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REVIEW

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A review of the current potential of European brown seaweed for the production of biofuels

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Abstract

Background In addition to the other uses for macroalgae, since the 1970s, there has been interest in using macroalgae as a source of biofuels, due to the high rates of productivity and intrinsic advantages over other biofuel crops such as not requiring land use or significant freshwater input. A wide range of conversion processes exist but anaerobic digestion was one of the first demonstrated and is still a widely proposed conversion pathway. To be economically viable and scalable within Europe, the industry will need to be based on a small number of fast growing, high-yielding European macroalgae species. There is a wide body of scientific work on the conversion of seaweeds to biofuel via anaerobic digestion.

Main text These studies demonstrate that the efficiency of this conversion pathway is highly variable between species, processing techniques, composition and digestor conditions. In this paper, we review this body of work specifically linking it to candidate species for European macroalgae bio-energy cultivation with the aim to promote the future development of the European macroalgal cultivation sector and allow for a better alignment with the requirements for biofuel production from macroalgae.

Conclusions Overall, anaerobic digestion of seaweed offers opportunities for large-scale energy production which avoids some of the issues that have faced previous generations of biofuels, but there are a number of key challenges to overcome to ensure wider adoption and economic viability. (1) Optimising the biomass production to ensure an economic and uniform feedstock with the composition optimised to increase desirable characteristics such as sugar content and the carbon and nitrogen ratio and to reduce inhibitory factors such as halogenated secondary metabolites, sulphur and heavy metals. (2) Improving conversion rates through co-digestion, pre-treatments and tailored microbial communities, using scalable and economically feasible technology. (3) Developing tailored microbial communities capable of utilising the diverse polysaccharides in seaweed feedstock and being tolerant of the saline conditions associated with them. Addressing these issues will deliver significant benefits towards the development of a bio-energy industry based on the anaerobic digestion of cultured seaweeds.

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Background

Worldwide, there are more than two hundred seaweed species with commercial value; however, only about ten are intensively cultivated [1]. In 2018, farmed seaweed represented 97.1% by volume of the total of 32.4 million tonnes of wild collected and cultivated aquatic algae combined. According to data from the Food and Agriculture Organisation [2], the world production of marine

macroalgae has more than tripled, up from 10.6 million tonnes in 2000 to 32.4 million tonnes in 2018. Seaweed farming is practised in a relatively small number of countries, dominated by countries in East and Southeast Asia. European macroalgae farming is currently at the nascent stage but has the potential to grow to 8 million tons per year by 2030 with positive environmental and socio-economic benefits [3]. While food use currently drives demand, macroalgae has received significant interest as a biomass feedstock. The use of macroalgae to produce biofuels is widely recognised as having advantages over terrestrial biomass [4]. There has been a surge in research interest in the use of macroalgae as an alternative feedstock to food crop-based starch and lignocellulosic biomass. This is mainly due to fast growth rates of macroalgae, high potential biomass yields, high carbohydrate content and nutrient requirements that can be fulfilled by wastewater or seawater [5]. Guo and McKinley [6] stated that cultivated yields of *Saccharina japonica* were 6.5 times more productive than sugarcane based on a wet tonnes per hectare per year basis (t ha^{-1} a^{-1} wet weight) but this did not compensate for difference in dry matter content between the feedstocks. For European kelp cultivation, a more realistic productivity of approximately 120 tonnes wet weight per hectare can be expected [7] with a dry solids content of 10%, which is roughly equivalent to European terrestrial crops on a per area basis [8]. Biomass yields of seaweed species can vary depending on species, seasonal and geographical variation, and growing conditions [9], with yields per unit area often greater than that of terrestrial plants [10]. The economics of biofuel production requires reliability of quality and quantity of feedstock that makes aquaculture production a preferred choice to build a scalable industry compared to wild harvest of seaweeds. However, there are only a limited number of species which are likely to be economically viable as a feedstock for biofuel production due to their high productivity, fast growth and high polysaccharide content [11]. As such, this review will focus on candidates for European aquaculture production, primarily brown phaeophyte macroalgae, commonly known as kelp, in the context of the wider European macroalgae landscape.

The need to develop sustainable and economically viable alternatives to traditional fossil-based fuels is now greater than ever due to the increasing threat of global warming, political pressure to reduce greenhouse gas emissions, energy security, concerns over fossil fuel depletion and volatile oil prices [12, 13]. Biofuel technologies have developed extensively over the last 50 years, mainly due to abundant feedstock availability and established biofuels conversion technologies [14]. Initial advances were led by sugarcane ethanol in Brazil in the 1970s, and more recently expanded to ethanol and methane production in the US and Europe via starch conversion and anaerobic digestion. The World Biogas Association states that anaerobic digestion has the potential to reduce global greenhouse gas emissions by 10–13% [15].

