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Review

Biofuel Production from Seaweeds: A Comprehensive Review

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Abstract: Seaweeds represent a promising and sustainable feedstock for biofuel production which raises increasing research interests. Their high availability, easy fermentable composition, and good degradation potential make them a suitable candidate for alternating fossil fuels as an advantageous energy resource. This comprehensive review aims to summarize and discuss data from the literature on the biochemical composition of seaweeds and its potential for biomethane and biohydrogen production, as well as to investigate the effect of the common pretreatment methods. Satisfactory yields comparable to terrestrial biomass could be obtained through anaerobic digestion; concerning dark fermentation, the challenge remains to better define the operating conditions allowing a stable production of biohydrogen. Finally, we propose a potential energy production scheme with the seaweed found by the Caribbean Islands of Guadeloupe and Martinique, as well as current techno-economic challenges and future prospects. An annual energy potential of 66 GWh could be attained via a two-stage biohythane production process, this tends to be promising in terms of energetic valorization and coastal management.

Keywords: macroalgae; biomethane; biohydrogen; *Sargassum*; anaerobic digestion

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1. Introduction

Seaweeds are multicellular, macroscopic, eukaryotic, and autotrophic organisms. They are taxonomically organized in three large and distinct groups, based especially on the color of the thallus: Chlorophyta (green algae), Rhodophyta (red algae), and Ochrophyta—Phaeophyceae (brown algae) [1]. They are present in the ocean and more particularly in coastal areas. Their distribution depends on geoclimatic conditions and various biotic or abiotic parameters. Phaeophyta and Rhodophyta include several commercially exploited alginophytes and carrageenophytes, respectively, such as *Laminaria*, *Macrocystis*, *Durvillaea*, *Ecklonia* and *Sargassum* or *Eucheuma*, *Kappahyccus*, *Chondrus*. Considered a valuable raw material for a wide range of value-added products and energy production, seaweed can be used in several fields, including human and plant health, cosmetics, agriculture, food, and construction [2–5].

The world population is expected to reach 9.8 billion by 2050. The growth of the world's population creates an urgent demand for energy, which currently consists mainly of fossil fuels [6]. In addition, the European Union adopts an ambition to displace petroleum-based fuels: "the decarbonization of the economy" has long been an important pillar of European energy policy. In this context, the European Union's target is to reduce its greenhouse gas (GHG) emissions by 80–95% by 2050, compared with their 1990 level, in order to contribute to limiting global warming to below 2 °C [7]. Globally, a growing number of countries have pledged to reach net-zero emissions by the midcentury [8]. Achieving these goals requires the exploration of energy from renewable sources. More specifically, the transition to "net zero" means that two-thirds of energy consumption should be covered by renewables, divided between bioenergy, wind, solar, hydroelectricity, geothermal and renewable marine energies. Nearly 70% of electricity generation is expected to come from solar photovoltaic and wind power [8]. Some countries have made efforts

to drive down their emissions by using renewable resources: Ireland became the world's first country to commit to divesting public money fully from fossil fuels (2017) [9]. France has set the objective of having its biogas production between 24 and 32 TWh of the higher heating value (HHV)/year in 2028 [10]. Iceland uses a combination of hydropower and geothermal power to meet almost all of its electricity needs [11]. Denmark is developing wind and solar power as well as bioenergy [12]. Germany is the leading producer of solar (45 GWh) and wind energy (90.5 TWh onshore, 19.5 TWh offshore) in the EU [13].

In terms of biofuel production, there are two main methods of biomass conversion: biochemical conversion and thermochemical conversion processes [14]. The former involves methanization/anaerobic digestion (AD) which is a versatile method that converts organic matter in an oxygen-free environment into biogas, a mixture of methane (60–70%) and CO_2 (30–40%) [15,16]. This renewable fuel can be combusted for combined heat and power generation, or purified for further injection into the gas grid. The latter one includes combustion, gasification and pyrolysis, among which gasification has many advantages: lower production of air pollutants, and the possibility of producing carbon-neutral or carbon-negative fuels, heat, cold, or power [17]. For wet and residual biomass processing, drying is typically required to obtain a desire range of moisture content appropriate for the process or to stabilize this biomass before its valorization, which could be energy-intensive. However, a recent study presented an alternative polygeneration system for bioenergy and biohydrogen production; no external energy is required with their cogeneration unit design [18].

Seaweeds used in terms of the production of bioenergy represent an idea that has received increasing attention in recent years. An analysis of published research results was conducted regarding selected keywords in these research areas until August 2022 based on Web of Science databases. The advanced research was used to cluster documents including the topic ("macroalgae" or "seaweeds") and ("biofuel" or "biogas" or "anaerobic digestion" or "biohydrogen"). A total of 1686 scientific records could be found since 1980. 97% (1639) of them have been published since 2010. The research field is mainly "energy fuels", "biotechnology applied microbiology", "environmental sciences ecology", "engineering" and "agriculture". We may notice that the research on "seaweeds" related to energy production remains at a nascent stage in the 2010s, where there are almost no review articles. However, it has gained significant attention in the last 10 years (Figure 1).

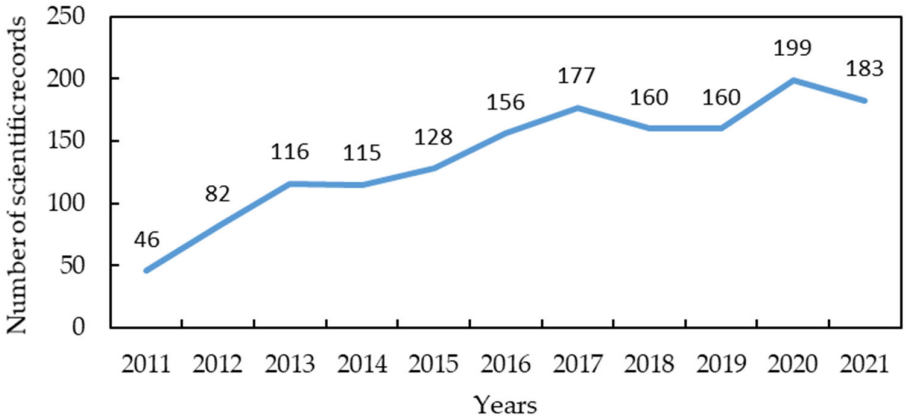

Figure 1. Record of the number of publications/articles in research works related to defined topics via Web of Science for the period 2011–2021.

Seaweeds are an attractive feedstock with various advantages: first, their wide availability offers an abundant supply of biomass for use in biogas plants [19]. As a promising feedstock for biogas production, high values of biomethane potential (BMP) have been obtained from the brown seaweed *Macrocystis* (0.39–0.41 m^3 CH_4/kg Volatile solids (VS)) [20], the red seaweed *Gracilaria* (0.28–0.4 m^3 CH_4/kg VS) [21], these values are comparable to the BMP of terrestrial crops such as sorghum (0.26–0.39 m^3 CH_4/kg VS),

sugarcane (0.23–0.3 m^3 CH_4/kg VS) [22]. Considering that their cultivation does not require arable land or the addition of fertilizers [4,23], they may offer a higher potential for large-scale biomass energy farms. Second, unlike the biomass used for the production of the so-called "second generation biofuels", the absence of woody and lignocellulosic biomaterials makes them highly degradable and fermentable, which is suitable for AD [24]. Furthermore, they can help mitigate greenhouse gas (GHG) emissions through photosynthesis. Previous work has shown that 961 kg of CO_2 can be removed by cultivation of one tons of dry seaweed [25].

In recent years, the unusual massive inundations of pelagic brown seaweed *Sargassum* in the Caribbean, West Africa, the Gulf of Mexico and Europe has had a strong impact on the local economy, tourists, and the environment. Such as contamination of the beaches, the eutrophication, introduction of nutrients to the marine-terrestrial ecotone, gaseous emissions of hydrogen sulfide and ammonia as a result of decomposition [26]. At the same time, the high volumes of *Sargassum* accumulated on coasts and beaches represent resources whose potential use for energy production is very interesting to explore.

Energy recovery from seaweeds via AD and/or dark fermentation processes has been discussed and reviewed by many authors, previous studies have almost exclusively focused on the energetic aspect (a synthesis of BMP, BHP values); the investigation towards seaweeds remains limited, with only a few works mentioned at the same time as the biomass generation and their characterizations. This paper can be considered as a step towards a more profound understanding of the biochemical conversion process, with a thorough illustration integrating the origin of the biomass, their morphology and biochemical composition, the pretreatment techniques frequently encountered, the associated by-products as well as the heavy metals issues. Moreover, the effects of operational parameters, considered as the most problematic by previous research, are also investigated.

In this review, we aim to present an extensive and updated overview of the potential use of seaweeds as a feedstock for methane and hydrogen production. A focus has been made to an invasive seaweed genera *Sargassum*. To our knowledge, no prior studies have examined the energy potential of beached *Sargassum* in the French West Indies. Furthermore, we analyze technical–economic challenges and propose future scientific investigations.

2. The Origin of the Biomass

2.1. Cultivation

Seaweeds play an essential role in the diet of a growing population. It has been reported that about 80% of harvested seaweed is used for human consumption [27]. They are consumed as sea vegetables, added to various food preparations for nutritional profile and taste improvement. On the other hand, industrial demand for seaweed extracts such as carrageenan and alginates show an increasing trend [28]. For all these reasons, the aquatic algae sector has been developing rapidly in recent times [29]. In 2019, aquaculture was estimated to contribute about 97% (35 million tons) of the global volume of seaweed production, with the remaining wild seaweeds accounting for less than 3% [27,30].

According to FAO data, aquaculture has produced 32.4 million tons of aquatic algae (97.1% of which is seaweed) worldwide, which represents an estimated ex-farm commercial value of USD 13.3 billion. This figure is three times higher compared with production at the beginning of the 21st century, which increased from 10.6 million tons in 2000 to 32.4 million tons in 2018. Major producing countries include China, Indonesia, South Korea and The Philippines (Figure 2). In the last 10 years, despite the slowdown in growth at a global level, the rapid growth of Indonesian production is most notable due to the rapid development of the cultivation of tropical red seaweed species (*Kappaphycus alvarezii* and *Eucheuma* spp.), which are used as raw material for the extraction of cell wall carrageenan.

