly correlates to the irradiation dosage [141]. In contrast, polar bonds in the biomass and the surrounding aqueous media are vibrated by microwave oven irradiation, creating a hot spot and internal heat inside the inhomogeneous material [142]. This particular heating property causes the particles to erupt, which enhances the disruption of polysaccharides in macroalgal biomass [143]. Yoon et al. [144] depolymerized the complex polysaccharides in *Undaria* sp. biomass using gamma irradiation pretreatment. A change in the concentration of reducing sugar was observed, rising from about 0.017 g L^{-1} in untreated biomass to about 0.048 g L^{-1} in the biomass exposed to 500 kGy of gamma radiation. Moreover, combining this pretreatment with acid hydrolysis (1% H_2SO_4, 121 °C, 180 min), the reducing sugars were increased up to five times. Similarly, Yuan and Macquarrie [145] used microwave heating as a pretreatment for drying the brown macroalga *Ascophyllum nodosum*. The biomass was then pulverized and the acid hydrolyzed (3.13% *w/v* biomass, 0.44 M H_2SO_4, 150 °C, 1 min) with the aid of microwave heating, resulting in the release of 127 mg monosaccharides g^{-1} dw of macroalgal biomass. *Saccharomyces cerevisiae* was used to immediately ferment the concentrated hydrolysate, which produced a yield of 60.7% and an ethanol titer of 5.57 g L^{-1}.

Ultrasonication is another physical pretreatment method that uses sound waves to create cavitation bubbles in a liquid medium. These bubbles collapse and generate shock waves that can disrupt the structure and composition of biomass [146]. Ultrasonication can enhance the solubilization of organic matter, degradation of hemicellulose, reduction of cellulose crystallinity, and enzymatic hydrolysis of polysaccharides in the biomass. Ultrasonication can also improve the extraction of valuable compounds from seaweeds, such as agar, carrageenan, fucoidan, and other phytochemicals. Ultrasonication, as well as other physical pretreatment methods, can be combined with other methods, such as chemical, thermal, or biological, to improve their efficiency and reduce their drawbacks [147].

6.1.2. Chemical Pretreatments

Chemical pretreatment techniques play a vital role in the processing of macroalgal biomass for various industrial applications, including bioethanol production. The three most significant chemical pretreatment methods are acid, alkaline, and steam explosion. Each method offers unique advantages in breaking down the complex macroalgal structures, facilitating the release of carbohydrates/sugars for subsequent hydrolysis. Understanding these pretreatment approaches is essential for harnessing the full potential of macroalgae as a sustainable resource for bioethanol production.

(a) Steam explosion pretreatment

Steam explosion or autohydrolysis was reported as one of the most popular and relatively feasible pretreatment techniques [148]. This method applies heat and pressure steam for a period of time without a catalyst, which is then abruptly decompressed to ambient pressure. As a result, the individual fibers within the biomass are separated, making them more accessible to acid or enzyme attack with relatively little material loss. This approach is typically used in conjunction with other pretreatment procedures, including acid or hot water pretreatments. Polysaccharides are changed into oligomers when macroalgal biomass is hydrothermally pretreated in the hot water process. For instance, *Padina tetrastromatica* was pretreated with hot water (121 °C, 45 min) and then xylanase (50 IU, pH 7, 30 °C, 6 h) from *Bacillus* sp. BT21 was used for enzymatic saccharification. The combined amount of released mannose, xylose, and glucose was 73.3 mg g^{-1} dw of seaweed biomass, 19% more than the untreated biomass [149]. Also, Soliman et al. [150] produced 510 mg sugars g^{-1} dw of *Sargassum latifolium* biomass using biological saccharification (at 80% efficiency) by *Trichoderma asperellum* RM1 at 30 °C for 21 days after hot water pretreatment (pH 5.5, 0.15 MPa, 120 °C, 15 min). While producing only trace amounts of inhibitive byproducts like carboxylic acid and furfural, this method is both cost-effective and eco-friendly due to the relatively high sugar recovery.

(b) Acid pretreatment

Polysaccharides are rapidly broken down into sugar monomers during acid pretreatment using diluted acids such as sulfuric acid (H_2SO_4), hydrochloric acid (HCl), or phosphoric acid (H_3PO_4). This kind of treatment improves the hydrolysis of cellulosic fractions into glucose in the forthcoming enzymatic process, increases the biomass porosity, and maximizes the conversion of polysaccharides into soluble sugars. The most common chemical process used to pretreat raw macroalgal biomass is diluted-acid hydrolysis, which is usually followed by enzymatic hydrolysis. However, for industrial use of acid and heat as catalysts, specialized methods and acid-resistant materials are needed in the pretreatment reactors. After this chemical preparation, ammonia or lime is added to raise the pH so that hydrolysis enzymes and microorganisms can act properly. Ammonia, a highly miscible neutralizer, can also be used to condition the hydrolyzate slurry overall and eliminate the need for solid-liquid separation phases [2]. The less expensive alternative to ammonia is lime, which is not a preferable neutralizer since it was reported to reduce the concentration of sugars by 13% and create side reactions at higher pH values. As an alternative to acid pretreatment, hot water pretreatment was suggested. For the pretreatment of 80,000 and 400,000 tons year^{-1} of dry brown macroalgae, Fasahati et al. [151] analyzed the capital cost, sugar yield, and operational cost of the hot water with acid pretreatment. They concluded that the hot water pretreatment method is more cost-effective than the acid thermal procedure (20% solid load, 50 °C, 30 min). However, it delivers a slightly lower concentration of sugars compared with acid pretreatment but requires less utility and capital cost.

(c) Alkaline pretreatment

The direct substitute for acid pretreatment is alkaline pretreatment, as a base such as 2% NaOH is used instead of using an acid as a catalyst. Few studies evaluated the use of bases for seaweed hydrolysis [132,152,153]. This could be because higher base concentrations are needed to produce catalysts with efficiencies comparable to those of acid

catalysts [154,155]. Additionally, hydroxide ions in bases interact with the hydrocolloids in seaweeds (agarophytes and carrageenophytes) at high temperatures and for extended periods of time to generate gels that are too viscous to ferment [129]. This makes using alkaline pretreatment difficult, especially for red seaweeds. However, this can be lessened by utilizing the biorefinery method, in which the hydrocolloids are removed before the residue is subjected to diluted alkaline hydrolysis.

While chemical pretreatment methods offer several advantages in biomass processing, they also come with certain disadvantages. These include negative environmental impacts due to using hazardous chemicals, elevated cost due to the cost of chemicals, safety measures, and waste disposal, high energy consumption because some chemical pretreatment processes require high energy inputs, such as heating or pressurization, and overreliance on chemical pretreatment may not align with long-term sustainability goals. Thus, balancing the advantages and disadvantages of chemical pretreatment is essential when considering its application in biomass conversion processes, as it requires careful consideration of the specific feedstock, process efficiency, environmental impact, and economic viability. In comparison to chemical methods, biological pretreatment can be highly desirable.

6.1.3. Biological Pretreatment

Biological pretreatment has been suggested as a safe and eco-friendly alternative for biofuel production from different biomass feedstocks. It involves the use of microorganisms and/or enzymes that can degrade cellulose, hemicellulose, and other biological compounds in the biomass, making it more accessible for further conversion [147]. In this regard, microorganisms have been reported as powerful cell factories for the economical manufacturing of several value-added products, including different enzymes [156,157]. Enzymes that break down different kinds of biomass, including seaweeds, are becoming more and more necessary for the effective handling of the feedstock. Several bacteria and fungi may break down seaweeds as the only carbon source by secreting specific enzymes such as alginase, amylase, and fucoidanase [158,159]. Fungal pretreatment using white-, soft-, or brown-rot fungi to break down cellulose and other macromolecules can enhance seaweed bioethanol production [160]. Bacterial pretreatment, using lignocellulose-degrading bacteria to solubilize and hydrolyze the biomass components, was reported to increase the biofuel yield from aquatic plants [147]. Enzymatic pretreatment, using cellulase, hemicellulase, and starch-degrading enzymes to catalyze the hydrolysis of polysaccharides in the biomass, was also reported to improve the ethanol yield from macroalgae and other aquatic plants [161]. Alginase is produced by a variety of organisms, including *Asteromyces cruciatus*, *Acrophialophora* sp., *Lindra thallassiae*, *Corollospora intermedia*, *Dendryphiella salina*, *Dendryphiella arenaria*, and *Setosphaeria rostrata*. Nonetheless, several fungi showed fucoidanase activity, including *Aspergillus niger*, *Aspergillus flavus*, *Curvularia lunata*, *Cladosporium salinae*, *Mucor* sp., *Penicillium purpurogenum*, *Dendryphiella arenaria*, and *Setospheria rostrata*. In general, marine algicolous fungi, including ascomycetes and Zygomycetes, have been recorded in high numbers living on or in healthy seaweeds, with an important role in the decomposition of marine algal materials due to high enzymatic activity [162].

6.1.4. Combined Pretreatment Method

The combination of various pretreatment methods has been found to be highly effective in improving the efficiency of processing lignocellulosic biomass. Numerous combinations, such as ball milling with microwave irradiation, alkali pretreatment alongside high-pressure homogenization, torrefaction combined with washing, acid treatment along with ionizing radiation, thermo-chemo-ozone pretreatment, and ozonation coupled with ultrasonication, are being explored as alternatives to traditional individual pretreatment techniques [147,163,164]. For instance, Patil et al. [165] investigated the efficacy of combined pretreatment using water hyacinth in different forms, like chopped, dried, and ground, as well as in combination with different chemicals/waste like NaOH, poultry waste, and primary sludge. Among these, the combined treatment exhibited the most

favorable potential for enhanced energy recovery compared to individual pretreatment. Notably, NaOH-treated dried, and ground water hyacinth demonstrated superior results when compared to that using dried and groundwater hyacinth alone as a control.

6.2. Hydrolysis

6.2.1. Acid and Alkaline Hydrolysis

After pretreatment, structural polysaccharides in the algal biomass are hydrolyzed chemically or enzymatically to liberate simple sugars. The sugar yield by acid saccharification is almost 50% of the total dry weight of the seaweed biomass, which is roughly 2.5 times higher than that of the enzymatic technique [16]. Table 2 shows the yield of fermentable sugars from various macroalgal biomass using different hydrolysis methods. However, the application of heat during acid or base treatment results in the generation of detrimental chemicals that can hinder the subsequent microbial fermentation stage. For instance, the mechanical pressing technique yielded less ethanol compared to hydrothermal pretreatment of *Ulva lactuca* due to these adverse chemical effects [16,166]. While heat treatment is necessary to solubilize laminarin, a similar finding was observed in *Saccharina latissima*, which had previously been heated and acidified [17]. In acid-hydrolyzed seaweed biomass, xylose and galactose are the precursors to caffeic acid, furfural, levulinic acid, and 5-hydroxymethylfurfural [167]. Furthermore, alkaline hydrolysis was reported to produce intermediate byproducts such as hydroxyacetone, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, and 2-hydroxy-3-methyl-2-cyclopenten-1-one, which can potentially act as fermentation inhibitors. Nevertheless, the impact of these breakdown products on fermentation processes remains uncertain. Karray et al. [168] documented that the hydrolysis of *Ulva rigida* yielded reducing sugars, amounting to 79% of the total carbohydrates on a weight-to-weight basis. This study highlighted that inhibitors resulting from the base-catalyzed hydrolysis were observed only at extremely high concentrations of the catalyst. As with acid catalyst hydrolysates, base catalyst hydrolysates also need to be neutralized before fermentation [153]. This is performed to give reducing sugars a pH value that allows microorganisms to ferment them into ethanol.

Cellulase-producing actinobacterial culture (BPSGA4) was supplied with seagrass substrate for ethanol production through saccharification. In *C. serrulata*, the actinobacterial strain BPSGA4 resulted in the highest bioethanol yield (0.78 mL/g), while *C. rotundata* showed the lowest (0.72 mL/g). Furthermore, the amount of cellulose was lower (23.1 and 21.5) following enzyme hydrolysis compared to acid hydrolysis (58.5 and 52.1) using *C. serrulata* and *C. rotundata*, respectively. Acid hydrolysis revealed that *C. serrulata* and *C. rotundata* both showed relatively high yields of bioethanol, 0.46 and 0.49 mL/g, respectively [21]. As of the present, there is no definitive evidence supporting the preference for base catalysts over acid catalysts in the hydrolysis of macroalgae. To determine which type of catalyst is more advantageous, conducting a techno-economic study could be essential to evaluate the cost-effectiveness and overall feasibility of using either acid or base catalysts for seaweed hydrolysis in bioethanol production.

6.2.2. Enzymatic Hydrolysis

Enzymatic hydrolysis provides several benefits over acid and alkaline hydrolysis, including more favorable circumstances and a larger sugar yield without the production of microbial inhibitory chemicals (Table 2). Despite these advantages, macroalgal enzymatic saccharification is still in its early stages of development. In addressing issues such as the enzyme's specificity towards different polysaccharides and the presence of multiple polysaccharide complexes within a single macroalgal species, substantial advancements are needed. In contrast to terrestrial plants, where the prevalent form of cellulose is monoclinic crystalline cellulose (I β), algal cells predominantly contain triclinic crystalline cellulose (I α). Due to the spatial arrangement of individual cellulose chains in relation to one another, hydrogen bonds in the polysaccharide form are weaker and looser [169,170]. Triclinic crystalline cellulose may, therefore, be more easily converted into reducing sugars than

monoclinic crystalline cellulose and is more accessible to endocellulases and exocellulases, as well as having more sites for attack on released fibrils. To successfully break down this polysaccharide, it is essential to choose the right enzymatic mixture for each type of algal biomass.