Biofuels and biomass derived energy can be divided into several generations, depending on the biomass feedstocks and the processing technology.

- First generation (1G) biofuels are associated with the conversion of food crops like wheat, corn, maize, rice, sugarcane, rapeseed among others, as energy sources. These biomasses have high sugar, starch or oil content [16].
- Second generation (2G) biofuels are derived from non-food wastes and lignocellulosic biomass that are generated as agriculture and forestry by-products. The main constraint with second generation feedstocks resides in the complex internal structures due to the presence of lignin that may hinder cellulose and hemicelluloses accessibility [17].
- Third generation (3G) biofuels feedstock derived from micro- and macroalgae are emerging as a renewable fuel source due to fast growth rates, potential for high biomass yields, low lignin content, high carbohydrate content, no competition for agriculture land and higher rate of CO_2 fixation than land crops [4, 18, 19].
- Fourth generation (4G) feedstock employs genetically modified algal biomass to obtain better hydrogen to carbon yields. They are expected to be carbon negative both at the level of the raw material and process technology [20].

Third generation biofuels from macroalgae are receiving increasing attention due to high biomass production per unit area facilitating process intensification [21], and the additional benefits of not competing with agricultural crops for land or freshwater and with high polysaccharide content [11]. Macroalgal forests are estimated to naturally cover 6.1–7.2 million km^2 [22] across the globe, as shown in Fig. 1, out of a potential 48 million km^2 suitable for macroalgae [23] allowing for the development of large scale aquaculture with minimal competition with existing natural habitats. In seaweed aquaculture, growth is dependent on the presence of suitable physical and chemical conditions and the selection of cultivation sites with suitable characteristics is essential for the successful establishment. Growth conditions of a site directly impact biomass yield and the composition of the crop which in turn controls the conversion efficiency of biomass to bioenergy [11]. The cultivation of macroalgae

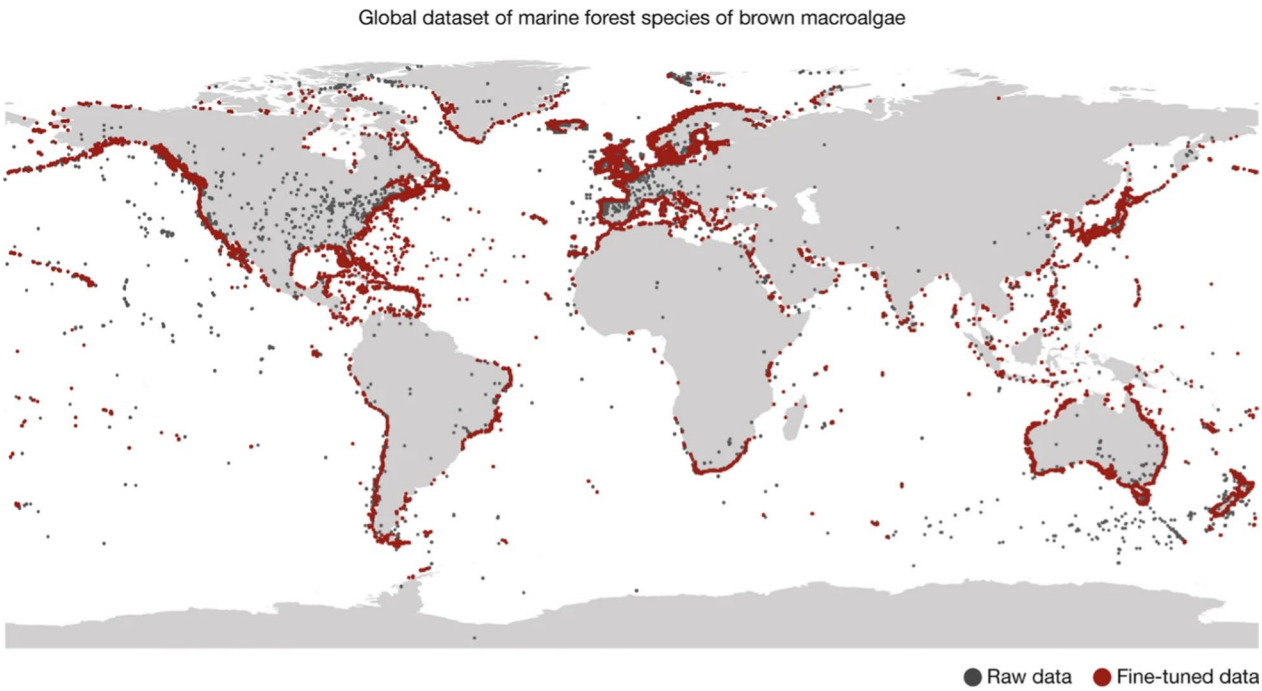

Fig. 1 Distribution of existing global marine macroalgae forests [27]

combined with fish and shellfish farming in the form of integrated multi-trophic aquaculture systems (IMTA), both onshore or offshore, can provide a more sustainable and significant source of seaweeds [24]. The integration of seaweed culture into fish farms reduces the environmental impact as fish farm nutrients can be sequestered by cultured seaweed which can subsequently be used as feedstock for biofuel production [17]. Detritus from seaweed farming results in additional carbon sequestration compared to the seaweed biomass through the incomplete degradation of detritus in marine sediments [25, 26].