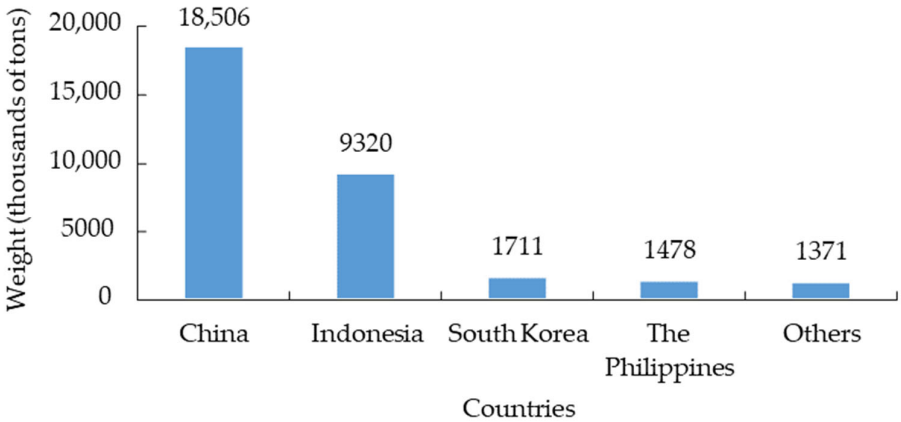

Figure 2. World aquatic algae production in 2018 (thousands of tons, live weight) [31].

2.2. Wild Seaweed

In 1969, the cultivation and wild collection of seaweed have similar contributions to world seaweed production, at about 1.1 million tons each. Five decades later, the aquaculture increased to about 35 million tons, whereas wild collection remains at a constant level; only a slight decline of 0.25 million tons has been found in all three groups of seaweeds, from 1990 [32]. There is no remarkable variation in wild seaweed production between 2009 and 2019 (Figure 3) [27]. Europe only contributes to 0.8% of world seaweed production in 2019, with a predominance of wild collection of over 95% [33]. The largest collector of wild seaweed in Europe is Norway (~150,000 tons per year), where in some harvesting areas, seaweed beds are harvested at an interval of 5 years [34]. France, Ireland, and the Russian Federation are also large producers in Europe [35].

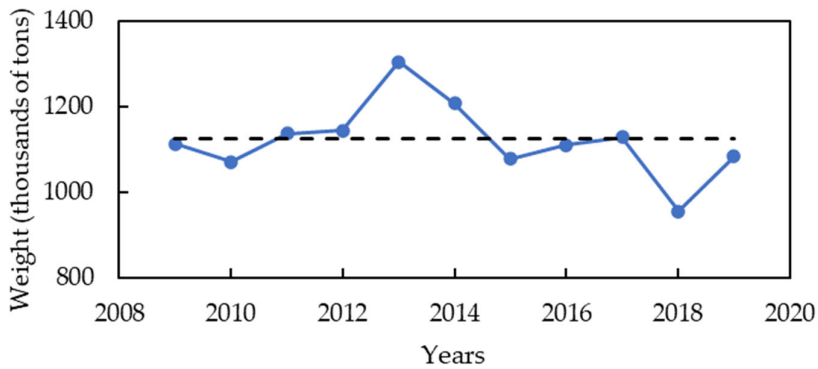

Figure 3. Global wild production of seaweed from 2009 to 2019 (thousands of tons, dotted line: average level, adapted from [27]).

Application

The cultivation of seaweeds is well developed in Asian countries as some species are intended for human consumption (e.g., brown seaweed *Undaria pinnatifida*, red seaweed *Porphyra* spp. (*Pyropia*), and green seaweed *Caulerpa* spp.) (Figure 4). The red seaweed Nori (*Pyropia* species) can be used for wrapping sushi, whereas the red seaweed *Eucheuma* can be used for food processing as well as for cosmetics. In Malaysia and Indonesia, seaweeds are eaten fresh as salad [36]. Outside of Asian countries, the production of seaweeds mainly serves the colloid market. This is the case in Chile, and in France, the brown species concerned are *Lessonia*, and *Laminaria digitata*, respectively [31]. In the future, the demand for seaweed products by western markets is expected to increase rapidly, due to the interest in alternative protein sources, dietary supplements and sustainable textural compounds [37].

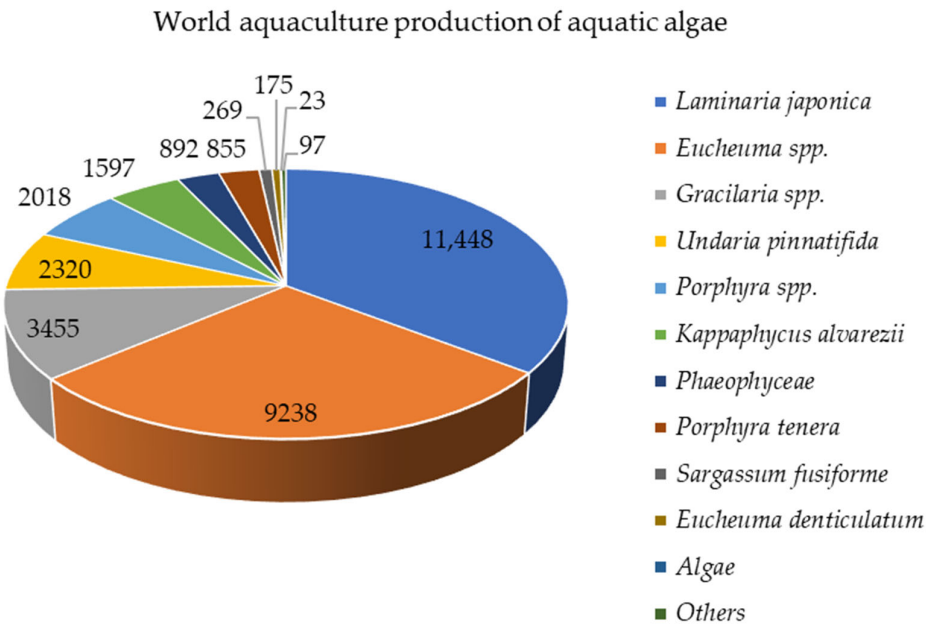

Figure 4. Global aquaculture production of aquatic algae in 2018 (thousands of tons, live weight) [31].

As seaweeds are largely explored for their food use, their related safety issues need to be addressed. Indeed, seaweeds have a higher level for minerals and trace elements than the surrounding water, due to their specific structural characteristics [27]. The concentration of metals is even three to ten times higher when they are in dried form [38]. This phenomenon is more pronounced for wild harvests which are more likely to be affected by industrial pollution. Therefore, it is of great importance to identify all possible hazards, to understand their occurrence and corresponding impact on food security, to monitor their levels in order to avoid all associated risks. However, only limited data are available in EU legislation, no standard has been developed for the maximum threshold of heavy metals (cadmium, lead, mercury, arsenic, etc.). France was the first European country to establish a specific evaluation of the use of seaweed for human consumption as non-traditional food substances. In total, 25 algae, including 3 microalgae, were listed as being suitable for food use [39]. In the future, attention should be drawn to this significant regulatory gap.

2.3. Drift Seaweed

The term "seaweed tides" describes the massive shoaling event of seaweed biomass. A possible explanation for the sudden beaching of huge algae could be climate change, and anthropogenic activities [40–42]. They are considered a nuisance by the decomposition and production of toxic vapors for tourism industry at coastal areas and marine ecosystems (inhibition of germination of seaweed zygotes, decrease in the growth rates of algal species, etc.). They are responsible for financial losses by resort operators to which the costs of removing and disposing of the thousands of tons of beached algae are added. Usually, seaweed inundation is characterized by the color of the seaweed (green, red or golden). Most of the inundation events can be attributed in particular to two genera: *Ulva*, responsible for green tides, and *Sargassum*, causing golden tides [40]. Green tides have been mainly reported in Europe, such as in Ireland [43], and on Brittany beaches [44]. In France, there are about 98,000 m^3/year of algal biomass, mainly *Ulva*, gathered during the summer along the Breton coastline, resulting in a necessary investment estimated at EUR 0.6–12 million per year, depending on area and equipment [44]. The most affected areas are Lannion Bay and St Brieuc Bay (Brittany, France) which could be remoted to the 1970s [45]. Up to now, attempts have been made to compost this biomass with ligneous materials to stabilize the seaweed. Otherwise, in terms of valorization, the AD of *Ulva* with manure pig seems to be the best solution at the moment [46], as *Ulva* alone has a low

methanogenic potential value due to its high-water content [47]. Allen et al. [48] obtained an optimum methane yield in the case of a *U. lactuca*/slurry co-digestion, applying a ratio of 25% *U. lactuca* and 75% slurry. However, it seems rather complicated to set up an AD unit with stranded algae as substrates. The process requires the installation of a biogas purification system to remove the significant production of H_2S [49]. In addition, to make the biomass suitable for AD, pretreatment steps such as rinsing, grinding, drying and storage are required, the corresponding cost is quite high. In the long term, more cost-effective treatment methods would be required, with the aim of achieving a higher methane yield. Another algal bloom involving a massive green tide of *Enteromorpha* (*Ulva*) *prolifera* occurred in 2008, covering 6 10^8 m^2 along the coast of Qingdao, a few weeks before the start of the Beijing Olympics. One million tons of algae were removed with the participation of more than 10,000 people [50]. The direct aquacultural losses were estimated at EUR 86 million [51]. Consequently, the gathered biomass was used as fertilizers and for biogas production. As for the *Sargassum* golden tides, events occur regularly in the summer in the Gulf of Mexico. Two related holopelagic species have been identified as responsible: *S. fluitans* et *S. natans*. This event was not observed by people in northwest Africa until 2011 [40]. During the inundation in 2015, approximately 10,000 tons of wet seaweed were noted daily on the beaches of the Caribbean Island [41]. Although more research is needed for the valorization of this biomass, it has been proven by a Mexico company that *Sargassum* can be converted into biogas, with a methane content up to 72%. It would also be conceivable to use *Sargassum* biogas as fuels for vehicles, with an additional cleaning process [52].

3. Characterization of Seaweeds

3.1. Morphology of Seaweeds

Seaweed thalli vary from a few millimeters to ~100 m, from thread-like filaments to multicellular complex thalli. They exhibit great variation in size, shape, and texture. They vary from small filamentous, cylindrical, flattened or foliaceous, siphonous to giant complex cladomothalli in red seaweeds. Brown Seaweed thalli are usually differentiated into: blades, floats, stipe, holdfast and thallus (Figure 5), with wide range of thallus organization from small filamentous forms, e.g., *Dictyota* or *Ectocarpus*, which are few millimeters, to intertidal aquatic plants, e.g., *Ascophyllum*, *Laminaria* and *Fucus*, to subtidal massive kelps and the largest *Macrocystis* [53,54]. Additional thallus morphologies include: sphere, fan, cup, and ball shaped, e.g., *Colpomenia*, *Padina*, etc.