The limited hydrolysis efficiency has hindered attempts to saccharify macroalgal biomass using seaweed-specific enzymes, like laminarinase, even though it has been proposed that hydrolysis efficiency could potentially be enhanced through additional pretreatment methods or the utilization of multi-enzyme complexes [52,171]. In this context, bioethanol yield of more than 3% was reported by Yanagisawa et al. [172] from the sequential enzymatic saccharification of glucan polysaccharide in Chigaiso seaweed (*Alaria crassifolia*). In this procedure, a secondary saccharification was carried out using residue-free hydrolysate from the primary saccharification. An enzyme mixture consisting of dextrozyme, liquozyme, rapinase, and viscozyme was used in another study to saccharify 5-cm chopped raw brown macroalgae (75% concentration) using 1% ascorbic acid [171]. After 5.5 h of incubation, the yield and sugar concentration were 8.8 g/L and 89.3%, respectively. Additionally, the fed-batch hydrolysis using the same enzyme technique produced 27.2 g/L of sugars with 80.6% saccharification efficiency after 16 h. Moreover, a combination of chemical and enzymatic hydrolysis can be used to extract mono-sugars from macroalgae. The generation of fermentable reducing sugars, such as D-galactose, D-glucose, D-mannuronate, D-xylose, L-fucose, L-glucuronate, and L-guluronate from brown macroalgae such as *Laminaria* sp. and *Saccharina* sp., was effectively accomplished using this method [173]. However, relatively high levels of heavy metals or even nitrogen, minerals, and sulfur in macroalgae (0.5–11% dw) may be released into the fermentation medium during the pretreatment and saccharification phases, regardless of the procedures chosen. This could affect the microbial community employed for fermentation, potentially necessitating the inclusion of a purification stage before proceeding with the fermentation process. This purification stage can be achieved by employing various materials, such as activated charcoal or lime, to mitigate the adverse effects of impurities or inhibitors introduced during the saccharification process [16,167].

6.3. Fermentation

The purpose of this stage is to effectively produce ethanol by using all of the sugars that may be produced from macroalgae through fermentation. Due to the complexity of lignocellulosic hydrolysate from seagrass, fermentation is more critical than macroalgae. The pretreated biomass can be fermented mainly using separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF), depending on the enzymatic fermentation method.

Table 2. Impact of different hydrolysis methods on releasing fermentable sugars from different macroalgal biomass. Modified from [174] with permission number 5622520595843.

Group	Algal Species	Substrate Concentration	Pretreatment Methods	Conditions of Process	Sugars (Yield)	Refs.
Red macroalgae	*Gelidium elegans*	200 g L^{-1}	Acidolysis	2.5% H_2SO_4, 120 °C, 40 min	Gal (0.238 g/g), Glu (0.243 g/g), Man (0.005 g/g), Xyl (0.010 g/g)	[175]
	G. amansii	120 g L^{-1}	Acidolysis + enzymatic hydrolysis	144 mM H_2SO_4, 150 °C, 10 min, 16 U/mL Viscozyme L and Celluclast 1.5 L (1:1), 45 °C, pH 5.0, 45 min	Gal (0.238 g/g), Glu (0.187 g/g)	[176]
	Gracilaria salicornia	168 g L^{-1}	Acidolysis Enzymatic hydrolysis	2% H_2SO_4, 120 °C, 30 min 5 g/L cellulase, 40 °C, 4 h, pH 5.0	RS (0.0043 g/g) RS (0.0138 g/g)	[133]
	G. lemaneiformis	30 g L^{-1} 5 g L^{-1}	Acidolysis Enzymatic hydrolysis	0.3 M HCl, 80 °C, 2 h 10 U/mL β-agarase, 55 °C, 2 h	RS (0.200 g/g) RS (0.896 g/g)	[177]

Table 2. *Cont.*

Group	Algal Species	Substrate Concentration	Pretreatment Methods	Conditions of Process	Sugars (Yield)	Refs.
Green macroalgae	*Ulva lactuca*	200 g L^{-1}	Acidolysis	1% H$_2$SO$_4$, 125 °C, 30 min	RS (0.180 g/g), Glu (0.152 g/g)	[178]
		100 g L^{-1}	Acidolysis	1% H$_2$SO$_4$, 125 °C, 30 min	Glu(0.041 g/g), Ara (0.087 g/g), Xyl (0.024 g/g)	[179]
		100 g L^{-1}	Acidolysis + enzymatic hydrolysis	7.5% H$_2$SO$_4$, 150 °C, 10 min, 0.3 mL/g commercial cellulase cocktail, 50 °C, pH 5.0, 96 h	Glu (0.082 g/g), Rha (0.070 g/g), Xyl (0.045 g/g), Gal (0.010 g/g)	[152]
		200 g L^{-1}	Enzymatic hydrolysis + acidolysis	Deionized water, 150 °C, 10 min, 0.3 mL/g cellulase, 50 °C, stirring 24 h, centrifugation, 12 M H$_2$SO$_4$, 30 °C, 1 h, 1 M H$_2$SO$_4$, 100 °C, 3 h	Glu (0.113 g/g), Rha (0.090 g/g), Xyl (0.029 g/g), Gal (0.007 g/g)	[180]
	U. reticulata	50 g L^{-1}	Acidolysis + enzymatic hydrolysis	0.5 M H$_2$SO$_4$, 120 °C, 90 min 50 IU/g Viscozyme L, 45 °C, 24 h	RS (0.609 g/g)	[181]
	Rhizoclonium spp.	300 g L^{-1}	Acidolysis + enzymatic hydrolysis	3% H$_2$SO$_4$, 95 °C, 1 h, 2.0 mL commercial enzyme cocktail (CELLIC® C TEC2), 50 °C, pH 6.3, 160 rpm, 24 h	Glu (0.558 g/g)	[182]
	Ulva (Enteromorpha) intestinalis	100 g L^{-1}	Acidolysis + enzymatic hydrolysis	270 mM H$_2$SO$_4$, 121 °C, 60 min, 16 U/mL Viscozyme L and Celluclast 1.5 L (1:1), 45 °C, pH 5.0, 150 rpm, 36 h	Glu (0.166 g/g), Xyl (0.076 g/g)	[183]
Brown macroalgae	*Saccharina* spp.	100 g L^{-1}	Grinding extraction	65 °C, Grinding for 1 h, 20 Volume diH$_2$O, pH 2.0	Man (0.261 g/g), Glu (0.047 g/g)	[184]
	Dilophus fasciola	1 g L^{-1} whole biomass	Acidolysis	5% H$_2$SO$_4$, 121 °C, 30 min	RS 31.98 g/L	[95]
		1 g L^{-1} free lipid biomass	Acidolysis	5% H$_2$SO$_4$, 121 °C, 30 min	RS 37.2 g/L	[95]
	Padina tetrastromatica	2 g L^{-1}	Acidolysis	1% H$_2$SO$_4$, 100 °C, 1 h	RS (0.045 g/g)	[185]
	Laminaria japonica	100 g L^{-1}	Acidolysis	0.15 M H$_2$SO$_4$, 121 °C, 60 min	Glu (0.300 g/g)	[186]
		50 g L^{-1}	Enzymatic hydrolysis	10 mL/g Cellulases mixture, (NS81016; Novozymes A/S) 45 °C, 24 h	Man (0.092 g/g), Glu (0.180 g/g)	[187]
		100 g L^{-1}	Acid hydrolysis Acidolysis+ enzymatic hydrolysis	0.2 M H$_2$SO$_4$, 121 °C, 20min Novozymes Biomass Kit, pH 5.5, 50 °C, 150 rpm, 18 h	RS (0.102 g/g) RS (0.293 g/g)	[188]
	Ascophylum nodosum	100 g L^{-1}	Acid hydrolysis Acidolysis + enzymatic hydrolysis	0.2 M H$_2$SO$_4$, 121 °C, 20min, Novozymes Biomass Kit, pH 5.5, 50 °C, 150 rpm, 18 h	RS (0.125 g/g) RS (0.156 g/g)	[188]
	Sargassum fulvellum (72%), *Hizikia fusiformis* (18%), *Undaria pinnatifida* (6.2%)	80 g L^{-1}	Acidolysis + enzymatic hydrolysis	138 mM H$_2$SO$_4$, 160 °C, 10 min, 16 unit/mL Viscozyme L (1.2 FBG/mL), 45 °C, 48 h	Gal (0.188 g/g), Glu (0.2 g/g), Man (0.037 g/g)	[189]

6.3.1. Separate Hydrolysis and Fermentation (SHF)

This method offers a broad spectrum of hydrolysis options, making it the most commonly used technique for bioethanol production. It also enables fermentation organisms to function at moderate temperatures, maximizing the use of sugars allows hydrolysis/saccharification to act at higher temperatures for improved performance. Furthermore, it allows for the utilization of catalysts and fermenting organisms under their optimal conditions for both hydrolysis and fermentation, respectively [129]. Before introducing an organism in the SHF process, the hydrolysis process must first be completed, and the reducing sugars are collected for fermentation, primarily by centrifugation or filtration. The same reactor may or may not be used for the two individual processes. In a study on *Gracilaria tenuistipitata*, SHF was utilized to acquire the reducing sugars after acid hydrolysis, which were then fermented by *Saccharomyces cerevisiae* [128]. The study showed a bioethanol yield of 0.042 g/g reducing sugars. In another study by Kumar et al. [190], bioethanol was produced from the *G. verrucosa* agar extraction pulp using

SHF. The recorded bioethanol yield was 0.43 g/g from the pulp's enzymatic hydrolysis, followed by fermentation using *S. cerevisiae*.

6.3.2. Simultaneous Saccharification and Fermentation (SSF)

In the SSF method, both yeast and hydrolysis are introduced into the reactor simultaneously and operate under the same set of conditions. Because both hydrolysis and fermentation occur concurrently in the same reactor, the SSF process is generally cost-effective [140]. However, it is important to note that achieving the optimal conditions for both processes simultaneously can be challenging at times, potentially affecting the overall efficiency. However, previous studies confirmed that SSF has a greater yield than SHF when optimum conditions are applied. For instance, maximal ethanol yields of 3.78 and 3.33 g/L were recorded from *Gelidium amansii* using SSF and SHF, respectively, using cellulase as the enzyme and *S. cerevisiae* (KCTC 7906) as a fermenting organism [129]. The conversion efficiencies of 84.9 and 74.7% were achieved for SSF and SHF, respectively. Using the SSF technique, ethanol was produced from rice straw fronds and showed a bioethanol yield of 38 g/L, with 84.7% conversion efficiency. Therefore, SSF appears to be the more favorable approach for bioethanol production from macroalgae.

6.3.3. Other Fermentation Methods

Simultaneous Saccharification and Co-fermentation (SSCF) and Consolidated Bioprocessing (CBP) are two less popular alternatives to SSF and SHF techniques. The creation of cellulose, biomass hydrolysis, and ethanol fermentation all proceed simultaneously in a single reactor as part of CBP processes [191,192]. These procedures employ a single microbe or a combination of microorganisms, which lowers capital expenses. For instance, *Clostridium thermocellum*, *Neurospora crassa*, *Fusarium oxysporum*, and *Paecilomyces* sp. are among the organisms that can be used in CBP [193]. For lignocelluloses, CBP combines self-cellulase production with substrate hydrolysis, fermentation of hexose and pentose sugars, and self-cellulase fermentation within the same reactor [194,195].