Main text

Converting algal biomass to energy

Conversion methods of algae biomass include biotechnological conversion—anaerobic digestion (biogas/methane), fermentation (bioethanol) and photobiological production of hydrogen; chemical conversion—extraction and transesterification (biodiesel) [19]; thermochemical conversion—gasification (syngas for heat and power generation), liquefaction (bio-oil/liquid fuel), pyrolysis (production of liquid bio-oil, syngas and charcoal), aqueous catalysis (biofuel precursors) and direct combustion (heat energy) [1, 10, 18, 19, 28–30]. For thermochemical conversion of seaweed, the presence of alkaline earth-metals in macroalgae, such as Mg and Ca, which have shown to promote decarboxylation over dehydration during gasification and pyrolysis improving hydrogen yields compared with terrestrial crops [31, 32]. However, gasification and pyrolysis processes require dry feedstocks with moisture contents less than 10%. Philippsen et al. [33] found that the use of non-renewable heating sources for drying seaweeds to less than 20% moisture had an energy return on investment (EROI) of less than 1. Similarly, Milledge et al. [34] noted that the most difficult challenge with valorising macroalgae is the high-water content (>90%) requiring either drying or dewatering with high associated energy costs. Inherently wet valorisation methods for seaweed include fermentation, catalysis for platform chemicals, hydrothermal carbonisation for hydrochar and bio-oil as well as anaerobic digestion for biogas. The EROI for ethanol fermentation from seaweed has been estimated as low as 1.78 with an EROI of 3 considered sustainable for bioenergy production. Brown et al. [35] estimated that integrating hydrothermal carbonisation with anaerobic digestion would result in an EROI of over 11. Though, the high K, Cl and S contents of the hydrochar make combustion technically challenging.

Among the most energy efficient pathways to acquiring biofuel from macroalgae is employing inherently wet processes such as anaerobic digestion to produce biogas with an EROI exceeding 3 [36, 37]. The production of biogas through anaerobic digestion provides substantial benefits over other types of bioenergy production

by trading reduced process inputs for slower conversion rates. Anaerobic digestion is an established technology and is considered a good method of choice for biomass such as seaweed that has a high-water content [34, 38, 39]. Anaerobic digestion is the anaerobic decomposition of biomass materials by symbiotic microbial processes into biogas. Biogas is a renewable gaseous fuel generated by anaerobic digestion of organic wastes and typically consists of 50–70% methane (CH_4), 30–45% carbon dioxide (CO_2) with trace impurities of < 2% hydrogen (H_2), < 3.5% hydrogen sulphide (H_2S), nitrogen (N_2) and is saturated with water vapour according to the reactor temperature and pressure [40, 41].

The degradation of macroalgae during anaerobic digestion occurs in individual steps carried out by different microorganisms, as shown in Fig. 2 [42]. Degradation is carried out by a complex microbial consortium in a four-stage process comprising:

(1) Hydrolysis—anaerobic bacteria such as *Bacteriocides*, *Clostridia*, *Bifidobacteria*, *Streptococci* and *Enterobacteriaceae* hydrolyse complex organic macromolecules into smaller, simpler molecules, e.g., carbohydrates to sugars, proteins to amino acids and lipids to fatty acids.
(2) Acidogenesis—acidogenic bacteria convert simple organic molecules to intermediate volatile fatty acids such as propionic and butyric acid in addition to hydrogen, carbon dioxide and lesser amounts of other metabolites, including ammonia, lactate and ethanol.
(3) Acetogenesis—acetogenic bacteria convert volatile fatty acids primarily to acetic acid, along with additional hydrogen, ammonia, and carbon dioxide.
(4) Methanogenesis—methanogenic archaea form methane via the anaerobic respiration of acetic acid and via the conversion of hydrogen and carbon dioxide by hydrogenotrophic archaea.