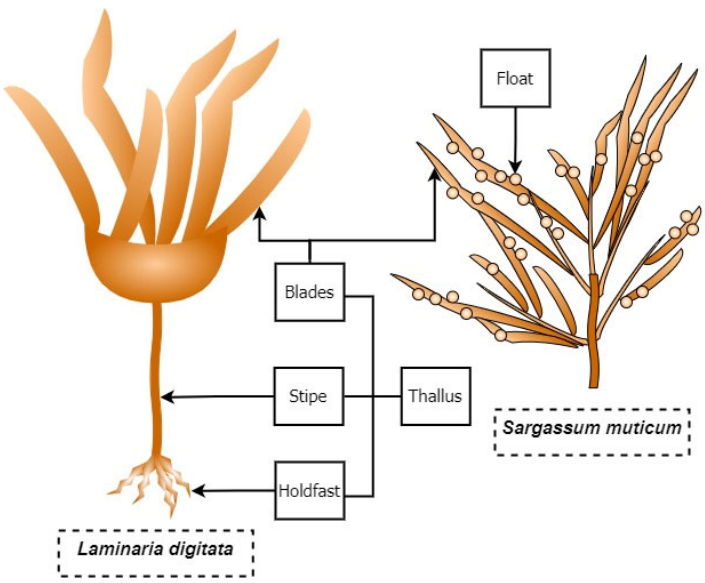

Figure 5. General morphology of brown seaweed (adapted from [55]).

The thallus is the place where photosynthesis occurs. A morphological modification can take place in case of strong water current, more resistant blades can be formed. The floats, also called air bladders or vesicles, are normally oval in shape with the primary function of providing buoyancy to the algae to float on the water surface. The stipe provides flexibility to the algae. The holdfast ensures the firm attachment of the algae to the substratum.

3.2. General Composition

Seaweeds are composed of proteins, a low percentage of lipids and a high percentage of carbohydrates mainly in the form of polysaccharides. They contain high levels of minerals: potassium, chlorine, sodium, calcium, magnesium, sulfur, phosphorus, iodine, iron, copper, manganese and many other trace elements, and also vitamins as well as phytohormones and pigments [56].

3.2.1. Algal Structure (Focus Carbohydrates)

The structural differences of algae can be found in the carbohydrates. For example, floridean starch, which serves as a storage polysaccharide, is found only in red algae [54], as well as cell wall anionic phycocolloids alginate, agar and carrageenan, which are widely used in the food industry as gelling and stabilizing agents. Alginate, sulfated polysaccharides rich in fucose (fucoidan, fucan), laminarin and mannitol are specific to brown algae: alginate processes several biological anti-bacterial, anti-aging and anti-inflammatory properties that make it an excellent candidate for cosmetic products [57–60]. As for sulfated polysaccharides rich in fucose interesting biological properties have been reported in the literature, we note its benefits for human health as an anti-inflammatory agent, immunomodulatory agent, and anti-tumor antioxidant agent [61–65]. Laminarins are known for their remarkable plant health benefits [66]. Concerning green algae, diverse applications of ulvan are already reported in the literature, such as therapeutic active agents [67], hydrogels [68] or the diet of humans [69].

Three groups of seaweeds have common components and their own sugars [53] (Figure 6).

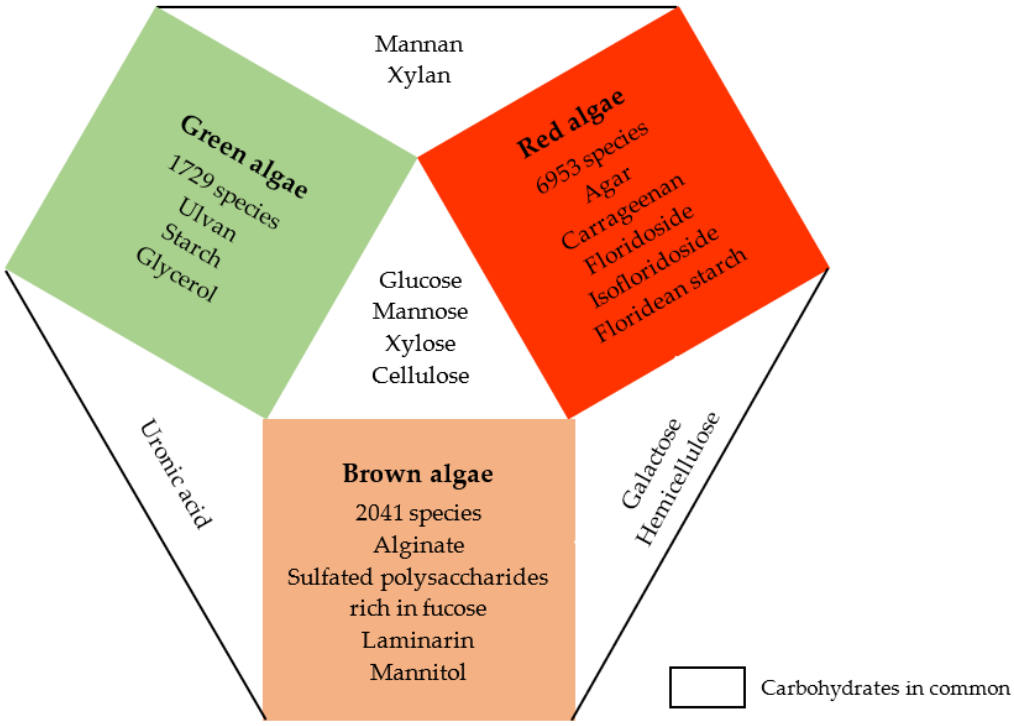

Figure 6. Cell wall polysaccharides and stored sugars present in three groups of seaweeds.

3.2.2. Biochemical Composition of Seaweeds

Biochemical composition can vary considerably depending on the species studied, the geographical region, the harvesting season, biotic and abiotic parameters. The pretreatment methods and processing of biomass (storage, drying) can also have an impact on the composition. Finally, it depends on the analytical methods used which may strongly influence the results of biochemical analyses.

Despite the richness of the literature and analytical tools, it does not seem rigorous to generalize the composition of seaweeds. In reality, even a rough estimation may help for future exploitation in terms of estimation of the energy potential and biomass valorization [56] (Table 1).

Table 1. Chemical composition of seaweeds (green, red and brown).

Compound	Green Algae	Red Algae	Brown Algae	Reference
Carbohydrates [1]	25–50%	30–60%	30–60%	[53,70]
Protein [1]	10–20%	10–25%	3–15%	[56,71,72]
Lipid [1]	1–4%	0.6–4%	0.4–2.4%	[56,73,74]
Mineral [1]	18–53%	26–48%	34–55%	[72]
Water content [2]	70–85%	70–80%	75–90%	[75]

[1] Dry weight, [2] fresh weight.

It appears that the three groups of seaweeds are similar in terms of carbohydrate and water content, as well as a low lipid content. However, a difference was noticed in the protein content, mainly that red algae have the highest protein content, and are especially known for their richness in essential amino acids; moreover, some red seaweeds have a protein composition close to Leguminosae such as soybeans, with a ratio of essential amino acids/total amino acids of about 35 percent. Regarding the mineral content, a slight advantage was found for brown and red algae. Otherwise, seaweeds differ in their mineral composition: brown algae are rich in iodine, they can accumulate from 1500 to 8000 ppm of I (based on dry weight) mostly in mineral form (iodide). Some red or green algae are high sources of calcium (*Phymatolithon calcareum*, *Lithothamnion coralliodes*). Regarding iron, we point out that the genus *Ulva* contains up to 12 times more iron than some legumes of the bean family [56].

On the other hand, seaweeds would be an excellent indicator or/and bio-adsorbent of heavy metals, especially in coastal areas, including copper, cadmium, lead, and zinc. However, attentions should be paid in case of cosmetic or food use, those products placed on the market must meet the criteria of heavy metal. Alternatively, the analysis of the composition has confirmed the high sugar content of seaweeds, which make it a suitable raw material for energy production.

4. Energy Production from Seaweeds

Marine seaweeds are used worldwide not only to produce colloidal chemicals, but also to produce renewable biofuels which are considered third-generation fuels [76].

In 2030, the physical potential of French biogas production from all cultivable seaweed biomass is about 9 TWh LHV/year by mobilizing all land and sea spaces; this corresponds to more than two times the French biogas production in 2011 [77].

In this section, we will explore possible alternatives to fossil fuels, particularly biogas and biohydrogen, which have attracted considerable research interest.

4.1. Biofuel Production

4.1.1. Bioethanol

Generally, the bioethanol production process consists of the transformation of polysaccharides into simple sugars, either by acid hydrolysis or by enzymatic means. It consists of three main steps: pretreatment, enzymatic hydrolysis/saccharification, and fermentation.

The last two steps can be performed simultaneously. The ethanol obtained is recovered by distillation and dehydration [53].

Seaweeds have the advantage of having very few lignocellulosic compounds, for this reason they are one of the best raw materials for bioethanol production. However, the different types of sugars present require the addition of specific and appropriate enzymes, hence it is a factor to consider when choosing the pretreatment methods. Otherwise, due to the high-water content of algal biomass, the potential for ethanol production from seaweed (*Sargassum horneri*) is limited (estimated at 29.6 kg/t raw material), which is comparable to that of sugarcane, although lower than that of many land crops such as barley, wheat, rice, for which the production rate is about 400 kg/t [78].

4.1.2. Biobutanol

For the production of biobutanol, it is the acetone-butanol-ethanol fermentation process (ABE) that can convert a wide variety of sugars (hexoses and pentoses) into simple alcohols, with the presence of *Clostridium* strains [79]. This process has two characteristic steps: acidogenesis and solventogenesis [80].

Huesemann et al. [81] investigated the potential of brown algae *Saccharina* spp. for biochemical conversion to butanol by *C. acetobutylicum*. A low yield of 0.12 g/g was obtained from the seaweed extract, and a triauxic was observed. The authors attributed this to the use of carbon sources. They found that product yields were limited by recalcitrant alginates and concluded that significant improvements are still needed to make the industrial-scale ABE process of seaweed economically feasible.

4.1.3. Bio-Oil

Bio-oil can be obtained from the thermochemical conversion of seaweeds, by pyrolysis, which is carried out at elevated temperature and under oxygen-limited conditions. A drying process is necessary for the biomass in order to increase the yield. Due to the difference in temperature and retention time, there are three main conventional variants of pyrolysis: conventional pyrolysis, fast pyrolysis, and flash pyrolysis. It can also be performed using HydroThermal Liquefaction (HTL), which is a promising process for biofuels production. It requires less energy and is performed under subcritical conditions (at temperatures of around 200–380 °C and pressures between 2 and 28 MPa) [53,82]. Compared with pyrolysis, the bio-oil produced by this process is lower in oxygen and moisture content thus more stable [79]. Along with bio-oil, other by-products such as biochar, soil conditioner, and chemicals can be produced.