With one or more inoculums, the SSCF process frequently involves the simultaneous fermentation of two or more substrates in a single system. Additionally, it can entail the simultaneous fermentation of sugars from several process streams [196]. With *K. alvarezii* as a feedstock, SSCF produced 64.3 g/L ethanol that was separated into a cellulose- and galactose-rich solid fraction [197]. Comparatively, the liquid and solid fractions were independently fermented with SSF in the same investigation, which provided bioethanol yields of 38 and 53 g/L, respectively [197]. The results showed that while SSCF is beneficial, using SSF individually will lead to significantly greater combined ethanol production from various fractions of the same biomass. However, it could increase the overall cost. Strains of *S. cerevisiae*, *E. coli*, *Z mobilis*, *P. tannophilus*, *C. shehatae*, and *P. stipitis* are a few co-fermenting microorganisms for SSCF [140]. Overall, SSCF offers several key advantages and serves various important purposes, including integration of saccharification and fermentation for different feedstocks, which results in improved overall efficiency in terms of time and resources at reduced costs. In addition, the co-fermentation of sugars as they are released during saccharification can enhance the overall conversion efficiency. Moreover, co-fermentation in SSCF can help mitigate the inhibitory effects of certain byproducts or high sugar concentrations, improving the fermentation process robustness. Overall, anaerobic SSCF can contribute to reducing the environmental footprint of bioethanol production by minimizing resource consumption and waste generation, making it an important technique in the quest for sustainable and environmentally friendly energy sources (Figure 2).
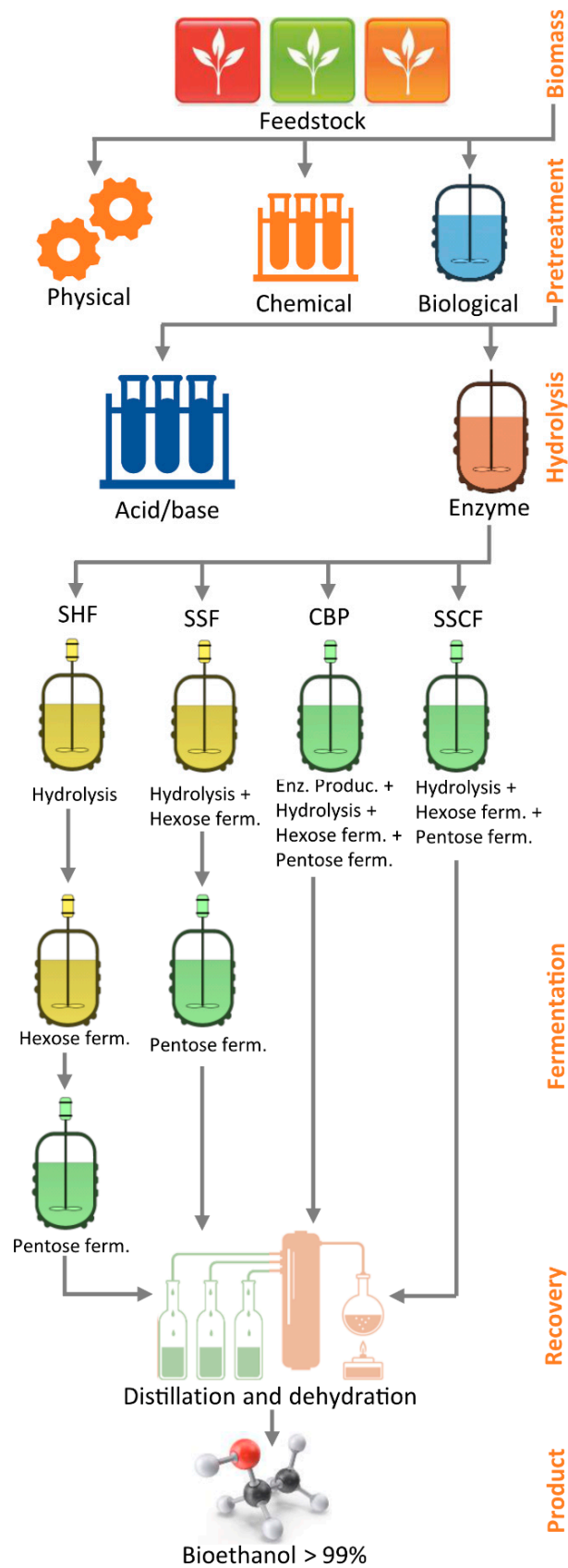
Figure 2. Schematic diagrams of different stages and available methods of ethanol production from macroalgae and/or lignocellulosic biomass.

6.4. Bioethanol Recovery

Following the fermentation process, bioethanol undergoes purification through distillation and dehydration techniques [198]. Distillation is the primary method used to extract ethanol from the fermentation broth (a mixture containing ethanol, solid biomass residue, residual sugars, and fermenting organisms). The vaporization of ethanol-water mixtures during the production process stands out as the most energy-intensive step due to the heat generated [199]. In analytical and small-scale research settings, bioethanol can be recovered using simple distillation units and rotary evaporators. In contrast, for commercial production, larger equipment such as distillation columns is employed [200], which might be combined with ethanol dewatering technologies such as using molecular sieves [151]. Dehydration techniques, including vacuum distillation, pressure swing, membranes, or molecular sieves, are utilized to generate >99% grade ethanol from fermentation broths distillation to a concentration of 95.6% ethanol (ethanol-water azeotrope) using distillation columns [198]. Molecular sieves, such as zeolite, operate by selectively adsorbing water molecules due to the size difference between water and ethanol molecules. They are well-suited for dehydration because they can be regenerated and reused through drying processes.

7. Co-Fermentation

Co-fermentation of marine biomass refers to the process of producing bioethanol from a mixture of different types of feedstocks simultaneously to produce bioethanol. For marine biomass in the present study, it refers to seaweeds and seagrass. In a broader context, it might include microalgae, fish waste, marine yeast, and seawater. Co-fermentation can improve the efficiency and stability of the bioprocess, as well as the quality and quantity of the final products [201]. It can also reduce the environmental impact of marine biomass disposal and contribute to the circular bioeconomy [202]. The bio-conversion of marine biomass has been discussed using different processes, such as co-digestion of seaweed and lipid-rich waste [203], co-digestion of microalgae and sewage sludge for biogas production [204], co-digestion of seaweed and cheese whey [205], co-pyrolysis of seaweeds with waste plastic [206], and hydrothermal co-liquefaction of seaweeds with lignocellulosic biomass [207]. However, there has been relatively less research on the co-fermentation of seaweeds with various feedstocks, indicating a need for further investigation in this area. In this context, SSCF is recognized as an advanced, next-generation method employed to enhance bioethanol production from different feedstocks. It achieves this by simultaneously breaking down cellulose and fermenting sugars. SSCF overcomes the limitations of marine biomass conversion, including challenges with no pentose utilization, low ethanol yield, and inhibition by fermentation parameters [208].

The concept of co-fermentation arises due to the need to ferment xylose, a sugar that normal wild-type yeast *Saccharomyces cerevisiae* cannot naturally ferment. To address this limitation, a genetically engineered *S. cerevisiae* was employed by introducing xylose-fermenting genes, enabling it to ferment both sugars [209]. In the SSCF, a single reactor is used to reduce the cost of using separate processes and eliminating enzyme feedback inhibition [210]. In contrast, SSF involves fermentation and hydrolysis at moderately high temperatures, with microbes capable of utilizing only glucose, requiring more processes (Section 6.3.2). Optimal cellulase enzyme activity occurs at around 50 °C, while yeast and bacteria typically function at moderate temperatures of around 30 °C and 37 °C, respectively. This temperature difference necessitates some modifications before implementing the process. Therefore, optimizing the process presents challenges, such as reducing the temperature for hydrolysis and increasing it for fermentation. Developing thermo-tolerance in a microbe or enhancing enzyme activity at a moderate temperature, approximately 34–37 °C, was discussed as a preferred approach for optimization [211]. While this may slightly extend the overall processing time, it remains more efficient than separate hydrolysis and fermentation processes. The operational parameters, such as pretreatment methods, temperature settings, and the presence of co-factors originating from the organisms employed,

have the potential to exert an impact on the SSCF conversion process, which has been comprehensively discussed by Sharma et al. [208].

Co-fermentation offers a significant advantage by enhancing the efficient utilization of diverse carbohydrates found in various marine biomasses. Different organisms excel in fermenting specific sugars more effectively. For instance, brown seaweeds are rich in alginate and mannitol, while green seaweeds contain more starch. The combination of these sources enables a more thorough conversion of sugars into ethanol. In a previous study, a waste mixture comprising ten species of red, brown, and green seaweeds from Gwangalli Beach was utilized as biomass feedstock for ethanol production. The result showed a maximum monosaccharide concentration of 30.2 g/L, which was employed for ethanol production. Fermentation involved both single and mixed yeasts, including non-adapted and adapted *Saccharomyces cerevisiae* KCTC 1126 and *Pichia angophorae* KCTC 17574, targeting galactose and mannitol, respectively. The most notable outcome was achieved through the co-culture of adapted strains, resulting in a maximum ethanol concentration of 13.5 g/L [212]. In a different study, mixed lignocellulosic biomass composed of *Ricinus communis*, *Saccharum officinarum*, and *Saccharum spontaneum* was used as a feedstock for bioethanol production that enhanced bioethanol yield to 62.01 g/L [213]. In addition, co-fermentation could play a crucial role in adjusting the carbon-to-nitrogen (C/N) ratio, a key factor influencing efficient bioethanol production during fermentation. Achieving the optimal C/N ratio is important for microbial growth and metabolism. The median C/N atomic ratio of benthic marine macroalgae and seagrasses is about 18:1. Benthic plants were reported to have much less N content, relative to C, than phytoplankton [214]. Thus, seaweed with higher carbohydrate content has better C/N ratios (20–40:1) for ethanol fermentation than protein-rich microalgae. Given the variability in carbohydrate and protein contents across different feedstocks, combining carbohydrate-rich and protein-rich feedstock becomes a strategic approach to fine-tune C/N ratios for effective fermentation. Despite proteins having lower hydrogen production potentials compared to carbohydrates, their presence is essential for maintaining a balanced C/N ratio conducive to microbial fermentation [213].

Co-fermentation can also facilitate synergistic effects between different microorganisms, improving ethanol yields. The enhanced microbial community can break down a wider range of substrates. Co-cultivation presents synergistic effects, which showed a higher fermentation rate compared to monocultures [215]. This approach requires a shorter fermentation period while providing an increased biofuel supply [212,216]. The co-culture system operates in harmony, ensuring process stability and superior performance compared to monocultures. It has successfully addressed the limitations of axenic cultures, offering economic and methodological advantages over enzymatically hydrolyzed cellulose by eliminating the need for reductants during the fermentation process [217]. It is worth noting that the co-fermentation of seaweeds with other feedstocks is a relatively unexplored area that demands in-depth research and exploration. Combining the co-fermentation of seaweeds with seagrass or other lignocellulosic biomass through integrated processes like wastewater treatment, aquaculture systems, and CO_2 sequestration (Figure 3) could provide a robust platform for augmenting bioethanol production. The absence of lignin in seaweeds is a critical factor that opens up intriguing possibilities in the realm of bioethanol production. When seaweeds are co-fermented with other lignin-containing feedstocks, such as seagrass or terrestrial biomass, it can create a cascading effect for continuous ethanol production. In this cascading method, ethanol production can be initiated using seaweeds while simultaneously conducting the hydrolysis of lignin-containing feedstocks. This concurrent approach allows for efficient utilization of both feedstocks, leveraging the absence of lignin in seaweeds to simplify hydrolysis and enhance overall ethanol production. This continuous process optimizes ethanol production and resource utilization while minimizing the need for complex and energy-intensive pretreatment steps. In addition, the consecutive utilization of various sugars can bolster the effectiveness of the cascading approach. For instance, a modified SSCF process was developed, which involved different

temperatures and duration conditions for C5 and C6 sugar fermentation [218]. This process reduced the enzyme dosage and process time while increasing ethanol yield from different lignocellulosic biomass. Overall, the study highlighted the potential for enhancing ethanol production through the co-fermentation of different biomass sources. In addition, low-cost feedstocks with high content of non-structural components (NSC), such as poplar chips, were reported to improve ethanol production without detoxification processes [219]. Moreover, extracting value-added products from seaweeds before fermentation can generate additional income and serve as a pretreatment step for the biomass [126]. Furthermore, the integration of different conversion routes could significantly impact the energy yield, necessitating a thorough assessment and research.
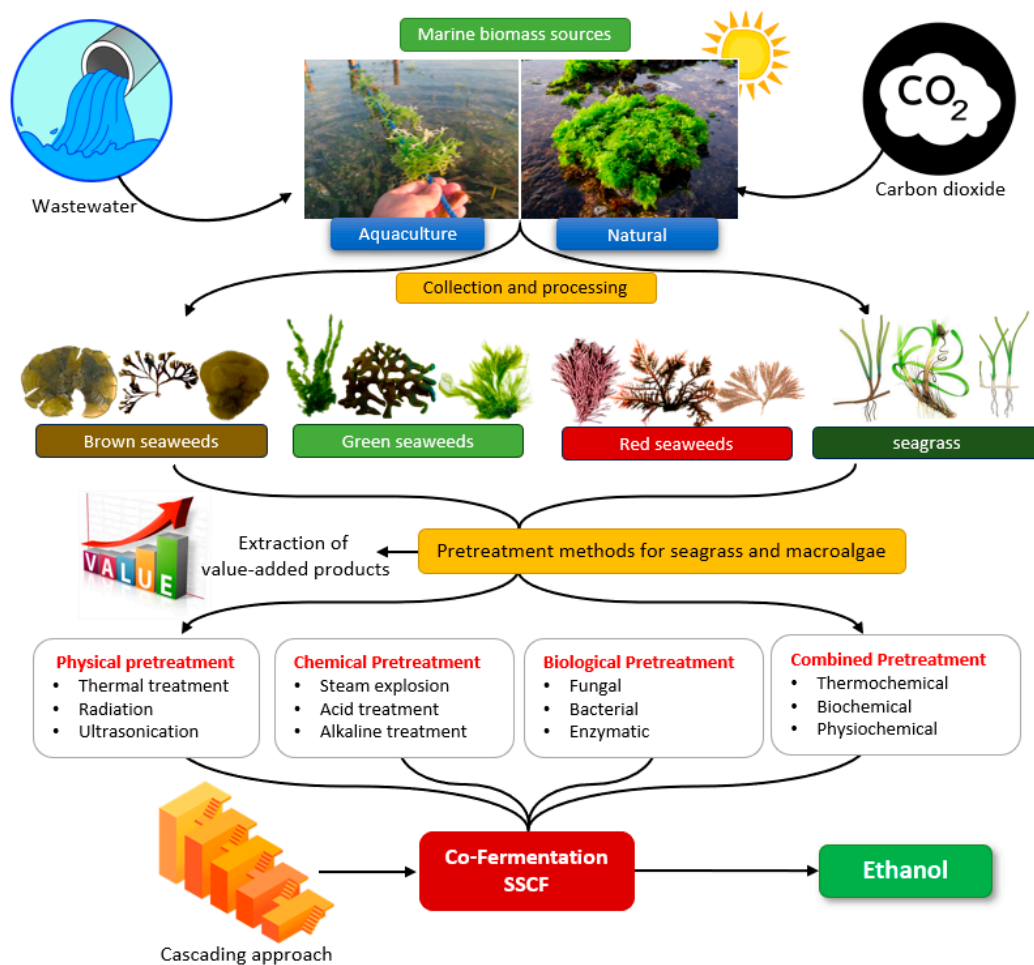

Figure 3. Suggested integrated route for enhanced bioethanol production from marine biomass, including seaweeds and seagrass, using simultaneous saccharification and co-fermentation (SSCF).