Various operational and environmental parameters affect anaerobic digestion, which correlate with the kinetics of the different digestion stages and biogas production [43]. The optimised process performance depends upon the balanced activity of each set of microbial consortia. If one stage, such as acidogenesis works too fast causing acidification, the other stage, such as methanogenesis due to their preference for higher pH, becomes rate limiting and vice versa. Methanogenesis is a critical step in the entire anaerobic digestion process as it is the slowest biochemical reaction of the process and under

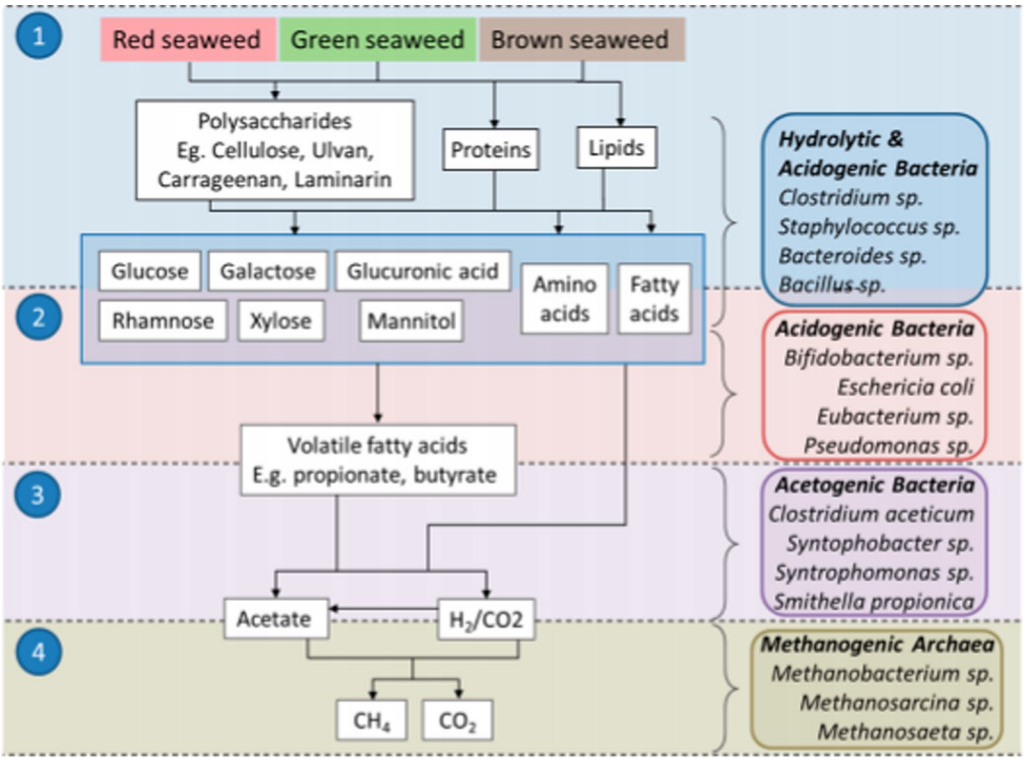

Fig. 2 Simplified schematic representation of the processes and microorganisms involved during anaerobic digestion of seaweed biomass. Schematic adapted by Nielsen et al. (2020) [41] from Maneein et al. (2018) [39]

most circumstances may be considered the rate limiting step of the overall reaction [40, 44, 45]. Slowly degrading substrates such as cellulose, fats, and proteins can make hydrolysis the rate limiting step instead. While deficiencies in macronutrients, such as nitrogen, phosphorus, potassium, calcium, magnesium and sodium, or trace elements, such as cobalt, iron, nickel, selenium and molybdenum, can limit microbial growth at any stage of the process they are thus are critical for the stable and optimum performance [4]. The optimisation of anaerobic digestion requires careful consideration of all constituent fractions to improve biogas yields as well as reduce operating and capital costs [46].