Yanik et al. [83] obtained lower yields (11–17%) of bio-oil production from seaweed than from lignocellulosic biomass (23–40%). Bio-oil production from seaweed biomass does not seem to be a viable option, either by pyrolysis or HTL, in terms of oil yield and quality (heating value), comparing to microalgal or terrestrial biomass. The relatively high water, nitrogen and ash content, as well as the presence of metals and inorganic ions, make seaweed an unsuitable candidate for bio-oil production.

4.1.4. Biodiesel

Composed of monoalkyl esters of long-chain fatty acids derived from bio-oils, biodiesel can be obtained by transesterification. It has many benefits, including respect for the environment, and high biodegradability. Milledge et al. [79] reviewed biodiesel production from seaweeds; low oil extraction yield values were obtained from *Ulva lactuca* (about 10%) and *Enteromorpha compressa* (about 11%). Seaweeds, therefore, seem less suitable for this production due to their low lipid content [73,84,85].

4.2. Biogas

Biogas is a mixture of gases composed mainly of methane, carbon dioxide and small quantities of hydrogen sulfide. It is a renewable energy source that is commonly produced

by anaerobic digestion, from raw materials such as agricultural waste, municipal waste, food waste, etc.

A typical AD scheme consists of four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Hydrolysis is the limiting step allowing the decomposition of complex organic matter. At the end of this step, simple materials such as amino acids and fatty acids are formed. In the acidogenesis stage, H_2, alcohol, ammonia, and volatile fatty acids (VFA) (acetic acid, propionic acid, butyric acid, isobutyric acid) are produced. Then, comes the acetogenesis which produces acetate, CO_2, and H_2. These are the main substrates of methane formation. Finally, the production of methane is done either from acetate by acetoclastic methanogenic bacteria, or by the reduction in CO_2 [86].

Seaweeds are considered a suitable substrate for biogas production owing to their high carbon-nitrogen ratio, low lipid content and lack of lignin. The huge amounts of stranded biomass represent an attractive feedstock for energy production and could be integrated into a biorefinery scheme.

4.2.1. Parameters Likely to Influence the Quality of AD

Biomethane production could be influenced by several factors, such as the chemical composition of seaweeds, pretreatment methods, experimental conditions, etc. They are well documented in the literature [20,87–90].

According to Jard et al. [72], low methane production can often be explained by the part of the algae that resists microbial attack. For example, the presence of colloid (agar-agar) [91], or a high proportion of insoluble fibers and polyphenols that are likely to make the algae less accessible and degradable for microbials. Furthermore, high salt concentrations can induce severe inhibition of methanogenic bacteria in the AD process [92]. On the contrary, the presence of easier fermentable components (sugars) is favorable to AD.

The above factors have been well studied in the literature. However, research interest in other parameters, such as sulfur content, inoculum/substrate ratio (ISR), seems sparse. Hence, we seek to elucidate the possible effects of these parameters on AD, and to estimate more precisely the value of BMP, in order to provide a more complete comprehensive description.

4.2.2. Effect of the Sulfur Content

In the presence of sulfates, an additional step is added to the essential steps of AD, namely, sulfate reduction. The bacteria responsible for this conversion, called sulfate-reducing microorganisms (SRMs), are able to use a wide variety of organic substrates such as VFAs, acetate and hydrogen for their metabolism, with the sulfur compound acting as the final electron acceptor in redox reactions that would be reduced to H_2S [47]. SRMs work in syntropy with other microorganisms, e.g., obligatory hydrogen-producing micro-organisms, making some thermodynamically unfavorable VFA conversion reactions possible [47]. However, there are possible competitive interactions between methanogenic archaea and sulfate-reducing bacteria that depend on the ratio of the amount of organic matter to the amount of available sulfate [47,93]. According to Hao et al. [94], no methanogenic activity occurs when a low ratio is applied; conversely, a high ratio leads to the predominance of methanogenesis.

As is well known, H_2S is a highly toxic gas that can damage most equipment, including combined heat and power engines [95]. Most manufacturers recommend that the H_2S concentration in gas heaters and stationary engines does not exceed 1000 ppm [96]. For this reason, monitoring the occurrence of H_2S and its concentration becomes important. Peu et al. [95] established a model that links H_2S production in biogas with the C/S ratio of the feedstock, which allows a prediction of the hydrogen sulfide concentration by statistical methods. When dealing with seaweeds as a substrate of AD, the problem of high S content should also be addressed. It is reported that the sulfur content could represent 2.9% of harvested seaweed dry mass [95]. This may vary according to species, and could be linked either with the natural presence of large quantity of sulphated polysaccharides,

or by the presence of residual water rich in sulphate, which highlight the importance to rinse before AD process [97]. Ghadiryanfar et al. [98] have reviewed the sulfur content of various seaweeds species. The sulfur content of seaweed is higher than that of land-based biomasses: with *Ulva* 3.1% (dry basis), *Macrocystis* and *Laminaria* 1%; for comparison, the levels in oat straw and miscanthus are extremely low (<0.02%).

4.2.3. Effect of the ISR

Defined as the ratio of VS from the inoculum (partially due to actively degrading biomass) to VS from the substrate, ISR is considered a key parameter in BMP tests.

ISR is strongly related to the limiting biological phenomena such as inhibition, acidification of the medium. It can play a role in the composition, concentration of VFAs produced and will have an impact on the metabolic pathways involved [99]. For a classical batch fermentation, an ISR of between 2 and 4 is usually applied [100]. In case the substrate is easily degradable, this ratio should be higher to avoid VFAs accumulation that is inhibitory to AD from a few grams per liter. To ensure optimal conditions, it is often recommended to test several ISRs. Only if at least two ISRs lead to the same BMP can it be assumed that no inhibition has occurred [100].

The choice of ISR value is well documented in the literature and can vary depending on the substrate: Chynoweth et al. [101] noticed an increase in constant rate when ISR increased from 0.92 to 1.88, for BMP tests on cellulose; however, no difference was observed in the methane yield. For microalgae biomass, Zeng et al. [102] noted a decrease in BMP (from 140.5 to 94.4 Nm^3 CH_4/kg VS) when ISR was reduced from 2.0 to 0.5, with *Microcystis* spp. as substrate. Regarding seaweeds, Costa et al. [103] worked with relatively low ISR values, on the green alga *Ulva* sp. ($0.17 < ISR < 0.85$), a decrease in BMP was observed when ISR varied from 0.35 to 0.17 (from 196 ± 9 to 167 ± 13 Nm^3 CH_4/kg VS); on the red alga *Gracilaria* sp. ($0.14 < ISR < 0.7$), the BMP value first increases and then decreases as a function of ISR. With *Sargassum* sp. ($0.01 < ISR < 0.04$), an increase was noticed on BMP (from 281 ± 7 to 541 ± 10 Nm^3 CH_4/kg VS) [104].

4.2.4. Variation of Methane Yield with Different Species and Components

Although the use of algal biomass as a renewable energy source seems potentially promising, it should be noted that the final methane yield varies from species to species and could be largely influenced by its biochemical composition. As mentioned above, the brown alga *Macrocystis* and red alga *Gracilaria* showed high BMP values. This is not the case for the brown alga *Sargassum* which represents a relatively low BMP value of 0.13–0.26 m^3/kg VS [21,72]. With respect to the effects of algal composition, to our knowledge, only one study has examined the different methane yield values obtained from *Sargassum* species [21]. The study was conducted in a continuous system with *S. fluitans* and *S. pteroleuron* as substrates, the BMP value of their tissue range from 0.12 to 0.19 m^3/kg VS. For *S. fluitans*, the maximal BMP value is about 0.2 m^3/kg VS of stipes compared with 0.15 m^3/kg VS of blades, whereas the test with *S. pteroleuron* stipes reached a minimal BMP value of 0.12 m^3/kg. The authors mentioned that the low BMP values obtained could be related to the insoluble fiber component, which is not available for methane bioconversion. It should be noted that seaweeds naturally have a difference in composition between tissue types, and that the sampling methods used may add another variability. All of these can impact the methane yield and should be rigorously considered prior to the fermentation process.

4.3. Biohydrogen

Hydrogen is a clean and renewable energy carrier with a high energy density (122 MJ/kg) [105]. Regarded as one of the promising fuels of the future, it represents a scientific, environmental, and ecological challenge. Its addition to methane allows for an increase in the thermal efficiency and a decrease in the polluting emissions during the combustion compared with the use of natural gas alone.

4.3.1. Biological Conversion Pathway

Three routes can lead to the production of biohydrogen: biophotolysis, photo fermentation and dark fermentation. Biophotolysis is the dissociation of water molecules to form hydrogen and oxygen in biological systems in the presence of light [106]. Photo fermentation, in turn, is a fermentation process which uses light energy and organic acids under nitrogen-deficient conditions for biohydrogen production [107]. Dark fermentation consists of an anaerobic microbial conversion of organic matter for biological hydrogen production [108].

For dark fermentation, two common pathways are involved in the production of biohydrogen from glucose as a degradation by-product: one produces acetate and the other produces butyrate, leading to a production rate of 4 mol H_2/mol glucose and 2 mol H_2/mol glucose, respectively [109,110]. Other types of pathways could be involved according to substrate/culture type and operational conditions.

The favorable environments for CH_4 and H_2 production are not the same; for H_2 it is rather acidic, whereas for CH_4 it is slightly basic. This induces a difference in the microorganisms involved. It is thus important to select the microflora if one wants to produce preferentially a gas rather than the other. The parameters to control are often: pH and temperature. In the case of hydrogen production, thermal pretreatment (10–30 min, T $\geq$ 60 °C) is usually applied to the digestate from AD units to enrich the spore-forming bacteria that mainly produce hydrogen (*Clostridia*), while eliminating the non-spore-forming microorganisms such as lactic bacteria and methanogenic archaea [111–114]. Regarding pH, a neutral or slightly acidic environment (5.5 to 7.3) is favorable for H_2 production. The optimal condition can vary depending on the substrate.