Overall, co-fermentation of marine biomass, encompassing seaweeds and seagrass, represents an innovative and promising approach to sustainable biofuel production and environmental stewardship by waste minimization and resource utilization. This integrated route can provide complementary processes such as wastewater treatment, aquaculture systems, and CO_2 sequestration, creating a multifaceted platform for enhanced bioethanol production through a cascading route. This integration maximizes resource utilization and mitigates environmental impacts, such as nutrient runoff and CO_2 emissions [220], contributing to overall sustainability. Furthermore, extracting value-added products from seaweeds before fermentation adds economic value and can serve as a pretreatment step, optimizing the efficiency of the co-fermentation process.

8. Conclusions

The present review discussed various strategies for simultaneous co-fermentation of 2nd and 3rd generation marine feedstocks (seagrass and seaweeds, respectively) and potential applications of co-fermentation systems for enhanced bioethanol production. Co-fermentation of macroalgae and seagrass, or other lignocellulosic feedstocks, might have synergistic action to overcome the challenges in the second-generation bioethanol production process. Drawing from contemporary research findings, it is a promising avenue for enhanced bioethanol yields and optimizing the utilization of various marine biomass resources when compared to processing these substrates separately. Future efforts to increase the efficiency of biofuel production from marine biomass should concentrate on reducing production costs and exploiting technological advancements. The focus should be placed on creating new strategies, highly active and stable catalysts, more efficient reactors, continuous operation bioreactors, and evaluation of the waste as well as the amount of greenhouse gas emissions produced. While co-fermentation of marine biomass holds great promise, there remains a need for further research studies to precisely quantify its energy yield and assess its feasibility on large scales. As research in this field continues to advance, co-fermentation emerges as a compelling solution at the intersection of renewable energy, environmental conservation, economic development, and sustainability.

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References

1. MMR Ethanol Market: Global Industry Analysis and Forecast (2023–2029). Available online: https://www.maximizemarketresearch.com/market-report/global-ethanol-market/25241/ (accessed on 25 September 2023).
2. Panahi, H.K.S.; Dehhaghi, M.; Aghbashlo, M.; Karimi, K.; Tabatabaei, M. Shifting Fuel Feedstock from Oil Wells to Sea: Iran Outlook and Potential for Biofuel Production from Brown Macroalgae (Ochrophyta; Phaeophyceae). *Renew. Sustain. Energy Rev.* **2019**, *112*, 626–642. [CrossRef]
3. Abdelsalam, I.; Elshobary, M.; Eladawy, M.M.; Nagah, M. Utilization of Multi-Tasking Non-Edible Plants for Phytoremediation and Bioenergy Source—A Review. *Phyton* **2019**, *88*, 69–90. [CrossRef]
4. Wargacki, A.J.; Leonard, E.; Win, M.N.; Regitsky, D.D.; Santos, C.N.S.; Kim, P.B.; Cooper, S.R.; Raisner, R.M.; Herman, A.; Sivitz, A.B. An Engineered Microbial Platform for Direct Biofuel Production from Brown Macroalgae. *Science* **2012**, *335*, 308–313. [CrossRef]
5. Elsayed, M.; Abomohra, A.; Ai, P.; Wang, D.; El-Mashad, H.; Zhang, Y. Biorefining of Rice Straw by Sequential Fermentation and Anaerobic Digestion for Bioethanol and/or Biomethane Production: Comparison of Structural Properties and Energy Output. *Bioresour. Technol.* **2018**, *268*, 183–189. [CrossRef]
6. Khan, M.I.; Shin, J.H.; Kim, J.D. The Promising Future of Microalgae: Current Status, Challenges, and Optimization of a Sustainable and Renewable Industry for Biofuels, Feed, and Other Products. *Microb. Cell Fact.* **2018**, *17*, 36. [CrossRef]
7. Abomohra, A.; Hanelt, D. Recent Advances in Micro-/Nanoplastic (MNPs) Removal by Microalgae and Possible Integrated Routes of Energy Recovery. *Microorganisms* **2022**, *10*, 2400. [CrossRef]
8. Almutairi, A.W. Full Utilization of Marine Microalgal Hydrothermal Liquefaction Liquid Products through a Closed-Loop Route: Towards Enhanced Bio-Oil Production and Zero-Waste Approach. *3 Biotech* **2022**, *12*, 209. [CrossRef]
9. Lü, J.; Sheahan, C.; Fu, P. Metabolic Engineering of Algae for Fourth Generation Biofuels Production. *Energy Environ. Sci.* **2011**, *4*, 2451–2466. [CrossRef]
10. Han, S.; Jin, W.; Chen, Y.; Tu, R.; Abomohra, A. Enhancement of Lipid Production of *Chlorella Pyrenoidosa* Cultivated in Municipal Wastewater by Magnetic Treatment. *Appl. Biochem. Biotechnol.* **2016**, *180*, 1043–1055. [CrossRef]

11. Barati, B.; Zeng, K.; Baeyens, J.; Wang, S.; Addy, M.; Gan, S.Y.; Abomohra, A. Recent Progress in Genetically Modified Microalgae for Enhanced Carbon Dioxide Sequestration. *Biomass Bioenergy* **2021**, *145*, 105927. [CrossRef]

12. Lin, H.; Qin, S. Tipping Points in Seaweed Genetic Engineering: Scaling up Opportunities in the Next Decade. *Mar. Drugs* **2014**, *12*, 3025. [CrossRef] [PubMed]

13. Radakovits, R.; Jinkerson, R.E.; Darzins, A.; Posewitz, M.C. Genetic Engineering of Algae for Enhanced Biofuel Production. *Eukaryot. Cell* **2010**, *9*, 486–501. [CrossRef] [PubMed]

14. Elshobary, M.E.; Zabed, H.M.; Qi, X.; El-Shenody, R.A. Enhancing Biomass and Lipid Productivity of a Green Microalga *Parachlorella Kessleri* for Biodiesel Production Using Rapid Mutation of Atmospheric and Room Temperature Plasma. *Biotechnol. Biofuels Bioprod.* **2022**, *15*, 122. [CrossRef] [PubMed]

15. Duarte, C.M.; Gattuso, J.P.; Hancke, K.; Gundersen, H.; Filbee-Dexter, K.; Pedersen, M.F.; Middelburg, J.J.; Burrows, M.T.; Krumhansl, K.A.; Wernberg, T.; et al. Global Estimates of the Extent and Production of Macroalgal Forests. *Glob. Ecol. Biogeogr.* **2022**, *31*, 1422–1439. [CrossRef]

16. Milledge, J.J.; Smith, B.; Dyer, P.W.; Harvey, P. Macroalgae-Derived Biofuel: A Review of Methods of Energy Extraction from Seaweed Biomass. *Energies* **2014**, *7*, 7194–7222. [CrossRef]

17. Adams, J.M.; Gallagher, J.A.; Donnison, I.S. Fermentation Study on *Saccharina latissima* for Bioethanol Production Considering Variable Pre-Treatments. *J. Appl. Phycol.* **2009**, *21*, 569. [CrossRef]

18. Dalena, F.; Senatore, A.; Iulianelli, A.; Di Paola, L.; Basile, M.; Basile, A. Ethanol from Biomass: Future and Perspectives. In *Ethanol*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 25–59.

19. Ravikumar, S. Production of Biofuel Ethanol from Pretreated Seagrass by Using *Saccharomyces cerevisiae*. *Indian J. Sci. Technol.* **2011**, *4*, 1087–1089. [CrossRef]

20. Uchida, M.; Miyoshi, T.; Kaneniwa, M.; Ishihara, K.; Nakashimada, Y.; Urano, N. Production of 16.5% *v/v* Ethanol from Seagrass Seeds. *J. Biosci. Bioeng.* **2014**, *118*, 646–650. [CrossRef]

21. Rajkumar, J.; Dilipan, E.; Ramachandran, M.; Panneerselvam, A.; Thajuddin, N. Bioethanol Production from Seagrass Waste, through Fermentation Process Using Cellulase Enzyme Isolated from Marine Actinobacteria. *Vegetos* **2021**, *34*, 581–591. [CrossRef]

22. McMillan, J.D.; Jennings, E.W.; Mohagheghi, A.; Zuccarello, M. Comparative Performance of Precommercial Cellulases Hydrolyzing Pretreated Corn Stover. *Biotechnol. Biofuels* **2011**, *4*, 29. [CrossRef]

23. Dobrowolski, A.; Nawijn, W.; Mirończuk, A.M. Brown Seaweed Hydrolysate as a Promising Growth Substrate for Biomass and Lipid Synthesis of the Yeast *Yarrowia lipolytica*. *Front. Bioeng. Biotechnol.* **2022**, *10*, 944228. [CrossRef]

24. Wi, S.G.; Kim, H.J.; Mahadevan, S.A.; Yang, D.-J.; Bae, H.-J. The Potential Value of the Seaweed Ceylon Moss (*Gelidium amansii*) as an Alternative Bioenergy Resource. *Bioresour. Technol.* **2009**, *100*, 6658–6660. [CrossRef] [PubMed]

25. Kim, H.; Song, H.; Jeong, M.; Sim, S.; Park, D.; Yang, J.; Singal, R.; Grimes, S.R.; Kim, H.; Song, H.; et al. Enzymatic Saccharification of *Salix viminalis* Cv. Q683 Biomass for Bioethanol Production. *Biotechniques* **2011**, *27*, 143–149.

26. Cosenza, V.A.; Navarro, D.A.; Ponce, N.M.A.; Stortz, C.A. Seaweed Polysaccharides: Structure and Applications. In *Industrial Applications of Renewable Biomass Products: Past, Present, Future*; Springer: Cham, Switzerland, 2017; pp. 75–116.

27. Ibraheem, O.; Ndimba, B.K. Molecular Adaptation Mechanisms Employed by Ethanologenic Bacteria in Response to Lignocellulose-Derived Inhibitory Compounds. *Int. J. Biol. Sci.* **2013**, *9*, 598–612. [CrossRef] [PubMed]

28. Pfeifer, L.; van Erven, G.; Sinclair, E.A.; Duarte, C.M.; Kabel, M.A.; Classen, B. Profiling the Cell Walls of Seagrasses from A (Amphibolis) to Z (Zostera). *BMC Plant Biol.* **2022**, *22*, 63. [CrossRef]

29. Torbatinejad, N.M.; Annison, G.; Rutherfurd-Markwick, K.; Sabine, J.R. Structural Constituents of the Seagrass *Posidonia australis*. *J. Agric. Food Chem.* **2007**, *55*, 4021–4026. [CrossRef]

30. Opsahl, S.; Benner, R. Decomposition of Senescent Blades of the Seagrass *Halodule wrightii* in a Subtropical Lagoon. *Mar. Ecol. Prog. Ser.* **1993**, *94*, 191. [CrossRef]

31. Syed, N.F.N.; Zakaria, M.H.; Bujang, J.S. Fiber Characteristics and Papermaking of Seagrass Using Hand-Beaten and Blended Pulp. *BioResources* **2016**, *11*, 5358–5380. [CrossRef]

32. Silva, J.; Dantas-Santos, N.; Gomes, D.L.; Costa, L.S.; Cordeiro, S.L.; Costa, M.S.S.P.; Silva, N.B.; Freitas, M.L.; Scortecci, K.C.; Leite, E.L. Biological Activities of the Sulfated Polysaccharide from the Vascular Plant *Halodule wrightii*. *Rev. Bras. Farmacogn.* **2012**, *22*, 94–101. [CrossRef]

33. Kolsi, R.B.A.; Fakhfakh, J.; Krichen, F.; Jribi, I.; Chiarore, A.; Patti, F.P.; Blecker, C.; Allouche, N.; Belghith, H.; Belghith, K. Structural Characterization and Functional Properties of Antihypertensive *Cymodocea nodosa* Sulfated Polysaccharide. *Carbohydr. Polym.* **2016**, *151*, 511–522. [CrossRef]

34. Barakat, K.M.; Ismail, M.M.; Abou El Hassayeb, H.E.; El Sersy, N.A.; Elshobary, M.E. Chemical Characterization and Biological Activities of Ulvan Extracted from *Ulva fasciata* (Chlorophyta). *Rend. Lincei. Sci. Fis. e Nat.* **2022**, *33*, 829–841. [CrossRef]

35. Gloaguen, V.; Brudieux, V.; Closs, B.; Barbat, A.; Krausz, P.; Sainte-Catherine, O.; Kraemer, M.; Maes, E.; Guerardel, Y. Structural Characterization and Cytotoxic Properties of an Apiose-Rich Pectic Polysaccharide Obtained from the Cell Wall of the Marine Phanerogam *Zostera marina*. *J. Nat. Prod.* **2010**, *73*, 1087–1092. [CrossRef]

36. Lv, Y.; Shan, X.; Zhao, X.; Cai, C.; Zhao, X.; Lang, Y.; Zhu, H.; Yu, G. Extraction, Isolation, Structural Characterization and Anti-Tumor Properties of an Apigalacturonan-Rich Polysaccharide from the Sea Grass *Zostera caespitosa* Miki. *Mar. Drugs* **2015**, *13*, 3710–3731. [CrossRef]

37. Cunha, J.T.; Soares, P.O.; Romaní, A.; Thevelein, J.M.; Domingues, L. Xylose Fermentation Efficiency of Industrial *Saccharomyces cerevisiae* Yeast with Separate or Combined Xylose Reductase/Xylitol Dehydrogenase and Xylose Isomerase Pathways. *Biotechnol. Biofuels* **2019**, *12*, 20. [CrossRef]

38. Katahira, S.; Mizuike, A.; Fukuda, H.; Kondo, A. Ethanol Fermentation from Lignocellulosic Hydrolysate by a Recombinant Xylose- and Cellooligosaccharide-Assimilating Yeast Strain. *Appl. Microbiol. Biotechnol.* **2006**, *72*, 1136–1143. [CrossRef] [PubMed]

39. Jin, Y.S.; Alper, H.; Yang, Y.T.; Stephanopoulos, G. Improvement of Xylose Uptake and Ethanol Production in Recombinant *Saccharomyces cerevisiae* through an Inverse Metabolic Engineering Approach. *Appl. Environ. Microbiol.* **2005**, *71*, 8249–8256. [CrossRef]

40. Oh, E.J.; Jin, Y.-S. Engineering of *Saccharomyces cerevisiae* for Efficient Fermentation of Cellulose. *FEMS Yeast Res.* **2020**, *20*, foz089. [CrossRef]

41. Sze, P. *A Biology of the Algae*, 2nd ed.; Wm, C., Ed.; Brown Publishers: Dubuque, IA, USA, 1993.

42. Lobban, C.S.; Harrison, P.J.; Duncan, M.J. *The Physiological Ecology of Seaweeds*; Cambridge University Press: New York, NY, USA, 1985.