Macroalgae sources and composition

Macroalgae are classified into three main phyla; Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae), depending on their chemical composition and evolutionary history [47]. In general, Rhodophyta is a phylum containing the highest number of species (approximately 6000 known species), followed by Chlorophyta and Phaeophyta with about 4500 and 2000 identified species, respectively [48]. The relative quantities of proteins, lipids and carbohydrates present in seaweed affect methane production potential and vary by species, season and production location [49]. Relative to terrestrial biomass, macroalgae have higher water and ash fractions, and less lignin relative to structural carbohydrates. Lipid fraction is generally smaller than in many microalgae but can be as high as 20% dry weight, while protein concentration is highly variable but can be nearly 50% of the dry mass in some cases. The high carbohydrate fraction includes a large variety of easily soluble saccharides, such as laminarin, mannitol (brown seaweeds), starch and mannan (green seaweeds) and carrageenans (red algae). Volatile organic compounds also play an essential role in chemical communications in macroalgae [50]. This can include terpenoids, furans, sulphur compounds, alkanes, alkenes, alcohols, aldehydes, ketones and esters that are affected by environmental factors, such as temperature, light, nutrition conditions and abiotic stresses [51]. The volatile solid content as a percentage of dry solids in brown and red seaweeds ranges from 44.6% to 73.8% compared to green seaweeds from 57% to 82.1% [52]. Despite the huge variety of seaweed species, less than 20 are relevant in terms of commercial cultivation [53]. Across Europe, several species have been successfully farmed on a "trial scale", including red seaweeds such as *Palmaria palmata, Asparagopsis armata* and *Porphyra umbilicalis* as well as the green seaweed *Ulva sp.* [54]. However, by far, the most success has been achieved with brown seaweeds, primarily *Alaria esculenta*, *Laminaria digitata* and *Saccharina latissima*. Combined in part due to their suitability as a biofuel source and successful farming trials [11], kelps are the most advanced seaweed phyla for large-scale production in Europe [55]. In the broader context of the European seaweed sector, green and red seaweeds may play a large part of the bioenergy sector in the future, though brown seaweeds should remain the primary focus for bioenergy and for anaerobic digestion.

Carbohydrates

Marine algae contain large fractions of polysaccharides, notably structural carbohydrates in the cell wall, but also myco-polysaccharides and storage polysaccharides that have numerous commercial applications as stabilisers, thickeners, emulsifiers and food [56]. Structural carbohydrates range between 30% and 50% of dry mass in brown algae, 30–60% in red algae and 25–50% in green algae [10]. This variation in composition allows a range of industrial applications. Furthermore, low lipid and high carbohydrate contents make specific macroalgae good candidates for alcohol-based fuels. The composition of the three phyla can be broadly summarised as

- Red macroalgae composition varies with species but generally consists of glucose-derived cellulose and galactose polysaccharides [57]. The cell walls contain two types of galactan long-chain polysaccharides, agar and carrageenan, which are valuable thickening agents used commercially in the food industry.
- Green macroalgae are mainly composed of cellulose and pectin, the main structural polysaccharides, in the cell wall in addition to starch as a food reserve [48].
- Brown macroalgae contain cellulose, alginate and fucoidan, as important structural polysaccharides that provide mechanical strength to cell walls. Storage sugars, laminarin and mannitol, can be constituted up to 55% of dry weight. Laminarin is a carbohydrate that can be hydrolysed into glucose by laminarase [endo-1,3(4)-b-glucanase] [58], while mannitol is a sugar alcohol that can be directly utilised by bacteria.

Protein

The protein content of seaweeds varies substantially more between the species of seaweeds within the taxonomic groups (red, green or brown seaweed) than between the taxonomic groups [47]. Typically, red and green varieties of seaweed are more protein-rich than brown, whereby red seaweeds species such as *Porphyra tenera, Palmaria palmata* and *Gracilaria* spp. contain some of the highest protein levels, at 47%, 35% and 33

wt.%, respectively. [59, 60]. Similarly, green seaweeds, such as *Ulva* spp., show protein levels of up to 35% dry weight. These levels are comparable to those found in high-protein crops such as soybeans that contain around 35 wt.% [61]. Brown seaweeds generally contain the lowest protein contents of the taxonomic groups, with the highest protein content of 24% noted in *Undaria pinnatifida*. Seasonal factors can affect the protein content in the fronds of seaweed species. For example, *Saccharina* sp., *Laminaria* sp. and *Alaria esculenta* were shown to have maximum levels during the period February to May, with the younger parts of the fronds of *Saccharina* and *Laminaria* being considerably richer than the older parts [62]. Curiously, despite the high sulphur content normally associated with seaweed, sulphur-containing amino acids are normally underrepresented [63].

Lipids

Lipids are a broad group of naturally occurring molecules that includes fatty acids, oils, fats, waxes, sterols, phospholipids, fat-soluble vitamins (such as vitamins A, D, E and K), mono-, di-, and triacylglycerols [60]. Major biological functions of lipids include energy storage, signalling and metabolic regulation and their role as structural components of cell membranes, lipid levels in macroalgae are species-specific and can range from less than 1% up to 20% of dry matter depending on season, environment, and age and growth stage of seaweed species [64]. Some species contain higher levels, such as green seaweed *Ulva rigida* at 12% and brown seaweed *Dictyota* spp. ranging from 12% to 20%. Kim et al. [65] reported that the maximum lipid content of fronds in *Saccharina* sp., *Laminaria* sp. and *Alaria esculenta* was generally found in winter, whereas the total lipid of *Fucus* sp. were most abundant in summer, with highest levels recorded in August. Furthermore, the composition varies within a single seaweed plant depending on the tissue that is sampled. A study by Tabassum et al. [66] on biomethane production in brown seaweeds by *A. nodosu*m, *L. digitata*, *L. hyperborea*, *S. latissima* and *Saccorhiza polyschides* showed significant variation in proximate, ultimate and biochemical composition of different parts of the thalli, i.e., stipe, bladder, frond and holdfast. The highest biomethane potential of 286 L CH_4 kg VS^{-1} was obtained from the stipe of *L. digitata* and the lowest value of 118 L CH_4 kg VS^{-1} from the holdfast of *L. hyperborea*. Accumulation of salt in the holdfast meant that biomethane performance was reduced compared to the stipe and frond parts of the thalli. Results showed that the most significant part for seaweed biogas production was the frond.