Theoretically, during the dark fermentation process, apart from the production of H_2, about two-thirds of the energy in the form of organic acids remains unexplored [115]. Towards a two-step biological process, the organic acids could be further converted to methane. The mixture of these two alternative fuels, called hythane, whose hydrogen concentration ranges from 10 to 25% (v/v), has been found to be capable to improve heat efficiency by facilitating the inflammation of methane [104]. There is growing research interest in this concept. Biohythane has been used in the automobile sector to replace methane, with several successful projects conducted in Montreal (Canada), California (USA), and Beijing (China) [116]. A major obstacle is the adaptation of the distribution system that is currently designed for methane. In addition, biohythane production requires a delicate balance between operational parameters such as pH, temperature, nutrients. Microbiome constructions should also be considered for process scale-up, as well as economic aspects. There are currently about 19,000 biogas plants in Europe [117]; this sector is promising but requires deeper investigations.

4.3.2. Thermochemical Conversion Pathway

Two major pathways are concerned: thermal gasification and supercritical water gasification (SCWG). Some studies suggest that they are more advantageous than biological ones mainly due to a faster conversion rate and higher carbon conversion efficiency [118,119]. Thermal gasification technology generally consists of four stages: drying (100–200 °C), pyrolysis (200–700 °C), combustion (700–1500 °C), and reduction (800–1000 °C) [120]. It allows an effective and efficient conversion of biomass to a uniform gaseous mixture called syngas, mainly comprising hydrogen (H_2, 30–40%), carbon monoxide (CO, 20–30%), methane (CH_4, 10–15%) and carbon dioxide (CO_2) [79]. This mixture can be further used for heat and power generation, H_2 production and liquid fuels synthesis [121]. The composition of the syngas depends on the nature of the biomass, the type of gasifier and other process parameters: such as steam to biomass mass ratios (S/B), gasification temperature, etc. [122]. However, when processing biomass with relatively high moisture content, a drying process is required, which reduces the overall efficiency of the whole process [118]. In this case, SCWG can be applied, with which one can directly handle wet biomass. The effect of different parameters on syngas production and system

efficiency should be considered: substrate concentration, reactor temperature, reforming options, for example. It is also crucial to better understand the mechanisms of char formation [123]. On the other hand, it is worth mentioning that Prestipino et al. [18] proposed an alternative solution for the treatment of wet residual biomass, and they achieved the highest hydrogen yield of 40.1 kg_{H2} per mass of dry biomass at S/B = 1.25, meaning that the system is able to cover the internal heat demands.

Studies on the gasification process of microalgal biomass are well documented [119,123–125]. Farobie et al. [126] investigated the potential of syngas and hydrochar production from macroalgae *U. lactuca*, they obtained hydrochar with the highest HHV value (22.93 MJ/kg) at 400 °C, comparable to low-ranked coals. With the maturity of the SCWG technique, gasification of macroalgae seems all the same promising. However, only a few works demonstrate the comparison of gasification between macroalgae et microalgae: in the study of Faraji et al. [127], *Chlorella vulgaris* shows the highest amount of H_2/CO for syngas production via gasification process, more appropriate than *Rhizoclonium*. We believe that this may be related to the difference in composition between the different species, especially the presence of more lipids and proteins in microalgae; more specific research is therefore needed.

4.4. Effects of Pretreatment Methods

Seaweeds are considered good candidates for AD and dark fermentation. However, the complex structures and the multiple types of polysaccharides present on the cell wall make this biomass difficult to access. A step of hydrolysis is therefore necessary to unlock the full potential of the methanogenesis. To improve the accessibility of the material and to facilitate hydrolysis, various pretreatment methods could be used. They generally have the following objectives: to weaken the recalcitrant part of the alga such as the crystalline structure and the polysaccharide matrix, to increase the contact surface, to reduce the crystallinity of the cellulose and the degree of polymerization of the complex structures and to break the bonds between the molecules [128].

In the literature, the most commonly used pretreatment methods are noted below: physical, chemical and biological pretreatment (Figure 7). The choice of methods is generally based on the algae species and the objectives sought (maximum production rate, yield, preference for gases, etc.). According to Barbot et al. [129], pretreatments can improve biomethane production with average values from 19 to 68%, sometimes even up to 140% [130]. This step represents 33% of the cost of equipment in the case of lignocellulosic biomass production and must therefore be carefully considered when assessing the feasibility and profitability of the process [128].

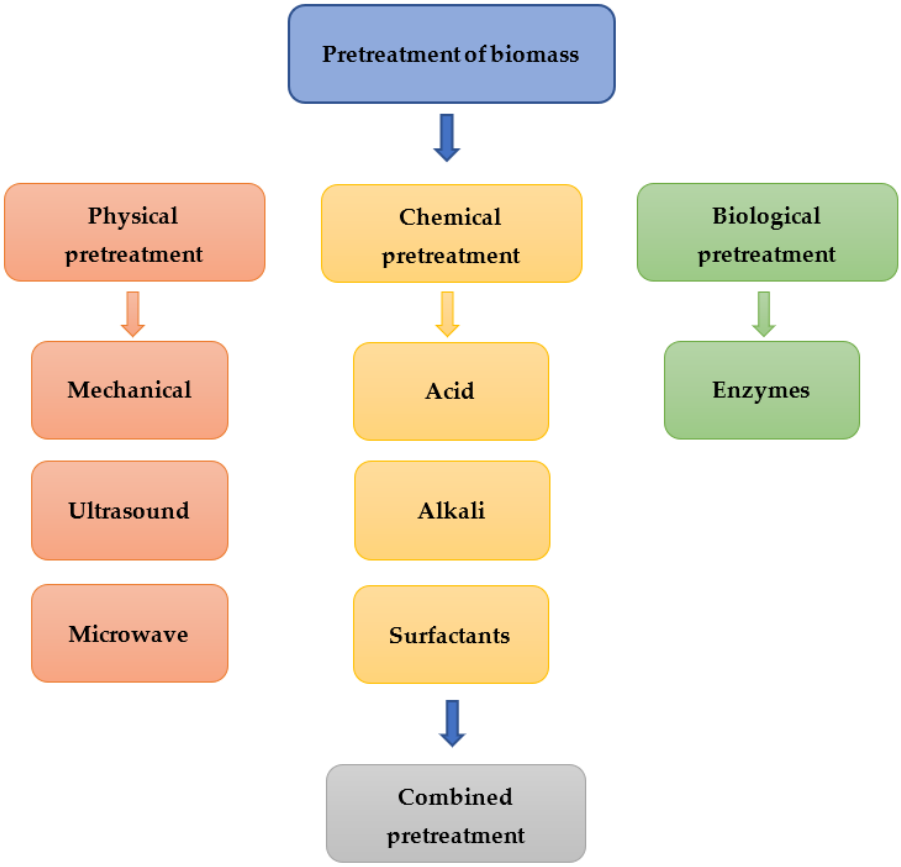

Figure 7. Different types of pretreatment methods.

4.4.1. Physical Pretreatment

Physical pretreatments aim at reducing particle size and crystallinity, increasing the contact surface, and thus the efficiency of possible downstream pretreatments [128]. It is considered as an essential step prior to chemical or biochemical pretreatment, for an improvement of the subsequent yields [131]. Three types are commonly considered, namely, mechanical treatment, microwave treatment and ultrasound treatment [132].

According to Gruduls et al. [89], the BMP of *F. lumbricalis* is increased approximately twofold with the practice of physical pretreatments, either by boiling or by microwave. However, a negative effect was noticed on green algae, with the same pretreatments performed. The authors attribute this to the presence of softer tissues, which may lead to significant evaporation of volatile solids. We argue that this observation remains at the laboratory scale, as in the industrial world, a closed pressurized environment normally prevents the loss of fermentable material.

Grinding represents an option to increase the degradation rate of the processes. The main interest of this pretreatment is to make the substrate much more easily degradable. The efficiency of this pretreatment depends strongly on the nature of the treated substrate. When the substrates have a high biodegradability, as is the case for carrots, potatoes, or meat (95% and 88%, respectively), the grinding effect is minor. In contrast, AD was improved by 10–20% in the case of sunflower seeds, hay and maple leaves as substrates [133]. As with seaweed, this process is often performed after washing and drying to increase the surface area to further enhance the hydrolysis. Briand et al. [134] obtained a gain in methane yield from ground *Ulva* samples compared with non-ground ones (0.177 m^3/kg VS versus 0.145 m^3/kg VS). Moreover, the powders are easier to handle and show less variability during fermentation tests.

4.4.2. Chemical Pretreatment

Chemical pretreatments require the addition of chemical agents such as acid, alkali or surfactants, and are often coupled with heat treatment. HCl, H_2SO_4, HNO_3, H_3PO_4 are common agents used in acid pretreatment, they can be performed either at low temperature with high concentration or at high temperature with diluted one [135]. Due to the toxicity and corrosion caused by concentrated acid, dilute acid becomes a more suitable option and is widely studied [135]. Sivagurunathan et al. [136] studied the effect of various acid pretreatment on the fermentation of red algae *G. amansii*. They revealed that only the H_2SO_4 pretreatment method had a significant effect on improving biohydrogen yield, resulting in a maximum hydrogen production of 0.052 m^3/kg dry biomass. Surfactants are often used in combination with other pretreatments. In the study of Kavitha et al. [137], the release of extracellular polymeric substance was stimulated by the addition of sodium dodecyl sulfate (SDS), which improved subsequent anaerobic biodegradability.

4.4.3. Biological Pretreatment

Biological pretreatment is gaining more and more attention for the disintegration of lignocellulosic resources. With low energy input and no chemical agent required, this eco-friendly process can be an alternative to traditional pretreatment methods that are sometimes conducted under harsh conditions. Through the synthesis of microbial extracellular enzymes (cellulase, hemicellulose, etc.), the microorganisms involved are able to break down complex structures [138]. Otherwise, they are used to treat algal biomass, by applying enzymolysis (e.g., glucoamylase from *Aspergillus niger*) before AD, Ding et al. [139] obtained a 23% increase (0.0083 m^3/kg VS) in biohydrogen production compared to untreated *L. digitata*. Passos et al. [140] studied the effect of enzymatic pretreatment of microalgal biomass on AD. They obtained an increased biomass solubilization of 126% and a methane yield of 15%, with the application of a mixture of enzyme (cellulase, glucohydrolase and xylanase).

4.4.4. Combined Pretreatment

Given the feasibility and cost-effectiveness of processes, it is often recommended, and sometimes necessary, to perform the pretreatments in a combined way.

Chikani-Cabrera et al. [141] evaluated the effect of different physical, chemical, and enzymatic pretreatments on the methane production from *Sargassum*. They obtained a maximum methane yield of 0.387 m^3 CH_4/kg VS with pretreatment of 2.5% hydrogen peroxide, followed by an enzymatic pretreatment, as well as the best biodegradability reaching 0.95%. Yin et al. [142] found in their study that high-temperature treatment with soda addition leads to better hydrogen yield (0.0175 m^3/kg TS_{added}), whereas acid-high temperature coupling leads to better energy conversion efficiency (35.4%).