43. Gao, G.; Burgess, J.G.; Wu, M.; Wang, S.; Gao, K. Using Macroalgae as Biofuel: Current Opportunities and Challenges. *Bot. Mar.* **2020**, *63*, 355–370. [CrossRef]

44. Elshobary, M.E.; Essa, D.I.; Attiah, A.M.; Salem, Z.E.; Qi, X. Algal Community and Pollution Indicators for the Assessment of Water Quality of Ismailia Canal, Egypt. *Stoch. Environ. Res. Risk Assess.* **2020**, *34*, 1089–1103. [CrossRef]

45. Ismail, M.M.; Ismail, G.A.; Elshobary, M.E. Morpho-Anatomical, and Chemical Characterization of Some Calcareous Mediterranean Red Algae Species. *Bot. Stud.* **2023**, *64*, 10. [CrossRef]

46. Knoll, A.H. The Multiple Origins of Complex Multicellularity. *Annu. Rev. Earth Planet. Sci.* **2011**, *39*, 217–239. [CrossRef]

47. Miyashita, K.; Mikami, N.; Hosokawa, M. Chemical and Nutritional Characteristics of Brown Seaweed Lipids: A Review. *J. Funct. Foods* **2013**, *5*, 1507–1517. [CrossRef]

48. Kim, H.; Ra, C.H.; Kim, S.-K. Ethanol Production from Seaweed (*Undaria pinnatifida*) Using Yeast Acclimated to Specific Sugars. *Biotechnol. Bioprocess Eng.* **2013**, *18*, 533–537. [CrossRef]

49. Yazdani, P.; Zamani, A.; Karimi, K.; Taherzadeh, M.J. Characterization of *Nizimuddinia zanardini* Macroalgae Biomass Composition and Its Potential for Biofuel Production. *Bioresour. Technol.* **2015**, *176*, 196–202. [CrossRef]

50. Ghadiryanfar, M.; Rosentrater, K.A.; Keyhani, A.; Omid, M. A Review of Macroalgae Production, with Potential Applications in Biofuels and Bioenergy. *Renew. Sustain. Energy Rev.* **2016**, *54*, 473–481. [CrossRef]

51. Gao, K.; McKinley, K.R. Use of Macroalgae for Marine Biomass Production and CO_2 Remediation: A Review. *J. Appl. Phycol.* **1994**, *6*, 45–60. [CrossRef]

52. Jung, K.A.; Lim, S.-R.; Kim, Y.; Park, J.M. Potentials of Macroalgae as Feedstocks for Biorefinery. *Bioresour. Technol.* **2013**, *135*, 182–190. [CrossRef] [PubMed]

53. Smith, R.G.; Bidwell, R.G.S. Mechanism of Photosynthetic Carbon Dioxide Uptake by the Red Macroalga, *Chondrus crispus*. *Plant Physiol.* **1989**, *89*, 93–99. [CrossRef]

54. Duarte, C.M. Reviews and Syntheses: Hidden Forests, the Role of Vegetated Coastal Habitats in the Ocean Carbon Budget. *Biogeosciences* **2017**, *14*, 301–310. [CrossRef]

55. Serrano, O.; Lovelock, C.E.; Atwood, T.B.; Macreadie, P.I.; Canto, R.; Phinn, S.; Arias-Ortiz, A.; Bai, L.; Baldock, J.; Bedulli, C.; et al. Australian Vegetated Coastal Ecosystems as Global Hotspots for Climate Change Mitigation. *Nat. Commun.* **2019**, *10*, 4313. [CrossRef] [PubMed]

56. Weathers, K.C.; Strayer, D.L.; Likens, G.E. *Fundamentals of Ecosystem Science*, 2nd ed.; Academic Press: Cambridge, MA, USA, 2021; ISBN 9780128127629.

57. FAO. *The State of Food and Agriculture 2021*; FAO: Rome, Italy, 2021.

58. McHugh, D.J. A Guide to the Seaweed Industry. *FAO Fish. Tech. Pap.* **2003**, *441*, 105.

59. Mišurcová, L.; Škrovánková, S.; Samek, D.; Ambrožová, J.; Machů, L. Health Benefits of Algal Polysaccharides in Human Nutrition. *Adv. Food Nutr. Res.* **2012**, *66*, 75–145. [PubMed]

60. Zhang, L.; Liao, W.; Huang, Y.; Wen, Y.; Chu, Y.; Zhao, C. Global Seaweed Farming and Processing in the Past 20 Years. *Food Prod. Process. Nutr.* **2022**, *4*, 23. [CrossRef]

61. Roesijadi, G.; Jones, S.B.; Snowden-Swan, L.J.; Zhu, Y. *Macroalgae as a Biomass Feedstock: A Preliminary Analysis*; Pacific Northwest National Lab. (PNNL): Richland, WA, USA, 2010.

62. Somerville, C.; Youngs, H.; Taylor, C.; Davis, S.C.; Long, S.P. Feedstocks for Lignocellulosic Biofuels. *Science* **2010**, *329*, 790–792. [CrossRef] [PubMed]

63. Roesijadi, G.; Copping, A.E.; Huesemann, M.H.; Forster, J.; Benemann, J.R. *Techno-Economic Feasibility Analysis of Offshore Seaweed Farming for Bioenergy and Biobased Products*; Battelle Pacific Northwest Div. Rep. Number PNWD-3931; Battelle Pacific Northwest Division: Richland, WA, USA, 2008; p. 115.

64. Mooney-McAuley, K.M.; Edwards, M.D.; Champenois, J.; Gorman, E. *Best Practice Guidelines for Seaweed Cultivation and Analysis: Public Output Report Report [WP1A5. 01] of the EnAlgae Project*; EnAlgae, Swansea University, Centre for Sustainable Aquatic Research: Swansea, UK, 2016.

65. de los Santos, C.B.; Krause-Jensen, D.; Alcoverro, T.; Marbà, N.; Duarte, C.M.; van Katwijk, M.M.; Pérez, M.; Romero, J.; Sánchez-Lizaso, J.L.; Roca, G.; et al. Recent Trend Reversal for Declining European Seagrass Meadows. *Nat. Commun.* **2019**, *10*, 3356. [CrossRef]

66. Papenbrock, J.; Teichberg, M. Editorial: Current Advances in Seagrass Research. *Front. Plant Sci.* **2023**, *14*, 1196437. [CrossRef]

67. Yue, S.; Zhou, Y.; Xu, S.; Zhang, X.; Liu, M.; Qiao, Y.; Gu, R.; Xu, S.; Zhang, Y. Can the Non-Native Salt Marsh Halophyte Spartina Alterniflora Threaten Native Seagrass (*Zostera japonica*) Habitats? A Case Study in the Yellow River Delta, China. *Front. Plant Sci.* **2021**, *12*, 643425. [CrossRef]

68. Han, Q.; Liu, D. Macroalgae Blooms and Their Effects on Seagrass Ecosystems. *J. Ocean Univ. China* **2014**, *13*, 791–798. [CrossRef]

69. El-Shaffai, A.A.; Hanafy, M.H.; Gab-Alla, A.A. Distribution, Abundance and Species Composition of Seagrasses in Wadi El-Gemal National Park, Red Sea, Egypt. *Indian J. Appl. Sci.* **2011**, *4*, 1–8. [CrossRef]

70. Waycott, M.; McMahon, K.; Mellors, J.; Calladine, A.; Kleine, D. *A Guide to Tropical Seagrasses of the Indo-West Pacific*; James Cook University: Townsville, Australia, 2004.

71. Group, A.P.; Chase, M.W.; Christenhusz, M.J.M.; Fay, M.F.; Byng, J.W.; Judd, W.S.; Soltis, D.E.; Mabberley, D.J.; Sennikov, A.N.; Soltis, P.S. An Update of the Angiosperm Phylogeny Group Classification for the Orders and Families of Flowering Plants: APG IV. *Bot. J. Linn. Soc.* **2016**, *181*, 1–20.

72. Hemminga, M.A.; Duarte, C.M. *Seagrass Ecology*; Cambridge University Press: Cambridge, UK, 2000; ISBN 0521661846.

73. Reynolds, P.L.; Duffy, E.; Knowlton, N. Seagrass and Seagrass Beds. Ocean Portal. 2018. Available online: https://www.dcbd.nl/sites/default/files/documents/SeagrassAndSeagrass%20Beds%20_%20SmithsonianOceanPortal.pdf (accessed on 26 September 2023).

74. Short, F.; Carruthers, T.; Dennison, W.; Waycott, M. Global Seagrass Distribution and Diversity: A Bioregional Model. *J. Exp. Mar. Bio. Ecol.* **2007**, *350*, 3–20. [CrossRef]

75. Green, E.P.; Edmund, P.; Short, F.T. *World Atlas of Seagrasses*; University of California Press: Oakland, CA, USA, 2003; p. 298.

76. Lee, H.; Golicz, A.A.; Bayer, P.E.; Jiao, Y.; Tang, H.; Paterson, A.H.; Sablok, G.; Krishnaraj, R.R.; Chan, C.-K.K.; Batley, J. The Genome of a Southern Hemisphere Seagrass Species (*Zostera muelleri*). *Plant Physiol.* **2016**, *172*, 272–283. [CrossRef] [PubMed]

77. Olsen, J.L.; Rouzé, P.; Verhelst, B.; Lin, Y.-C.; Bayer, T.; Collen, J.; Dattolo, E.; De Paoli, E.; Dittami, S.; Maumus, F. The Genome of the Seagrass *Zostera marina* Reveals Angiosperm Adaptation to the Sea. *Nature* **2016**, *530*, 331–335. [CrossRef]

78. Aquino, R.S.; Landeira-Fernandez, A.M.; Valente, A.P.; Andrade, L.R.; Mourao, P.A.S. Occurrence of Sulfated Galactans in Marine Angiosperms: Evolutionary Implications. *Glycobiology* **2005**, *15*, 11–20. [CrossRef] [PubMed]

79. Ellis, M.; Egelund, J.; Schultz, C.J.; Bacic, A. Arabinogalactan-Proteins: Key Regulators at the Cell Surface? *Plant Physiol.* **2010**, *153*, 403–419. [CrossRef] [PubMed]

80. Ma, Y.; Zeng, W.; Bacic, A.; Johnson, K. AGPs through Time and Space. *Annu. Plant Rev. Online* **2018**, *1*, 767–804.

81. Pfeifer, L.; Classen, B. The Cell Wall of Seagrasses: Fascinating, Peculiar and a Blank Canvas for Future Research. *Front. Plant Sci.* **2020**, *11*, 588754. [CrossRef] [PubMed]

82. Klap, V.A.; Hemminga, M.A.; Boon, J.J. Retention of Lignin in Seagrasses: Angiosperms That Returned to the Sea. *Mar. Ecol. Prog. Ser.* **2000**, *194*, 1–11. [CrossRef]

83. Martone, P.T.; Estevez, J.M.; Lu, F.; Ruel, K.; Denny, M.W.; Somerville, C.; Ralph, J. Discovery of Lignin in Seaweed Reveals Convergent Evolution of Cell-Wall Architecture. *Curr. Biol.* **2009**, *19*, 169–175. [CrossRef]

84. Kaal, J.; Serrano, O.; del Río, J.C.; Rencoret, J. Radically Different Lignin Composition in Posidonia Species May Link to Differences in Organic Carbon Sequestration Capacity. *Org. Geochem.* **2018**, *124*, 247–256. [CrossRef]

85. ReMEDIES Seagrass Cultivation Lab—Save Our Seabed. Available online: https://saveourseabed.co.uk/seagrass-cultivation-lab/ (accessed on 4 September 2023).