Macroalgae characteristics and effects on anaerobic digestion

The chemical composition of seaweed feedstocks differs markedly, as shown in Table 1, and the amount of maximum biomethane potential varies from one substrate to another. Several factors affect the potential of feedstocks for biomethane production. Important feedstock parameters include: total solids (TS) and volatile solids (VS) content, nutrient content, carbon fraction, carbon-to-nitrogen ratio (C/N) and the presence of inhibitory substances [50]. This is particularly true for the digestion of cultivated macroalgae due to variations in their composition between species, locations and time of harvest [67]. Storing seaweed harvests via ensiling can reduce the compositional variation through the year, though still results in higher variation in composition compared to terrestrial crops, which must be considered for commercial applications [68]. It should also be noted that the macroalgae polysaccharides of alginate, carrageenan and laminarin are composed of repeating uronic acids. Uronic acids differ in structure to that of simple sugars in that the hydroxyl group furthest from the carbonyl group has been oxidized to a carboxylic acid. Enzymatic hydrolysis of uronic acid-containing polysaccharides, therefore, requires a neutralising group for carboxylic acid group to facilitate glycosidic bond cleavage [69]. As such, different microbial functionalities are required for their hydrolysis into monomers for further conversion, which is considered a major limitation of macroalgae anaerobic digestion [34]. For instance, carrageenan and fucoidan contain sulphate ester groups that require specialised desulphatase enzymes to remove sulphate groups before polysaccharide hydrolysis can occur [70]. This low biodegradability using terrestrial inocula is not limited to the solid polysaccharide fraction. Salgado-Hernandez et al. [71] found that the biodegradability index of the liquid fraction of only 39% compared with the solid fraction of 25%. The low biodegradability of seaweed has also been attributed to a combination of inhibitors present in seaweed, including but not limited to sulphur compounds, phenolics, halogenated hydrocarbons, high salt contents and heavy metals [72].

Carbon/nitrogen ratio

The molar compositional ratio of carbon to nitrogen (C/N ratio) in a feedstock is one of the most important parameters for assessing feedstock suitability for anaerobic digestion due to its effects on the stability of anaerobic digesters and the maximising of methane output. Nitrogen is essential for the microbial production of proteins and enzymes and insufficient quantities (high C/N ratio) lead to a nutrient-limited environment with reduced

Table 1 Seaweed characteristics as they relate to anaerobic digestion

Seaweed species	Proximate analysis			Ultimate analysis					C:N ratio	References
	TS (% of w.w.)	VS (% of w.w.)	Ash (%TS)	C (%VS)	H (%VS)	N (%VS)	O (%VS)	S (%VS)		
Alaria esculenta	18.7	11.9	36.4	29.3	4.2	1.9	28.2	–	15.5	[52]
Laminaria digitata	14.2	10.3	27.2	34.2	4.8	1.5	32.3	–	22.3	[52]
	9.5	7.6	1.7	48.0	6.3	3.1	42.6	–	15.5	[73]
	17.7	14.4	18.31	33.45	4.71	1.22	42.31	–	27.42	[74]
	13.7	1.3	18.9	–	–	–	–	–	–	[75]
Laminaria digitata (fresh)	7.78	72.0	–	27.28	3.77	3.17	37.54	–	8.6	[76]
Laminaria digitata (dried)	98.7	69.0	–	30.11	4.73	2.16	37.54	–	13.9	[76]
Laminaria hyperborea (fresh)	6.2	69.1	–	25.62	3.58	1.30	35.16	–	19.7	[76]
Laminaria hyperborea (dried)	97.9	63.2	–	28.67	4.26	1.02	35.16	–	28.1	[76]
Saccharina latissima	15.5	10.1	34.9	29.1	3.8	1.2	31.0	–	24.0	[52]
Saccharina latissima	9.2	5.3	42.80	27.85	3.58	1.80	24.02	–	15.84	[77]
	–	–	–	31.5	4.0	2.2	28.4	–	14.3	[78]
	–	–	24.2	31.3	3.7	2.4	36.3	0.7	13.0	[34]
Saccorhiza polyschides	15.3	13.1	14.0	36.1	5.0	1.6	44.3	–	23.2	[52]