4.5. By-Products Generation and Detoxification Techniques

The employment of pretreatment methods, especially thermal and thermo-chemical one [74,143], could lead to a generation of byproducts that are generally divided into three groups: furans, weak acids (acetate, formic acid, levulinic acid) and phenolic compounds [74]. Their formation depends in particular on the temperature and the duration of pretreatment [144]. Furfural and 5-(hydroxymethyl)furfural (5-HMF) are mostly formed at low pH, whereas phenolic compounds from lignin are preponderant at high pH [74,145–147]. Given the lack of lignin in algal biomass, the generation of furan derivatives is more likely, hydrolysates of some common carbohydrates could be responsible, such as cellulose, agar and starch [74,148]. They are thought to cause inhibition to the biomethane and biohydrogen production process, by damaging microbial cells and prolonging the lag phase [149,150]. Thus, in order to ensure that the fermentation process runs smoothly, it is sometimes necessary to carry out a detoxification step. To do this, we note in the literature the addition of chemical agents (such as $Ca(OH)_2$, CaO for example), bio-adsorbents (bacteria, yeast, fungi, etc.), or by the implementation of extraction processes [128]. An increase in the concentration of the inoculum or the sequential addition of

byproducts can be helpful as these compounds can be transformed into fewer inhibitory compounds or further degraded [151,152].

Dark fermentation is more sensitive to by-products than AD. Although the early stages of both processes are similar, AD is more complete without heat damage; thus, the microorganisms show better adaptability to environmental changes.

Von Sivers et al. [153] evaluated the economical aspect of bioethanol production from willow hydrolysate, the cost of detoxification was estimated at 22% of the total cost. In the future, more cost-effective and economically feasible methods should be proposed.

The main steps of the AD and dark fermentation process, as well as the generation of possible by-products are summarized below (Figure 8):

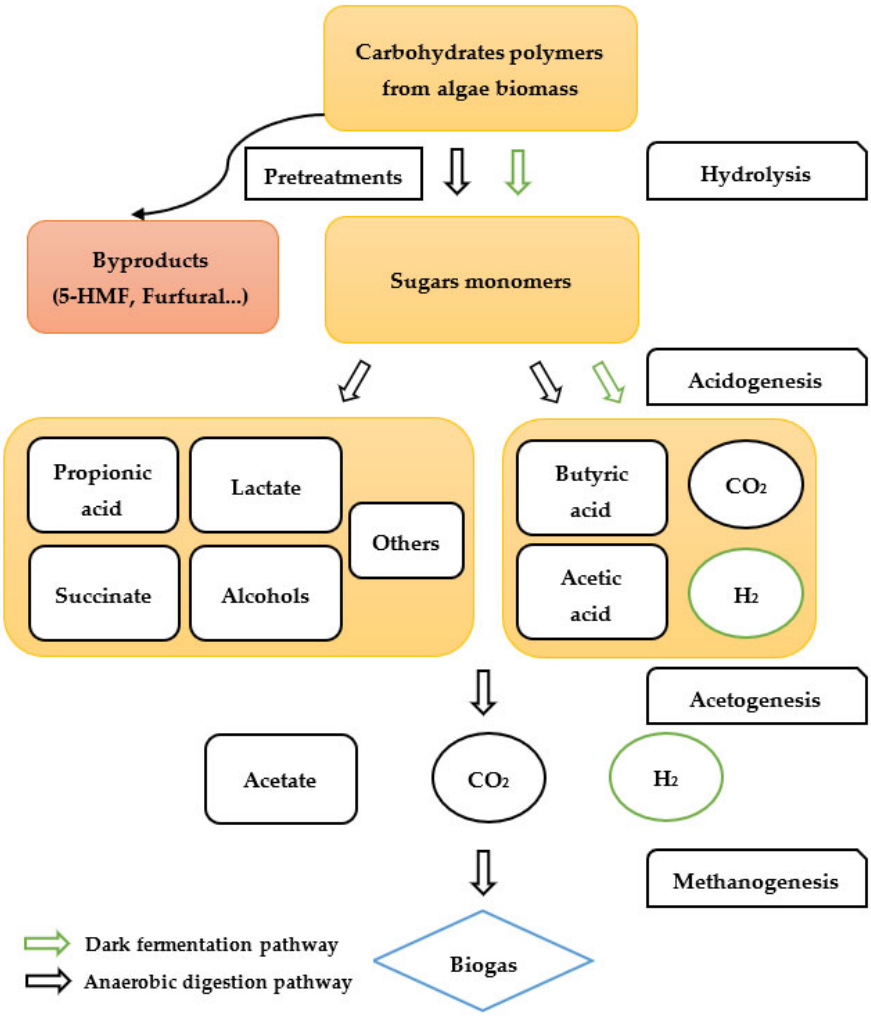

Figure 8. Scheme of carbohydrate polymers degradation through dark fermentation and AD bioprocesses (adapted from [47,86,154–156]).

4.5.1. Biomethane Potential and Experimental Conditions

The BMP is the amount of methane produced by an organic substrate during its biodegradation under anaerobic conditions. It is an important parameter to evaluate during the AD process. The BMP value can vary depending on the algae species, the pretreatment methods used and the fermentation modality (batch or continuous). Three parameters affecting the quality of biogas production were used to characterize the continuous fermentation: working temperature, hydraulic retention time (HRT) defined as the average time that liquid and soluble compounds remain in a reactor, organic loading rate (OLR) defined as the amount of organic waste introduced per unit volume of the

digester per day. Results from the literature are summarized in Table 2 ('B' refers to batch fermentation and 'C' refers to continuous fermentation).

Table 2. Examples of biomethane production and operational conditions with seaweeds as raw material.

Groups	Seaweeds	Pretreatment	Condition	Methane Yield	Ref.
Green algae	*Ulva* sp.	Ground and centrifuged	B	0.148 m^3/kg VS	[49]
		Non-washed	B	0.11 m^3/kg VS	[134]
		Washed	B	0.094 m^3/kg VS	
		Non-ground dried	B	0.145 m^3/kg VS	
		Ground dried	B	0.177 m^3/kg VS	
		Ground	C (HRT: 15 days OLR: 1.8 kg VS m^{-3} day^{-1} T: 35 °C)	0.203 m^3/kg VS	
		Ground	C (HRT: 20 days OLR: 1.7 kg VS m^{-3} day^{-1} T: 35 °C)	0.182 m^3/kg VS	
		Washed, dried, milled	B	0.191 m^3/kg VS	[157]
	Ulva lactuca	Macerated	B	0.271 m^3/kg VS	[158]
		Fresh	B	0.183 m^3/kg VS	[159]
		Washed and dried	B	0.25 m^3/kg VS	
		Washed, cut, and ensiling	B	0.256 m^3/kg VS	[160]
	Chaetomorpha linum	Frozen, washed, chopped	B	0.166 m^3/kg VS	[161]
Red algae	*Gracilaria* spp.	Frozen	C (HRT: 15 days OLR: 1.6 kg VS m^{-3} day^{-1} T: 35 °C)	0.28–0.4 m^3/kg VS	[21]
	Gracilaria gracilis	Non-pretreated	B	0.0818 m^3 biogas/kg TS	[162]
		Cut by a Hollander beater	B	0.1718 m^3 biogas/kg TS	
	Gracilaria vermiculophylla	Frozen, washed, chopped	B	0.132 m^3/kg VS	[161]
		Washed, maceration (cut, crushed by a mortar)	B	0.481 ± 0.009 m^3/kg VS	[163]

Table 2. *Cont.*

Groups	Seaweeds	Pretreatment	Condition	Methane Yield	Ref.
	Palmaria palmata	Raw alga (dried, chopped)	B	0.308 m^3/kg VS	[164]
		Dried, chopped then maceration (20 °C)	B	0.328 m^3/kg VS	
		Dried, chopped then thermal treatment (120 °C)	B	0.296 m^3/kg VS	
		Dried, chopped then thermal treatment (160 °C)	B	0.269 m^3/kg VS	
		Dried, chopped then thermal treatment (180 °C)	B	0.268 m^3/kg VS	
		Dried, chopped then thermal treatment (200 °C)	B	0.211 m^3/kg VS	
		Dried, chopped then thermal treatment (160 °C) + NaOH	B	0.282 m^3/kg VS	
		Dried, chopped then thermal treatment (160 °C) + HCl	B	0.268 m^3/kg VS	
Brown algae	*Sargassum*	Frozen	C (HRT: 15 days OLR: 1.6 kg VS m^{-3} day^{-1} T: 35 °C)	0.12–0.19 m^3/kg VS	[21]
	S. muticum	Washed	B	0.177 m^3/kg VS	[165]
		Non-washed	B	0.225 m^3/kg VS	
		Dried	B	0.13 m^3/kg VS	[72]
		Dried, ground/chopped	B	0.166–0.208 m^3/kg VS	[166]
	S. natans VIII	Frozen and freeze-dried	B	0.145 m^3/kg VS	[167]
	S. natans I	Frozen and freeze-dried	B	0.066 m^3/kg VS	
	S. fluitans	Frozen and freeze-dried	B	0.113 m^3/kg VS	
	A. nodosum	Chopped and frozen	B	0.28 m^3 biogas/kg VS	[168]
		Chopped and frozen	C (HRT: 24 days OLR: 1.75 kg VS m^{-3} day^{-1} T: 35 °C)	0.11 m^3/kg VS	
		Cut, 15 min mechanical pretreatment	B	0.169 m^3/kg VS	[169]
		Washed, cut, and ensiling	B	0.237 m^3/kg VS	[160]

Table 2. *Cont.*

Groups	Seaweeds	Pretreatment	Condition	Methane Yield	Ref.
	Saccorhiza polyschides	Washed, dried, milled	B	0.255 m^3/kg VS	[157]
	Nizimuddinia zanardini	Washed, cut, and ensiling	B	0.277 m^3/kg VS	[160]
		Washed, dried	B	0.117 m^3/kg VS	[170]
		Washed, dried, autoclaved (30 min, 121 °C)	B	0.143 m^3/kg VS	
	Fucus vesiculosus	Washed, dried, thermochemical pretreatment (200 mol/m^3 HCl, 24 h, 80 °C)	B	0.113 m^3/kg VS	[130]
	Laminaria digitata	Oven drying (24 h, 104 °C) then pulverized with a blender	B	0.141 m^3/kg VS	[171]
		Washed with hot water then macerated	B	0.282 m^3/kg VS	[172]
		Washed, cut, and ensiling	B	0.354 m^3/kg VS	[160]
	Saccharina latissima	Frozen, defrosted, cut, ground	B	0.223 m^3/kg VS	[173]
		Frozen, defrosted, cut, ground then steam explosion (10 min, 130 °C)	B	0.268 m^3/kg VS	
		Washed, cut, and ensiling	B	0.33 m^3/kg VS	[160]

B: batch fermentation, C: continuous fermentation, HRT: hydraulic retention time, OLR: organic loading rate.