86. Yue, S.; Zhang, X.; Xu, S.; Zhang, Y.; Zhao, P.; Wang, X.; Zhou, Y. Reproductive Strategies of the Seagrass *Zostera japonica* under Different Geographic Conditions in Northern China. *Front. Mar. Sci.* **2020**, *7*, 574790. [CrossRef]

87. Subhashini, P.; Raja, S.; Thangaradjou, T. Establishment of Cell Suspension Culture Protocol for a Seagrass (*Halodule pinifolia*): Growth Kinetics and Histomorphological Characterization. *Aquat. Bot.* **2014**, *117*, 33–40. [CrossRef]

88. Potouroglou, M.; Pedder, K.; Wood, K.; Scalenghe, D. What to Know About Seagrass, the Ocean's Overlooked Powerhouse 2022. Available online: https://www.wri.org/insights/understanding-seagrass?utm_campaign=wridigest&utm_source=wridigest-2022-08-09&utm_medium=email&utm_content=image (accessed on 1 October 2023).

89. Barakat, K.M.; El-Sayed, H.S.; Khairy, H.M.; El-Sheikh, M.A.; Al-Rashed, S.A.; Arif, I.A.; Elshobary, M.E. Effects of Ocean Acidification on the Growth and Biochemical Composition of a Green Alga (*Ulva fasciata*) and Its Associated Microbiota. *Saudi J. Biol. Sci.* **2021**, *28*, 5106–5114. [CrossRef] [PubMed]

90. El-Khodary, G.M.; El-Sayed, H.S.; Khairy, H.M.; El-Sheikh, M.A.; Qi, X.; Elshobary, M.E. Comparative Study on Growth, Survival and Pigmentation of Solea Aegyptiaca Larvae by Using Four Different Microalgal Species with Emphasize on Water Quality and Nutritional Value. *Aquac. Nutr.* **2020**, *27*, 615–629. [CrossRef]

91. Pal, A.; Kamthania, M.C.; Kumar, A. Bioactive Compounds and Properties of Seaweeds—A Review. *Open Access Libr. J.* **2014**, *1*, 1–17. [CrossRef]

92. Wells, M.L.; Potin, P.; Craigie, J.S.; Raven, J.A.; Merchant, S.S.; Helliwell, K.E.; Smith, A.G.; Camire, M.E.; Brawley, S.H. Algae as Nutritional and Functional Food Sources: Revisiting Our Understanding. *J. Appl. Phycol.* **2017**, *29*, 949–982. [CrossRef]

93. Olsson, J.; Toth, G.B.; Albers, E. Biochemical Composition of Red, Green and Brown Seaweeds on the Swedish West Coast. *J. Appl. Phycol.* **2020**, *32*, 3305–3317. [CrossRef]

94. Mæhre, H.K.; Malde, M.K.; Eilertsen, K.; Elvevoll, E.O. Characterization of Protein, Lipid and Mineral Contents in Common Norwegian Seaweeds and Evaluation of Their Potential as Food and Feed. *J. Sci. Food Agric.* **2014**, *94*, 3281–3290. [CrossRef]

95. Elshobary, M.E.; El-Shenody, R.; Abomohra, A.E. Sequential Biofuel Production from Seaweeds Enhances the Energy Recovery: A Case Study for Biodiesel and Bioethanol Production. *Int. J. Energy Res.* **2020**, *45*, 6457–6467. [CrossRef]

96. Cherry, P.; O'Hara, C.; Magee, P.J.; McSorley, E.M.; Allsopp, P.J. Risks and Benefits of Consuming Edible Seaweeds. *Nutr. Rev.* **2019**, *77*, 307–329. [CrossRef]

97. Kumari, P.; Kumar, M.; Gupta, V.; Reddy, C.R.K.; Jha, B. Tropical Marine Macroalgae as Potential Sources of Nutritionally Important PUFAs. *Food Chem.* **2010**, *120*, 749–757. [CrossRef]

98. Plaza, M.; Cifuentes, A.; Ibáñez, E. In the Search of New Functional Food Ingredients from Algae. *Trends Food Sci. Technol.* **2008**, *19*, 31–39. [CrossRef]

99. Dewsbury, B.M.; Bhat, M.; Fourqurean, J.W. A Review of Seagrass Economic Valuations: Gaps and Progress in Valuation Approaches. *Ecosyst. Serv.* **2016**, *18*, 68–77. [CrossRef]

100. Touchette, B.W. Seagrass-Salinity Interactions: Physiological Mechanisms Used by Submersed Marine Angiosperms for a Life at Sea. *J. Exp. Mar. Bio. Ecol.* **2007**, *350*, 194–215. [CrossRef]

101. Pradheeba, M.; Dilipan, E.; Nobi, E.P.; Thangaradjou, T.; Sivakumar, K. *Evaluation of Seagrasses for Their Nutritional Value*; NISCAIR-CSIR: New Delhi, India, 2011.

102. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A.T.; Smith, F. Colorimetric Method for Determination of Sugars and Related Substances. *Anal. Chem.* **1956**, *28*, 350–356. [CrossRef]

103. Han, S.; Xing, Z.; Jiang, H.; Li, W.; Huang, W. Biological Adaptive Mechanisms Displayed by a Freshwater Plant to Live in Aquatic and Terrestrial Environments. *Environ. Exp. Bot.* **2021**, *191*, 104623. [CrossRef]

104. Kamenev, G.M. Fatty Acids as Markers of Food Sources in a Shallow-Water Hydrothermal Ecosystem (Kraternaya Bight, Yankich Island, Kurile Islands). *Mar. Ecol. Prog. Ser.* **1995**, *120*, 231–241.

105. Burton, T.; Lyons, H.; Lerat, Y.; Stanley, M.; Rasmussen, M.B. *A Review of the Potential of Marine Algae as a Source of Biofuel in Ireland*; Sustainable Energy Authority of Ireland (SEAI): Dublin, Ireland, 2009.

106. Lüning, K. *Seaweeds: Their Environment, Biogeography, and Ecophysiology*; John Wiley & Sons: Hoboken, NJ, USA, 1990; ISBN 0471624349.

107. Lahaye, M.; Robic, A. Structure and Functional Properties of Ulvan, a Polysaccharide from Green Seaweeds. *Biomacromolecules* **2007**, *8*, 1765–1774. [CrossRef]

108. Stiger-Pouvreau, V.; Bourgougnon, N.; Deslandes, E. Carbohydrates from Seaweeds. In *Seaweed in Health and Disease Prevention*; Elsevier: Amsterdam, The Netherlands, 2016; pp. 223–274.

109. Karsten, U.; Barrow, K.D.; King, R.J. Floridoside, L-Isofloridoside, and D-Isofloridoside in the Red Alga *Porphyra Columbina* (Seasonal and Osmotic Effects). *Plant Physiol.* **1993**, *103*, 485–491. [CrossRef]

110. Stengel, D.B.; Connan, S.; Popper, Z.A. Algal Chemodiversity and Bioactivity: Sources of Natural Variability and Implications for Commercial Application. *Biotechnol. Adv.* **2011**, *29*, 483–501. [CrossRef]

111. Yu, S.; Blennow, A.; Bojko, M.; Madsen, F.; Olsen, C.E.; Engelsen, S.B. Physico-chemical Characterization of Floridean Starch of Red Algae. *Starch-Stärke* **2002**, *54*, 66–74. [CrossRef]

112. Karsten, U.; West, J.A.; Zuccarello, G.C.; Nixdorf, O.; Barrow, K.D.; King, R.J. Low Molecular Weight Carbohydrate Patterns in the Bangiophyceae (Rhodophyta). *J. Phycol.* **1999**, *35*, 967–976. [CrossRef]

113. Karsten, U.; Michalik, D.; Michalik, M.; West, J.A. A New Unusual Low Molecular Weight Carbohydrate in the Red Algal Genus Hypoglossum (Delesseriaceae, Ceramiales) and Its Possible Function as an Osmolyte. *Planta* **2005**, *222*, 319–326. [CrossRef]

114. Lobban, C.S.; Wynne, M.J. *The Biology of Seaweeds*; University of California Press: Oakland, CA, USA, 1981; Volume 17, ISBN 0520045858.

115. Adams, J.M.M.; Toop, T.A.; Donnison, I.S.; Gallagher, J.A. Seasonal Variation in *Laminaria digitata* and Its Impact on Biochemical Conversion Routes to Biofuels. *Bioresour. Technol.* **2011**, *102*, 9976–9984. [CrossRef]

116. Song, M.; Duc Pham, H.; Seon, J.; Chul Woo, H. Marine Brown Algae: A Conundrum Answer for Sustainable Biofuels Production. *Renew. Sustain. Energy Rev.* **2015**, *50*, 782–792. [CrossRef]

117. Davis, T.A.; Volesky, B.; Mucci, A. A Review of the Biochemistry of Heavy Metal Biosorption by Brown Algae. *Water Res.* **2003**, *37*, 4311–4330. [CrossRef]

118. Graiff, A.; Ruth, W.; Kragl, U.; Karsten, U. Chemical Characterization and Quantification of the Brown Algal Storage Compound Laminarin—A New Methodological Approach. *J. Appl. Phycol.* **2016**, *28*, 533–543. [CrossRef]

119. Nøkling-Eide, K.; Langeng, A.-M.; Åslund, A.; Aachmann, F.L.; Sletta, H.; Arlov, Ø. An Assessment of Physical and Chemical Conditions in Alginate Extraction from Two Cultivated Brown Algal Species in Norway: *Alaria esculenta* and *Saccharina latissima*. *Algal Res.* **2023**, *69*, 102951. [CrossRef]

120. Huang, X.; Huang, L.; Li, Y.; Xu, Z.; Fong, C.W.; Huang, D.; Han, Q.; Huang, H.; Tan, Y.; Liu, S. Main Seagrass Beds and Threats to Their Habitats in the Coastal Sea of South China. *Chin. Sci. Bull.* **2006**, *51*, 136–142. [CrossRef]

121. Larkum, A.W.D.; Orth, R.J.; Duarte, C.M. Seagrasses: Biology, Ecology and Conservation. *Phycologia* **2006**, *45*, 5.

122. Hasegawa, N.; Hori, M.; Mukai, H. Seasonal Shifts in Seagrass Bed Primary Producers in a Cold-Temperate Estuary: Dynamics of Eelgrass *Zostera marina* and Associated Epiphytic Algae. *Aquat. Bot.* **2007**, *86*, 337–345. [CrossRef]

123. Touchette, B.W.; Burkholder, J.M. Overview of the Physiological Ecology of Carbon Metabolism in Seagrasses. *J. Exp. Mar. Biol. Ecol.* **2000**, *250*, 169–205. [CrossRef] [PubMed]

124. Marbà, N.; Krause-Jensen, D.; Alcoverro, T.; Birk, S.; Pedersen, A.; Neto, J.M.; Orfanidis, S.; Garmendia, J.M.; Muxika, I.; Borja, A.; et al. Diversity of European Seagrass Indicators: Patterns within and across Regions. *Hydrobiologia* **2013**, *704*, 265–278. [CrossRef]

125. Popper, Z.A.; Michel, G.; Hervé, C.; Domozych, D.S.; Willats, W.G.T.; Tuohy, M.G.; Kloareg, B.; Stengel, D.B. Evolution and Diversity of Plant Cell Walls: From Algae to Flowering Plants. *Annu. Rev. Plant Biol.* **2011**, *62*, 567–590. [CrossRef] [PubMed]

126. El-Hefnawy, M.E.; Alhayyani, S.; El-Sherbiny, M.M.; Abomohra, A.; Al-Harbi, M. Endogenous Bioethanol Production by Solid-State Prefermentation for Enhanced Crude Bio-Oil Recovery through Integrated Hydrothermal Liquefaction of Seaweeds. *J. Clean. Prod.* **2022**, *355*, 131811. [CrossRef]

127. Osman, M.E.H.; Abo-Shady, A.M.; Elshobary, M.E.; Abd El-Ghafar, M.O.; Abomohra, A. Screening of Seaweeds for Sustainable Biofuel Recovery through Sequential Biodiesel and Bioethanol Production. *Environ. Sci. Pollut. Res.* **2020**, *27*, 32481–32493. [CrossRef]

128. Chirapart, A.; Praiboon, J.; Puangsombat, P.; Pattanapon, C.; Nunraksa, N. Chemical Composition and Ethanol Production Potential of Thai Seaweed Species. *J. Appl. Phycol.* **2014**, *26*, 979–986. [CrossRef]

129. Kim, H.M.H.; Wi, S.G.S.; Jung, S.; Song, Y.; Bae, H.-J.H. Efficient Approach for Bioethanol Production from Red Seaweed *Gelidium amansii*. *Bioresour. Technol.* **2015**, *175*, 128–134. [CrossRef]

130. Adams, J.M.M.; Schmidt, A.; Gallagher, J.A. The Impact of Sample Preparation of the Macroalgae *Laminaria digitata* on the Production of the Biofuels Bioethanol and Biomethane. *J. Appl. Phycol.* **2015**, *27*, 985–991. [CrossRef]

131. Bledzki, A.K.; Sperber, V.E.; Faruk, O. *Natural and Wood Fibre Reinforcement in Polymers*; iSmithers Rapra Publishing: Shawbury, UK, 2002; Volume 13, ISBN 1859573592.