methane yields. Excess nitrogen (low C/N ratio) inhibits methanogens by high ammonia concentrations due to the degradation of the protein-rich feedstocks [79]. The manageable C/N ratio for anaerobic digestion is considered to be in the range of 20–30, with an optimum at around 25 [40]. Seasonal variation in the protein content leads to increased N and lower C/N ratios during winter and spring, while rising as summer progresses from May to August [17]. Brown seaweeds have a low concentration of protein, with a C/N ratio of 26 having been recorded in *A. nodosum*, 24 in *S. latissima*, 21 in *Himanthalia elongata* [52], 22 for *S. muticum* [80], 27 for *L. digitata* and 23 for *Saccorhiza polyschides*. *U. lactuca* has a C/N ratio of 10–20 in nature dependent on season but can be cultivated to obtain a ratio of more than 30 [81]. In a study by Bruhn et al. [82] *U. lactuca* was cultivated in Saccharina sp., Laminaria sp. and Alaria esculenta in ponds and it was found that the C/N ratios varied from 7.9 to 24.4. A controlling factor in the C/N ratio was suggested as incoming irradiance, with more light causing seaweed to accumulate more carbon and carbohydrates leading to an increase in the C/N ratios. The authors found that nitrogen-starved *U. lactuca* produced more biomethane than nitrogen-replete biomass and that a critical value of N of 2.17% of total solids was recorded for maximum growth [82]. Romagnoli et al. [83] recorded a C/N ratio of 29.3 for *U. intestinalis*, an abundantly available macroalgae in the Baltic Sea. The variation in the C/N ratio can be approximated between genera with brown seaweeds typically having near-ideal C/N ratios compared to red and green seaweeds with excess nitrogen for anaerobic digestion.

Macroalgae pre-treatments and biogas yields

In anaerobic digestion, the hydrolysis phase has been identified as the rate limiting step that can be improved by substrate pre-treatment [84]. Many different pre-treatment technologies have been reviewed [19, 20, 29, 40, 43, 85] and can be classified into physical, chemical, biological and physio-chemical dependent on the different forces or energies used in the pre-treatment process. Experimental and implementation work should focus on technologies for pre-treatment and conditioning of macroalgae biomass as they have a direct impact on the methane fermentation process [86]. Pre-treatment is an important step in the biorefinery process of macroalgae due to their complex composition. Pre-treatment technologies facilitate the conversion of complex substrates into simpler monomers debottlenecking the hydrolysis stage and are a crucial step in the biorefinery process [39]. The pre-treatment of macroalgae has been thoroughly investigated for achieving improved methane yields when using many macroalgae species (see Table 2) as it greatly affects the technical, economic and environmental sustainability of macroalgal biogas production [87].

Initial pre-treatments: washing and/or drying (dewatering)

Washing reduces the ash content of seaweeds by the removal of specific compounds such as K_2O and Na_2O. This increases the ratio of VS to TS and, therefore, increasing BMP per unit mass [82]. Washing macroalgae in freshwater also can increase the BMP by reducing the inhibition of methane production by anaerobic digestion associated with high salinity (accumulation of sodium and potassium cations) [88]. Washing seaweed may remove salts and impurities but the organic matter may also be lost. Therefore, selecting the optimal pre-treatment time and temperature may allow a removal of salts with minimum organic matter loss [4]. Drying or dewatering of macroalgae biomass depends on the type of conversion process (wet or dry) [19]. However, even for wet processes, dewatering algae to a water content by 20–30% can be beneficial in stabilising the biomass and lower energy consumption during transportation. Alvarado-Morales et al. [89] considered drying an energy intensive process when carrying out an energy analysis of seaweed-based biofuel production.

Edward et al. [76] stated that washed and dried *Laminaria digitata* showed the highest biomethane potential (BMP) with a yield of 141.45 L CH_4 kg^{-1} VS, compared to 93.35 L CH_4 kg^{-1} VS for washed and fresh *L. digitata* biomass. Adams et al. [90] found that unwashed *L. digitata* generated a higher concentration of ethanol than washed biomass; however, in contrast, washed samples gave higher methane yields. Therefore, the prewashing step may be considered more suitable for the anaerobic digestion conversion route. A study by Milledge et al. [91] to examine the effect of washing *Sargassum muticum* with freshwater showed no statistical difference in methane yield between washed and unwashed samples. Mono-digestion experiments on the green seaweed *Ulva lactuca* by Allen et al. [92] demonstrated that a combination of washing and drying yielded the best results with a yield of 250 L CH_4 kg^{-1} VS. Bruhn et al. [82] found that drying *U. lactuca* resulted in a five–ninefold increase in specific methane production compared to wet biomass, but this increase might also be associated with reduction in particle size during drying. A study by Briand et al. [93] demonstrated the action of washing and grinding pre-treatments on the development of *Ulva* sp. methane digestion with non-washed biomass having a higher methane yield of 110 L CH_4 kg^{-1} VS than washed at 94 L CH_4 kg^{-1} VS. Washing the algae resulted in the loss of some easily digestible soluble metabolites slowing down the start of the process and decreasing the methane yield. Grinding of the biomass facilitated a rapid hydrolysis and