Previous studies suggest a difference in methane yields between seaweed species mainly due to their biochemical composition. Rinzema et al. [174] studied sodium inhibition of acetoclastic methanogens in granular sludge. They found that at neutral pH, a sodium concentration of 10 kg/m^3 Na^+ caused a 50% inhibition relative to the maximum specific acetoclastic methanogen activity of granular sludge, and that this inhibition can be more pronounced at pH levels near 8. Therefore, seaweeds samples are usually washed prior to AD. Mechanical pretreatments are almost employed in all studies as they are considered useful for improving methane potential. By simple actions such as cutting, grinding, chopping, the size of the substrate is reduced and the exchange area with microorganisms is increased, which facilitates the release of fermentable substrate [19]. Some authors have also proposed oven drying that would decrease the water activity and facilitate *a posteriori* the transport. The scale-up of this process is limited by its cost and therefore remains at the laboratory scale. Solar drying could be a more promising and sustainable preservation method that deserves attention. On the other hand, freezing allows the preservation of products over a long period of time because it slows down the development of microorganisms. However, it may alter the structure and composition. It is therefore necessary to find a compromise. In terms of BMP values, the pretreatment methods tested can lead to different values ranging from 0.1–0.5 m^3/kg VS. Thermal treatments generally improve the methane yield, but the harshness of process should be considered, as refractory compounds or aromatic compounds can sometimes be formed under harsh conditions (extremely high temperature), which leads to a decrease in BMP [164,175,176]. Table 3 below shows only the pretreatment leading to the best BMP value (data based on Table 2).

Table 3. Improvement of BMP of seaweeds through the employed pretreatment methods.

Seaweeds	Pretreatment Methods	BMP	Ref.
Ulva sp.	Ground	+0.032 m^3/kg VS (+22%)	[134]
Ulva lactuca	Washed and dried	+0.067 m^3/kg VS (+37%)	[159]
Gracilaria gracilis	Hollander beater	+0.09 m^3/kg TS (+110%)	[162]
Nizimuddinia zanardini	Autoclaved	+0.026 m^3/kg VS (+22%)	[170]
Saccharina latissima	Steam explosion	+0.045 m^3/kg VS (+20%)	[173]

4.5.2. Biohydrogen Potential and Experimental Conditions

In the case of production of hydrogen, we talk about the biohydrogen potential (BHP). Similar to BMP, the value of BHP can be related to algal species and the substrate pretreatment methods [135]. However, this time we also focus on pretreatments for inoculum: temperature and duration of thermal pretreatment, as well as pH control (Table 4).

Table 4. Examples of biohydrogen production and operational conditions with seaweeds as a raw material.

Groups	Seaweeds	Substrate Pretreatment	Inoculum Pretreatment	Condition	pH	Hydrogen Yield	Ref.
Green algae	*Ulva reticulata*	Washed, dried, disperser	102 °C, 30 min	B	5.5 ± 0.1	0.045 m^3/kg COD	[177]
		Washed, dried, disperser, 21.6 mg/L tween 80	102 °C, 30 min	B		0.063 m^3/kg COD	
	Chaetomorpha antennina	Washed, microwave disintegration, 15 min	100 °C, 30 min	B	—	0.063 m^3/kg COD	[178]
Red algae	*Gelidium amansii*	Washed, ammonium dodecyl sulfate + microwave disintegration	100 °C, 30 min	B	7	0.0745 m^3/kg COD	[136]
		121 °C, 1% H_2SO_4, 30 min	90 °C, 30 min	B		0.0528 ± 0.0002 m^3/kg TS	
		121 °C, 1% HNO_3, 30 min		B		0.016 ± 0.0009 m^3/kg TS	
		121 °C, 1% HCl, 30 min		B		0.0224 ± 0.0004 m^3/kg TS	
		121 °C, 1% H_3PO_4, 30 min		B		0.014 ± 0.0004 m^3/kg TS	
		121 °C, water, 30 min		B		0.0272 ± 0.0003 m^3/kg TS	

Table 4. *Cont.*

Groups	Seaweeds	Substrate Pretreatment	Inoculum Pretreatment	Condition	pH	Hydrogen Yield	Ref.
		Washed, dried, ground, sieved, then 150 °C, 2% H_2SO_4, 15 min	90 °C, 10 min	B	>5.5	0.518 m^3 kg^{-1} VS day^{-1}	[179]
		Washed, milled, then 164 °C, 12.7% S/L, 0.5% H_2SO_4 *	90 °C, 20 min	B	>5.3	0.037 m^3/kg TS	[148]
Brown algae	*Laminaria japonica*	Non-pretreated	90 °C, 20 min	B	5.5	0.0714 m^3/kg TS	[180]
		Washed, dried and ground	90 °C, 20 min	C (HRT: 6 days OLR: 3.4 kg COD m^{-3} day^{-1} T: 35 °C)		0.0613 ± 0.002 m^3/kg TS	[181]
		Washed, dried with a ball mill at 120 °C for 30 min	65 °C, 20 min	B	7.5	0.028 m^3/kg TS	[182]
		Washed, dried and ground, 93 °C, 4.8% HCl, 23 min *	90 °C, 20 min	B	5.5	0.1596 m^3/kg TS	[183]
		Washed, dried and ground, 170 °C, 20 min	90 °C, 20 min	B	5.5	0.1096 m^3/kg COD_{added}	[184]
		Washed, dried and ground, 11.7 V/cm, 30 min * C_{sub} 2%, Washed, oven dried, 105 °C, 4 h, then autoclaved 121 °C, 30 min	2 V/cm, 10 min	B	5.5	0.1027 m^3/kg TS	[185]
			80 °C, 20 min	B	6	0.08345 ± 0.00696 m^3/kg TS	[186]
		Washed, oven dried, 105 °C, 4 h, ball milling		B	7.0 ± 0.1	0.01 ± 0.00121 m^3/kg TS	
		Washed, oven dried, 105 °C, 4 h, then autoclaved 121°C, 30 min		B		0.06668 ± 0.00568 m^3/kg TS	
		Washed, oven dried, 105 °C, 4 h, then ultrasonic cell breaker, 20 kHz		B		0.02356 ± 0.00456 m^3/kg TS	
		Washed, oven dried, 105 °C, 4 h, then HCl 1000 mol/m^3, 30 min		B		0.04365 ± 0.00687 m^3/kg TS	
		Washed, oven dried, 105 °C, 4 h, then NaOH 1000 mol/m^3, 30 min		B		0.015 ± 0.00389 m^3/kg TS	

Table 4. *Cont.*

Groups	Seaweeds	Substrate Pretreatment	Inoculum Pretreatment	Condition	pH	Hydrogen Yield	Ref.
	Sargassum sp.	Dried, milled, autoclaved 121 °C, 1 bar, 15 min	Precultured *C. saccharolyticus*	B	7.0–7.2	0.0913 ± 0.0033 m^3/kg VS	[104]
	Padina tetrastromatica	Washed, dried, cut, milled then 1% HCl, 100 °C, 2 h	60 °C, 10 min	B	6 ± 0.5	0.76 m^3/kg VS	[112]
		Washed, dried, cut, milled then 1% HNO_3, 100 °C, 2 h		B		0.68 m^3/kg VS	
		Washed, dried, cut, milled then 1% H_2SO_4, 100 °C, 2 h		B		1.56 m^3/kg VS	
		Washed, dried, cut, milled then 2% KOH, 100 °C, 2 h		B		0.84 m^3/kg VS	
		Washed, dried, cut, milled then 2% NaOH, 100 °C, 2 h		B		1.1 m^3/kg VS	
	Laminaria digitata	Washed, cut	100 °C, 30 min	B	6.00 ± 0.05	0.097 m^3/kg VS	[113]
	Saccharina japonica	Washed, dried, 80 °C, 24 h, milled, sifted, then 2% NaOH, 121 °C, 30 min	5 kGy ionizing irradiation	B	-	0.0175 m^3/kg TS	[142]

B: batch fermentation, C: continuous fermentation, C_{sub}: substrate concentration, *: optimal condition found by response surface methodology, S/L: Solid/Liquid.

The BHP values range from 0.01 to 1.6 m^3/kg VS; this large variation may be due to the simplified heat-treated inoculum and thus presents more uncertainty in H_2 yield. Like for the BMP test, thermal and thermo-chemical pretreatment would be required to overcome the natural physicochemical barriers of the algae biomass and enhance the solubilization of carbohydrate polymers into soluble sugars (i.e., glucose, xylose, arabinose, and galactose) [74]. However, the type and concentration of chemical agents may lead to different extraction efficiencies and, therefore, different BHP values. Otherwise, a 10–30 min thermal treatment of inoculum at a temperature above 80 °C is usually applied to eradicate the non-spore-forming microorganisms when allowing some acidogenic H_2-producing bacteria such as *Clostridium* sp. to sporulate. Table 5 below shows only the pretreatment leading to the best BHP value (data based on Table 4).

Table 5. Improvement of BHP of seaweeds through the employed pretreatment methods.