132. Offei, F.; Mensah, M.; Thygesen, A.; Kemausuor, F. Seaweed Bioethanol Production: A Process Selection Review on Hydrolysis and Fermentation. *Fermentation* **2018**, *4*, 99. [CrossRef]

133. Ge, L.; Wang, P.; Mou, H. Study on Saccharification Techniques of Seaweed Wastes for the Transformation of Ethanol. *Renew. Energy* **2011**, *36*, 84–89. [CrossRef]

134. Enquist-Newman, M.; Faust, A.M.E.; Bravo, D.D.; Santos, C.N.S.; Raisner, R.M.; Hanel, A.; Sarvabhowman, P.; Le, C.; Regitsky, D.D.; Cooper, S.R. Efficient Ethanol Production from Brown Macroalgae Sugars by a Synthetic Yeast Platform. *Nature* **2014**, *505*, 239–243. [CrossRef] [PubMed]

135. Cho, Y.; Kim, H.; Kim, S.K. Bioethanol Production from Brown Seaweed, Undaria pinnatifida, Using NaCl Acclimated Yeast. *Bioprocess Biosyst. Eng.* **2013**, *36*, 713–719. [CrossRef] [PubMed]

136. Ma, N.L.; Teh, K.Y.; Lam, S.S.; Kaben, A.M.; Cha, T.S. Optimization of Cell Disruption Methods for Efficient Recovery of Bioactive Metabolites via NMR of Three Freshwater Microalgae (Chlorophyta). *Bioresour. Technol.* **2015**, *190*, 536–542. [CrossRef]

137. Kim, Y.; Kim, D.; Kim, T.; Shin, M.-K.; Kim, Y.J.; Yoon, J.-J.; Chang, I.S. Use of Red Algae, Ceylon Moss (*Gelidium amansii*), Hydrolyzate for Clostridial Fermentation. *Biomass Bioenergy* **2013**, *56*, 38–42. [CrossRef]

138. Schultz-Jensen, N.; Thygesen, A.; Leipold, F.; Thomsen, S.T.; Roslander, C.; Lilholt, H.; Bjerre, A.B. Pretreatment of the Macroalgae *Chaetomorpha Linum* for the Production of Bioethanol—Comparison of Five Pretreatment Technologies. *Bioresour. Technol.* **2013**, *140*, 36–42. [CrossRef]

139. Percival, E.; McDowell, R.H. *Chemistry and Enzymology of Marine Algal Polysaccharides*; Academic Press: Cambridge, MA, USA, 1967.

140. Sarkar, N.; Ghosh, S.K.; Bannerjee, S.; Aikat, K. Bioethanol Production from Agricultural Wastes: An Overview. *Renew. Energy* **2012**, *37*, 19–27. [CrossRef]

141. Torun, M. Radiation Pretreatment of Biomass. *Appl. Ioniz. Radiat. Mater. Process.* **2017**, *2*, 447–460.

142. El-Mesery, H.S.; Mao, H.; Abomohra, A. Applications of Non-Destructive Technologies for Agricultural and Food Products Quality Inspection. *Sensors* **2019**, *19*, 846. [CrossRef]

143. Hu, Z.; Wen, Z. Enhancing Enzymatic Digestibility of Switchgrass by Microwave-Assisted Alkali Pretreatment. *Biochem. Eng. J.* **2008**, *38*, 369–378. [CrossRef]

144. Yoon, M.; Choi, J.; Lee, J.-W.; Park, D.-H. Improvement of Saccharification Process for Bioethanol Production from *Undaria* sp. by Gamma Irradiation. *Radiat. Phys. Chem.* **2012**, *81*, 999–1002. [CrossRef]

145. Yuan, Y.; Macquarrie, D.J. Microwave Assisted Acid Hydrolysis of Brown Seaweed Ascophyllum Nodosum for Bioethanol Production and Characterization of Alga Residue. *ACS Sustain. Chem. Eng.* **2015**, *3*, 1359–1365. [CrossRef]

146. Kassim, M.A.; Meng, T.K.; Kamaludin, R.; Hussain, A.H.; Bukhari, N.A. Bioprocessing of Sustainable Renewable Biomass for Bioethanol Production. In *Value-Chain of Biofuels—Fundamentals, Technology, and Standardization*; Elsevier: Amsterdam, The Netherlands, 2022; pp. 195–234. [CrossRef]

147. Alam, S.N.; Khalid, Z.; Guldhe, A.; Singh, B.; Korstad, J. Harvesting and Pretreatment Techniques of Aquatic Macrophytes and Macroalgae for Production of Biofuels. *Environ. Sustain.* **2021**, *4*, 299–316. [CrossRef]

148. Sahay, S. Impact of Pretreatment Technologies for Biomass to Biofuel Production. In *Substrate Analysis for Effective Biofuels Production*; Springer: Berlin, Germany, 2020; pp. 173–216. [CrossRef]

149. Parab, P.; Khandeparker, R.; Amberkar, U.; Khodse, V. Enzymatic Saccharification of Seaweeds into Fermentable Sugars by Xylanase from Marine Bacillus Sp. Strain BT21. *3 Biotech* **2017**, *7*, 1–7. [CrossRef] [PubMed]

150. Soliman, R.M.; Younis, S.A.; El-Gendy, N.S.; Mostafa, S.S.M.; El-Temtamy, S.A.; Hashim, A.I. Batch Bioethanol Production via the Biological and Chemical Saccharification of Some Egyptian Marine Macroalgae. *J. Appl. Microbiol.* **2018**, *125*, 422–440. [CrossRef]

151. Fasahati, P.; Woo, H.C.; Liu, J.J. Industrial-Scale Bioethanol Production from Brown Algae: Effects of Pretreatment Processes on Plant Economics. *Appl. Energy* **2015**, *139*, 175–187. [CrossRef]

152. van der Wal, H.; Sperber, B.L.H.M.; Houweling-Tan, B.; Bakker, R.R.C.; Brandenburg, W.; López-Contreras, A.M. Production of Acetone, Butanol, and Ethanol from Biomass of the Green Seaweed *Ulva lactuca*. *Bioresour. Technol.* **2013**, *128*, 431–437. [CrossRef]

153. Tan, I.S.; Lee, K.T. Solid Acid Catalysts Pretreatment and Enzymatic Hydrolysis of Macroalgae Cellulosic Residue for the Production of Bioethanol. *Carbohydr. Polym.* **2015**, *124*, 311–321. [CrossRef]

154. Tan, I.S.; Lee, K.T. Enzymatic Hydrolysis and Fermentation of Seaweed Solid Wastes for Bioethanol Production: An Optimization Study. *Energy* **2014**, *78*, 53–62. [CrossRef]

155. Tan, Y.; Fang, M.; Jin, L.; Zhang, C.; Li, H.-P.; Xing, X.-H. Culture Characteristics of the Atmospheric and Room Temperature Plasma-Mutated Spirulina Platensis Mutants in CO_2 Aeration Culture System for Biomass Production. *J. Biosci. Bioeng.* **2015**, *120*, 438–443. [CrossRef]

156. Hamedi, J.; Mohammadipanah, F.; Panahi, H.K.S. Biotechnological Exploitation of Actinobacterial Members. In *Halophiles: Biodiversity and Sustainable Exploitation*; Springer: Berlin, Germany, 2015; pp. 57–143.

157. Dehhaghi, M.; Kazemi Shariat Panahi, H.; Guillemin, G.J. Microorganisms' Footprint in Neurodegenerative Diseases. *Front. Cell. Neurosci.* **2018**, *12*, 466. [CrossRef]

158. Wainwright, M.; Sherbrock-Cox, V. Factors Influencing Alginate Degradation by the Marine Fungi: *Dendryphiella salina* and *D. arenaria*. *Bot. Mar.* **1981**, *24*, 489–492. [CrossRef]

159. El-Shishtawy, R.M.; Mohamed, S.A.; Asiri, A.M.; Gomaa, A.M.; Ibrahim, I.H.; Al-Talhi, H.A. Saccharification and Hydrolytic Enzyme Production of Alkali Pre-Treated Wheat Bran by Trichoderma Virens under Solid State Fermentation. *BMC Biotechnol.* **2015**, *15*, 37. [CrossRef] [PubMed]

160. Agbor, V.B.; Cicek, N.; Sparling, R.; Berlin, A.; Levin, D.B. Biomass Pretreatment: Fundamentals toward Application. *Biotechnol. Adv.* **2011**, *29*, 675–685. [CrossRef] [PubMed]

161. Bohutskyi, P.; Bouwer, E. Biogas Production from Algae and Cyanobacteria through Anaerobic Digestion: A Review, Analysis, and Research Needs. In *Advanced Biofuels and Bioproducts*; Springer: New York, NY, USA, 2013; pp. 873–975.

162. Gomaa, M.; Hifney, A.F.; Fawzy, M.A.; Issa, A.A.; Abdel-Gawad, K.M. Biodegradation of *Palisada perforata* (Rhodophyceae) and *Sargassum* sp.(Phaeophyceae) Biomass by Crude Enzyme Preparations from Algicolous Fungi. *J. Appl. Phycol.* **2015**, *27*, 2395–2404. [CrossRef]

163. Hu, Y.; Wang, S.; Wang, Q.; He, Z.; Abomohra, A.; Cao, B. Influence of Torrefaction Pretreatment on the Pyrolysis Characteristics of Seaweed Biomass. *Cellulose* **2019**, *26*, 8475–8487. [CrossRef]

164. Peng, J.; Abomohra, A.; Elsayed, M.; Zhang, X.; Fan, Q.; Ai, P. Compositional Changes of Rice Straw Fibers after Pretreatment with Diluted Acetic Acid: Towards Enhanced Biomethane Production. *J. Clean. Prod.* **2019**, *230*, 775–782. [CrossRef]

165. Patil, J.H.; AntonyRaj, M.; Gavimath, C.C. Study on Effect of Pretreatment Methods on Biomethanation of Water Hyacinth. *Int. J. Adv. Biotechnol. Res.* **2011**, *2*, 143–147.

166. Nikolaison, L.; Dahl, J.; Bech, K.S.; Bruhn, A.; Rasmussen, M.B.; Bjerre, A.B.; Nielsen, H.B.; Ambus, P.; Rost, K.A.; Kadar, Z. Energy Production Fom Macroalgae. In Proceedings of the 20th European Biomass Conference, Milan, Italy, 18–22 June 2012; pp. 18–22.

167. Meinita, M.D.N.; Hong, Y.-K.; Jeong, G.-T. Detoxification of Acidic Catalyzed Hydrolysate of *Kappaphycus alvarezii* (Cottonii). *Bioprocess Biosyst. Eng.* **2012**, *35*, 93–98. [CrossRef] [PubMed]

168. Karray, R.; Hamza, M.; Sayadi, S. Evaluation of Ultrasonic, Acid, Thermo-Alkaline and Enzymatic Pre-Treatment on Anaerobic Digestion of Ulva Rigida for Biogas Production. *Bioresour. Technol.* **2015**, *187*, 205–213. [CrossRef] [PubMed]

169. Atalla, R.H.; Vanderhart, D.L. Native Cellulose: A Composite of Two Distinct Crystalline Forms. *Science* **1984**, *223*, 283–285. [CrossRef]

170. Daroch, M.; Geng, S.; Wang, G. Recent Advances in Liquid Biofuel Production from Algal Feedstocks. *Appl. Energy* **2013**, *102*, 1371–1381. [CrossRef]

171. Choi, D.; Sim, H.S.; Piao, Y.L.; Ying, W.; Cho, H. Sugar Production from Raw Seaweed Using the Enzyme Method. *J. Ind. Eng. Chem.* **2009**, *15*, 12–15. [CrossRef]

172. Yanagisawa, M.; Nakamura, K.; Ariga, O.; Nakasaki, K. Production of High Concentrations of Bioethanol from Seaweeds That Contain Easily Hydrolyzable Polysaccharides. *Process Biochem.* **2011**, *46*, 2111–2116. [CrossRef]

173. Jang, J.-S.; Cho, Y.; Jeong, G.-T.; Kim, S.-K. Optimization of Saccharification and Ethanol Production by Simultaneous Saccharification and Fermentation (SSF) from Seaweed, *Saccharina japonica*. *Bioprocess Biosyst. Eng.* **2012**, *35*, 11–18. [CrossRef] [PubMed]

174. Zhang, K.; Zhang, F.; Wu, Y.R. Emerging Technologies for Conversion of Sustainable Algal Biomass into Value-Added Products: A State-of-the-Art Review. *Sci. Total Environ.* **2021**, *784*, 147024. [CrossRef]

175. Hessami, M.J.; Cheng, S.F.; Ambati, R.R.; Yin, Y.H.; Phang, S.M. Bioethanol Production from Agarophyte Red Seaweed, Gelidium Elegans, Using a Novel Sample Preparation Method for Analysing Bioethanol Content by Gas Chromatography. *3 Biotech* **2019**, *9*, 1–8. [CrossRef]

176. Ra, C.H.; Jeong, G.-T.; Kim, S.-K. Hyper-Thermal Acid Hydrolysis and Adsorption Treatment of Red Seaweed, *Gelidium amansii* for Butyric Acid Production with PH Control. *Bioprocess Biosyst. Eng.* **2017**, *40*, 403–411. [CrossRef]

177. Xu, X.-Q.; Su, B.-M.; Xie, J.-S.; Li, R.-K.; Yang, J.; Lin, J.; Ye, X.-Y. Preparation of Bioactive Neoagaroligosaccharides through Hydrolysis of Gracilaria Lemaneiformis Agar: A Comparative Study. *Food Chem.* **2018**, *240*, 330–337. [CrossRef]

178. Bruhn, A.; Dahl, J.; Nielsen, H.B.; Nikolaisen, L.; Rasmussen, M.B.; Markager, S.; Olesen, B.; Arias, C.; Jensen, P.D. Bioenergy Potential of *Ulva lactuca*: Biomass Yield, Methane Production and Combustion. *Bioresour. Technol.* **2011**, *102*, 2595–2604. [CrossRef]

179. Potts, T.; Du, J.; Paul, M.; May, P.; Beitle, R.; Hestekin, J. The Production of Butanol from Jamaica Bay Macro Algae. *Environ. Prog. Sustain. Energy* **2012**, *31*, 29–36. [CrossRef]

180. Bikker, P.; van Krimpen, M.M.; van Wikselaar, P.; Houweling-Tan, B.; Scaccia, N.; van Hal, J.W.; Huijgen, W.J.J.; Cone, J.W.; López-Contreras, A.M. Biorefinery of the Green Seaweed *Ulva lactuca* to Produce Animal Feed, Chemicals and Biofuels. *J. Appl. Phycol.* **2016**, *28*, 3511–3525. [CrossRef] [PubMed]

181. Anh, H.T.L.; Kawata, Y.; Tam, L.T.; Thom, L.T.; Ha, N.C.; Hien, H.T.M.; Thu, N.T.H.; Huy, P.Q.; Hong, D.D. Production of Pyruvate from Ulva Reticulata Using the Alkaliphilic, Halophilic Bacterium Halomonas sp. BL6. *J. Appl. Phycol.* **2020**, *32*, 2283–2293. [CrossRef]

182. Saleh, S.; Salk, M.; Pamukçu, O. Estimating Curie Point Depth and Heat Flow Map for Northern Red Sea Rift of Egypt and Its Surroundings, from Aeromagnetic Data. *Pure Appl. Geophys.* **2013**, *170*, 863–885. [CrossRef]

183. Nguyen, T.H.; Sunwoo, I.Y.; Ra, C.H.; Jeong, G.-T.; Kim, S.-K. Acetone, Butanol, and Ethanol Production from the Green Seaweed *Enteromorpha intestinalis* via the Separate Hydrolysis and Fermentation. *Bioprocess Biosyst. Eng.* **2019**, *42*, 415–424. [CrossRef]

184. Huesemann, M.H.; Kuo, L.-J.; Urquhart, L.; Gill, G.A.; Roesijadi, G. Acetone-Butanol Fermentation of Marine Macroalgae. *Bioresour. Technol.* **2012**, *108*, 305–309. [CrossRef]

185. Radha, M.; Murugesan, A.G. Enhanced Dark Fermentative Biohydrogen Production from Marine Macroalgae *Padina tetrastromatica* by Different Pretreatment Processes. *Biofuel Res. J.* **2017**, *4*, 551–558. [CrossRef]

186. Ventura, J.-R.S.; Jahng, D. Improvement of Butanol Fermentation by Supplementation of Butyric Acid Produced from a Brown Alga. *Biotechnol. Bioprocess Eng.* **2013**, *18*, 1142–1150. [CrossRef]

187. Hou, X.; From, N.; Angelidaki, I.; Huijgen, W.J.J.; Bjerre, A.-B. Butanol Fermentation of the Brown Seaweed *Laminaria digitata* by Clostridium Beijerinckii DSM-6422. *Bioresour. Technol.* **2017**, *238*, 16–21. [CrossRef]

188. Obata, O.; Akunna, J.; Bockhorn, H.; Walker, G. Ethanol Production from Brown Seaweed Using Non-Conventional Yeasts. *Bioethanol* **2016**, *2*, 134–145. [CrossRef]

189. Sunwoo, I.Y.; Hau, N.T.; Ra, C.H.; Jeong, G.-T.; Kim, S.-K. Acetone–Butanol–Ethanol Production from Waste Seaweed Collected from Gwangalli Beach, Busan, Korea, Based on PH-Controlled and Sequential Fermentation Using Two Strains. *Appl. Biochem. Biotechnol.* **2018**, *185*, 1075–1087. [CrossRef] [PubMed]

190. Kumar, S.; Gupta, R.; Kumar, G.; Sahoo, D.; Kuhad, R.C. Bioethanol Production from *Gracilaria verrucosa*, a Red Alga, in a Biorefinery Approach. *Bioresour. Technol.* **2013**, *135*, 150–156. [CrossRef] [PubMed]

191. Fokum, E.; Zabed, H.M.; Ravikumar, Y.; Elshobary, M.E.; Chandankere, R.; Zhang, Y.; Yun, J.; Qi, X. Co-Fermentation of Glycerol and Sugars by *Clostridium beijerinckii*: Enhancing the Biosynthesis of 1,3-Propanediol. *Food Biosci.* **2021**, *41*, 101028. [CrossRef]

192. Ahring, B.K.; Jensen, K.; Nielsen, P.; Bjerre, A.B.; Schmidt, A.S. Pretreatment of Wheat Straw and Conversion of Xylose and Xylan to Ethanol by Thermophilic Anaerobic Bacteria. *Bioresour. Technol.* **1996**, *58*, 107–113. [CrossRef]

193. Gupta, A.; Verma, J.P. Sustainable Bio-Ethanol Production from Agro-Residues: A Review. *Renew. Sustain. Energy Rev.* **2015**, *41*, 550–567. [CrossRef]

194. Lynd, L.R.; Van Zyl, W.H.; McBride, J.E.; Laser, M. Consolidated Bioprocessing of Cellulosic Biomass: An Update. *Curr. Opin. Biotechnol.* **2005**, *16*, 577–583. [CrossRef]

195. Lynd, L.R.; Weimer, P.J.; Van Zyl, W.H.; Pretorius, I.S. Microbial Cellulose Utilization: Fundamentals and Biotechnology. *Microbiol. Mol. Biol. Rev.* **2002**, *66*, 506–577. [CrossRef]

196. Das Neves, M.A.; Kimura, T.; Shimizu, N.; Nakajima, M. State of the Art and Future Trends of Bioethanol Production. *Dyn. Biochem. Process Biotechnol. Mol. Biol.* **2007**, *1*, 1–14.

197. Hargreaves, P.I.; Barcelos, C.A.; da Costa, A.C.A.; Pereira, N., Jr. Production of Ethanol 3G from *Kappaphycus alvarezii*: Evaluation of Different Process Strategies. *Bioresour. Technol.* **2013**, *134*, 257–263. [CrossRef]

198. Baeyens, J.; Kang, Q.; Appels, L.; Dewil, R.; Lv, Y.; Tan, T. Challenges and Opportunities in Improving the Production of Bio-Ethanol. *Prog. Energy Combust. Sci.* **2015**, *47*, 60–88. [CrossRef]

199. Alvarado-Morales, M.; Boldrin, A.; Karakashev, D.B.; Holdt, S.L.; Angelidaki, I.; Astrup, T. Life Cycle Assessment of Biofuel Production from Brown Seaweed in Nordic Conditions. *Bioresour. Technol.* **2013**, *129*, 92–99. [CrossRef] [PubMed]

200. Aitken, D.; Bulboa, C.; Godoy-Faundez, A.; Turrion-Gomez, J.L.; Antizar-Ladislao, B. Life Cycle Assessment of Macroalgae Cultivation and Processing for Biofuel Production. *J. Clean. Prod.* **2014**, *75*, 45–56. [CrossRef]

201. Faisal, S.; Zaky, A.; Wang, Q.; Huang, J.; Abomohra, A. Integrated Marine Biogas: A Promising Approach towards Sustainability. *Fermentation* **2022**, *8*, 520. [CrossRef]

202. Nguyen, T.; Sperou, N.; Su, P.; Zhang, W. Marine Biorefinery: An Environmentally Sustainable Solution to Turn Marine Biomass and Processing Wastes into Value-Added Products and Profits. *Biochemist* **2022**, *44*, 22–27. [CrossRef]

203. Abomohra, A.; Faisal, S.; Ebaid, R.; Huang, J.; Wang, Q.; Elsayed, M. Recent Advances in Anaerobic Digestion of Lipid-Rich Waste: Challenges and Potential of Seaweeds to Mitigate the Inhibitory Effect. *Chem. Eng. J.* **2022**, *449*, 137829. [CrossRef]

204. Shin, S.-R.; Lee, M.-K.; Im, S.; Kim, D.-H. Effect of Seaweed Addition on Enhanced Anaerobic Digestion of Food Waste and Sewage Sludge. *Environ. Eng. Res.* **2019**, *24*, 449–455. [CrossRef]

205. Jung, H.; Kim, J.; Lee, C. Continuous Anaerobic Co-Digestion of Ulva Biomass and Cheese Whey at Varying Substrate Mixing Ratios: Different Responses in Two Reactors with Different Operating Regimes. *Bioresour. Technol.* **2016**, *221*, 366–374. [CrossRef]

206. Abomohra, A.; Sheikh, H.M.A.; El-Naggar, A.H.; Wang, Q. Microwave Vacuum Co-Pyrolysis of Waste Plastic and Seaweeds for Enhanced Crude Bio-Oil Recovery: Experimental and Feasibility Study towards Industrialization. *Renew. Sustain. Energy Rev.* **2021**, *149*, 111335. [CrossRef]

207. Yuan, C.; Wang, S.; Cao, B.; Hu, Y.; Abomohra, A.; Wang, Q.; Qian, L.; Liu, L.; Liu, X.; He, Z.; et al. Optimization of Hydrothermal Co-Liquefaction of Seaweeds with Lignocellulosic Biomass: Merging 2 Nd and 3 Rd Generation Feedstocks for Enhanced Bio-Oil Production. *Energy* **2019**, *173*, 413–422. [CrossRef]

208. Sharma, S.; Nair, A.; Sarma, S.J. Biorefinery Concept of Simultaneous Saccharification and Co-Fermentation: Challenges and Improvements. *Chem. Eng. Process.-Process Intensif.* **2021**, *169*, 108634. [CrossRef]

209. Ha, S.J.; Galazka, J.M.; Kim, S.R.; Choi, J.H.; Yang, X.; Seo, J.H.; Glass, N.L.; Cate, J.H.D.; Jin, Y.S. Engineered *Saccharomyces cerevisiae* Capable of Simultaneous Cellobiose and Xylose Fermentation. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 504–509. [CrossRef] [PubMed]

210. Ojeda, K.; Sánchez, E.; El-Halwagi, M.; Kafarov, V. Exergy Analysis and Process Integration of Bioethanol Production from Acid Pre-Treated Biomass: Comparison of SHF, SSF and SSCF Pathways. *Chem. Eng. J.* **2011**, *176–177*, 195–201. [CrossRef]

211. Olofsson, K.; Rudolf, A.; Lidén, G. Designing Simultaneous Saccharification and Fermentation for Improved Xylose Conversion by a Recombinant Strain of *Saccharomyces cerevisiae*. *J. Biotechnol.* **2008**, *134*, 112–120. [CrossRef] [PubMed]

212. Sunwoo, I.Y.; Kwon, J.E.; Nguyen, T.H.; Ra, C.H.; Jeong, G.-T.; Kim, S.-K. Bioethanol Production Using Waste Seaweed Obtained from Gwangalli Beach, Busan, Korea by Co-Culture of Yeasts with Adaptive Evolution. *Appl. Biochem. Biotechnol.* **2017**, *183*, 966–979. [CrossRef]

213. Althuri, A.; Gujjala, L.K.S.; Banerjee, R. Partially Consolidated Bioprocessing of Mixed Lignocellulosic Feedstocks for Ethanol Production. *Bioresour. Technol.* **2017**, *245*, 530–539. [CrossRef] [PubMed]

214. Atkinson, M.J.; Smith, S.V. C:N:P Ratios of Benthic Marine Plants1. *Limnol. Oceanogr.* **1983**, *28*, 568–574. [CrossRef]

215. Patel, S.K.S.; Kumar, P.; Mehariya, S.; Purohit, H.J.; Lee, J.-K.; Kalia, V.C. Enhancement in Hydrogen Production by Co-Cultures of Bacillus and Enterobacter. *Int. J. Hydrogen Energy* **2014**, *39*, 14663–14668. [CrossRef]

216. El-Sheekh, M.; Elshobary, M.; Abdullah, E.; Abdel-Basset, R.; Metwally, M. Application of a Novel Biological-Nanoparticle Pretreatment to *Oscillatoria acuminata* Biomass and Coculture Dark Fermentation for Improving Hydrogen Production. *Microb. Cell Fact.* **2023**, *22*, 34. [CrossRef]

217. Laurinavichene, T.; Laurinavichius, K.; Shastik, E.; Tsygankov, A. Long-Term H2 Photoproduction from Starch by Co-Culture of *Clostridium butyricum* and *Rhodobacter sphaeroides* in a Repeated Batch Process. *Biotechnol. Lett.* **2018**, *40*, 309–314. [CrossRef]

218. Sharma, A.K.; Swain, M.R.; Singh, A.; Mathur, A.S.; Gupta, R.P.; Tuli, D.; Puri, S.K.; Ramakumar, S.S.V. US20190276857A1—Method for Second Generation Ethanol Production from Lignocellulosic Biomass—Google Patents. U.S. Patent US20190276857A1, 12 September 2021.

219. Hörhammer, H.; Dou, C.; Gustafson, R.; Suko, A.; Bura, R. Removal of Non-Structural Components from Poplar Whole-Tree Chips to Enhance Hydrolysis and Fermentation Performance. *Biotechnol. Biofuels* **2018**, *11*, 222. [CrossRef]

220. Johnston, K.G.; Abomohra, A.; French, C.E.; Zaky, A.S. Recent Advances in Seaweed Biorefineries and Assessment of Their Potential for Carbon Capture and Storage. *Sustainability* **2023**, *15*, 13193. [CrossRef]