Seaweeds	Pretreatment Methods	BHP	Ref.
Ulva reticulata	Tween 80	+0.018 m^3/kg COD (+40%)	[177]
Chaetomorpha antennina	ALS	+0.0115 m^3/kg COD (+18%)	[178]
Gelidium amansii	1% H_2SO_4, 121 °C, 30 min	+0.0256 m^3/kg TS (+94%)	[136]
Laminaria japonica	Autoclaved	+0.07345 m^3/kg dry sample (+735%)	[186]
Padina tetrastromatica	1% H_2SO_4, 100 °C, 2 h	+1.56 m^3/kg VS (-)	[112]

4.6. Remarks on BMP and BHP Evaluation/Assays

In this section, we would like to point out some of the problems encountered in existing research, together with the limitations of some studies and potential research to explore:

1. The majority of previous research has applied mechanical pretreatment to reduce the size of algal biomass, whether by chopping, cutting, grounding, milling, or even Hollander beating. Although the size of samples after pretreatments has been specified, only a few studies demonstrate the preservation methods used before pretreatment. It is clear that a fresh sample does not result in the same loss of VS during the pretreatment process as a frozen sample. The question then becomes how best to define this loss of fermentable substrate and how to compensate in case of a considerable loss. Moreover, the various mechanical pretreatment methods could result in a loss of water content and therefore the VS value is biased, which can potentially impact the gas yield results. We highly recommend that these points be considered and worth mentioning.
2. Most studies mentioned the pretreatment methods used for inoculum without its characterization (TS, VS, even pH, alkalinity, etc.). However, we considered that the efficiency of pre-treatment strongly depends on the initial property of the inoculum. The choice of temperature and duration may differ between treated inoculums. Moreover, a detailed description of the seed inoculum would facilitate the comparison of different studies performed under various conditions.
3. When exploring the BMP and BHP of the substrate, only a few studies within the literature have demonstrated the temperature and pressure conditions of the gas produced. In some circumstances, this may be problematic, for example: when comparing tests conducted under mesophilic and thermophilic conditions, the results would be unusable without conversion to normal condition (298 K, 101,325 Pa).
4. In the BMP test, methane yields were calculated by dividing the corrected methane volume (standard pressure and temperature) by the weight of sample (VS) added to each bottle. In this case, to minimize the effect of endogenous gas production (gas produced by the inoculum on total gas production), an important point is to increase the amount of substrate. However, some studies deal with a low amount of substrate, which may decrease the reliability of the test [104,112].
5. When choosing pretreatment methods, most studies aimed to achieve maximum methane/hydrogen yield. However, the economic aspect is hardly mentioned: the balance between energy input and output, the profitability of the process and the feasibility of industrialization. These issues remain to be addressed in the future.

5. Study Example: Potential Energy Estimation of *Sargassum* in the French West Indies (Guadeloupe and Martinique)

The *Sargassum* genera are distributed in tropical and subtropical oceans, they play an essential role in maintaining the ecological balance, providing food, protecting invertebrates from predation, they also serve as nurseries for fish [82], approximately 4 10^6–10^7 tons of biomass could be found annually in the Sargasso Sea [187]. It is a perennial genus of about ten centimeters to several meters long (up to 8 m for *S. muticum*), fixed by a discoid-conical

holdfast. Stipes of 1–20 cm long arise from this basal disc are stem-like with ramifications that are variable according to the species [188], and they are covered with small visible thorns (<1 mm) [189]. Air bladders (vesicles) are normally present in a swollen and berry-like form. They usually grow on rocks, boulders and hard substrata [54]. However, two typical stranded species have an entirely pelagic lifecycle; they are unattached and only in drift which reproduce asexually by fragmentation of the thallus [190].

Since 2011, the coastlines of Guadeloupe and Martinique have experienced regular inundations of seaweed; two species are identified as predominant: *S. fluitans* and *S. natans*. The cause remains to be elucidated, it is mainly attributed to the modification of the marine currents and the consequent rejection of nutrients by the rivers of America and Africa. These invasive algae are considered to pose a threat to the ecosystem and local economic productivity, especially in areas where tourism remains the pillar sector. An amount of EUR 8.5 M is allocated by financial partners to counter the threat of *Sargassum* seaweed. Although the potential of *Sargassum* has been proved by several studies, current commercial exploitation seems limited [167]. In Guadeloupe and Martinique, this biomass is mainly valorized through composting and the production of biomaterials.

Since fermentation processes have fewer restrictions for substrates, unlike drying, pyrolysis, or combustion, generally no costly process is required. We would like to propose a scheme, in order to estimate the theoretical energy through the existing hydrogen and AD processes. This is a rough estimate considering the achievable annual harvest, without consideration of the energy requirement.

In 2018, 116,000 m^3 of *Sargassum* seaweed were collected on the Guadeloupean coast and 41,000 t were collected on the Martinique coast [191,192]. Considering an average density of 250 kg/m^3 of wet biomass [193], a dry weight rate of 30% [193,194], 21,000 t of dry biomass can be obtained. A VS/TS ratio of 0.53 has been applied based on samples 'mixed *Sargassum*' collected from Shark Bay [167]. To our knowledge, only one study has focused on the brown alga *Sargassum* with a two-stage biohythane production [104]. With the process described in this study, 1,012,830 Nm^3 H_2 and 6,021,330 Nm^3 CH_4 could be produced, the total energy produced is estimated at 66 GWh/year (Figure 9).

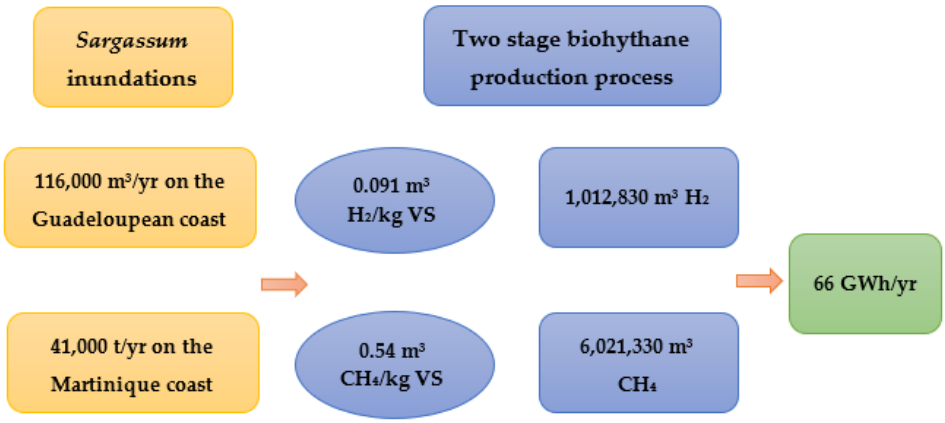

Figure 9. Potential energy generation through a two-stage biohythane production (adapted from [102]).

6. Challenges, Constraints, Future Scope

Seaweed biomass has a good potential as a feedstock for energy production, more specifically biogas and biohydrogen production. Their high availability, easy fermentable composition, and good degradation potential make them a promising and sustainable candidate to alternate fossil fuels as an advantageous energy resource.

AD is a cost-effective and environmentally friendly process suitable for energy production from biomass with high-water content. The average conversion yield (0.2~0.3 Nm^3/kg) reported for seaweed was found to be satisfactory compared to other terrestrial biomasses. The presence of highly recalcitrant hydrocolloids, as well as inhibitory phenolic compounds,

may somehow limit the biogas yield, which remains a major concern for further developments. As for biohydrogen production, variable BHP values have been obtained with studies based mainly on a laboratory scale, further investigations regarding optimization of the operating conditions would be necessary for stable industrial H_2 production.

Moreover, the possible presence of heavy metals, marine biotoxins, together with the generation of by-products (i.e., furfural, 5-HMF) during fermentation process, may hinder the further exploitation of seaweeds in several industrial sectors. Otherwise, some technological barriers still exist, such as techniques permitting a reliable prediction and localization of seaweed stranding, optimization of the process efficiency, cultivation of macro-algae on a larger scale and at a lower cost. A better comprehension of seasonal variations in chemical composition would also be necessary for further exploitation. Efforts should be made to propose more economical and sustainable preservation methods to improve coastal management.

A SWOT analysis was conducted to identify strengths, weaknesses, opportunities, and threats related to biogas and biohydrogen production from seaweed biomass (Table 6).

Table 6. SWOT analysis of biogas and biohydrogen production from seaweed biomass.

	Helpful	**Harmful**
Internal	Strengths	Weaknesses
	• High availability from cultivation and beach-cast biomass • Easy fermentable composition mainly composed of carbohydrates • Relatively high BMP yield (average 0.2~0.3 Nm^3/kg) comparable to terrestrial biomasses	• Chemical composition diversity • Presence of sand, epiphytes which may damage equipment and pumps • Presence of hydrocolloids and phenolic compounds • Presence of sulfur and heavy metals (cadmium, lead, mercury, arsenic, etc.)
External	Opportunities	Threats
	• Successful trials of methane production from *Sargassum* by Mexican society 'Nopalimex' • Financial government supports available in the battle against beach-cast seaweed (Japan for the Eastern Caribbean, France for the French West Indies, etc.) • Existing international collaboration between industrial actors and academic partners (SAVE-C, Sargassum joint call, etc.)	• Logistical constraints • Significant gaps in regulations concerning hazards in seaweed. • Limited data available for large-scale energy recovery from stranded biomass • Adequate pretreatments required to enhance the BMP of *Sargassum* whose practical yield is considerably below the theoretical maximum

7. Conclusions

This review has provided insight into the production of biofuels based on seaweeds, with a scope on seaweed composition, its biomethane and biohydrogen potential under different pretreatment methods. From the origin of the biomass, its morphology and initial composition, the pretreatment techniques applied during the process, to its final bioconversion, we attempt to establish a cause-effect relationship. The SWOT analysis helps to assess the feasibility of seaweed bioconversion and to identify possible multi-disciplinary partnerships between stakeholders. The main outcomes are as follows:

(1) Seaweed composition may vary according to the species studied, the geographical region, the harvesting season, biotic/abiotic parameters, the pretreatment methods, the processing of biomass (storage, drying), and analytical methods. Its characterization prior to the bioconversion process is essential.

(2) Attention should be devoted to the presence of heavy metals, marine biotoxins and by-products (i.e., furfural, 5-HMF) during the fermentation process; they can be an obstacle to the further exploitation and valorization of seaweeds and should therefore be carefully considered.

(3) AD and dark fermentation are promising processes suitable for energy production frommacroalgal biomass, with a relatively high yield of BMP (average 0.2~0.3 Nm^3/kg) obtained in a manner comparable to terrestrial biomasses. The aim of dark fermentation will be to obtain a stable hydrogen production by adjusting the operating conditions.
(4) Both gasification and anaerobic digestion considered promising methods, the choice of one process over the other should be based on energy balance and economic competitiveness.
(5) *Sargassum* invasions pose a threat for coastal communities, which at the same time represent an opportunity for energy production, estimation brings a total of 66 GWh of energy per year in the French West Indies.

In the future, more research is needed to raise scientific and technical challenges related to the energetic valorization of seaweeds, furthermore, to evaluate the feasibility of large-scale energy production. From a bio-economic point of view, the different means of energy valorization can be integrated into an efficient biorefinery approach which allows the utilization of macroalgal biomass to the fullest extent.

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Abbreviations

AD	Anaerobic digestion
ALS	Ammonium lauryl sulfate
BHP	Biohydrogen potential
BMP	Biomethane potential
COD	Chemical oxygen demand
EU	European Union
LHV	Lower heating value
OLR	Organic loading rate
TS	Total solids
HRT	Hydraulic retention time
VFA	Volatile fatty acids
VS	Volatile solids
5-HMF	5-(Hydroxymethyl)furfural
SCWG	Supercritical water gasification
SDS	Sodium dodecyl sulfate
SRMs	Sulfate-reducing microorganisms

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