Table 2 Impact of species and pre-treatments on the methane yields of anaerobic digestion of European seaweeds

Seaweed species	Location details	Pre-treatments	Inoculum/ Co-digestion	Inoculum to Sludge ratio	Process conditions	Methane yield	References
Phaeophyta							
Alaria esculenta	Cork, South of Ireland July	Macerated to < 4 mm	Combination of lab-scale reactors processing grass silage, dairy slurry & macroalgae	2:1	Batch, 37 °C, 30 days	226.0 L CH_4 kg^{-1} VS	[52]
Alaria esculenta	Trondheim, Norway Aug/Oct 2014 & May 2015	Stored at 10 °C in sea-water tanks. Epiphytes removed manually. Stored at − 80 °C. Cut to ≤ 1 cm^2	Wastewater sludge (mixed primary and waste active sludge)	n/k	Batch, 37 °C, 60 days	Between 187 N mL CH_4 g^{-1} VS (Aug. 2014) and 254 N mLCH_4 g^{-1} VS (May 2015)	[9]
Laminaria digitata	USA	Coarsely shredded	Digester slurry	n/k	Batch, 35 °C, 30 days	280 mL CH_4 g^{-1} VS	[98]
Laminaria digitata	Aberystwyth, Wales Jan–Dec. 2008	Frozen, dried at 70–80 °C. Milled to produce flour to < 1.0 mm	Digested sludge	n/k	Batch, 35 °C, 36 days	254.14 L CH_4 kg^{-1} VS (July) 196.33 L CH_4 kg^{-1} VS (March)	[149]
Laminaria digitata (Fronds)	Aberystwyth, Wales July 2009 & Jan. 2010	Unwashed Washed with tap water up to 1 min Oven-dried 70 °C, 72 h Frozen at 20 °C, oven dried 70 °C, 72 h Shock frozen with liquid nitrogen, freeze-dried Material milled to < 1 mm	Digested sludge	n/k	Batch, 35 °C, 35 days	257.7 mL CH_4 g^{-1} VS (Freeze-dried, washed)	[90]
Laminaria digitata at 15, 24 & 41%	Denmark	Stored at − 18 °C after collection Washed with tap water, chopped for 30 min. to ~ 10 × 10 mm	Cattle manure	n/a	5L reactors, semi-continuous, 80 days Mesophilic (35 °C) Thermophilic (50 °C)	165 L CH_4 kg^{-1} VS mesophilic (41% *L. digitata*) 198 L CH_4 kg^{-1} VS thermophilic (41% *L. digitata*)	[112]
Laminaria digitata	Co. Sligo, Ireland Sept. 2011	Washed, dried at room temp. for 48 h. Milled to < 1.0 mm	Co-digested with Cattle manure	1:1	Batch, 35 °C, 109 days	246 mLCH_4 g^{-1} VS	[101]

Table 2 (continued)

Seaweed species	Location details	Pre-treatments	Inoculum/ Co-digestion	Inoculum to Sludge ratio	Process conditions	Methane yield	References
Laminaria digitata (Frond only)	Culler Coats, Tyne & Wear Dec. 2013	Washed. Fronds roughly chopped to approx. 10 mm. Two pre-treatments, fresh and dried Fresh: fronds roughly chopped to approx. 10 mm, macerated to consistency of thick slurry (< 2 mm) Dried: feedstock dried at 104 °C for 24 h, milled to max. 1 mm	Lab scale mesophilic digester	3:1	Batch, 35 °C, 32 days	141.45 L CH_4 kg^{-1} VS (washed & dried) 93.35 L CH_4 kg^{-1} VS (washed & fresh)	[76]
Laminaria digitata	Cork, South of Ireland August	Macerated to < 4 mm	Lab-scale reactors processing grass silage, dairy slurry & macroalgae	2:1	Batch, 35 °C, 35 days	218 L CH_4 kg^{-1} VS	[52]
Laminaria digitata + dairy slurry	West Cork, South of Ireland	Washed with tap water, macerated to < 4 mm, stored at − 20 °C	Originating from digester treating grease trap waste and slurry	2:1	Batch 37 °C, 30 days	232 L CH_4 kg^{-1} VS (66.6% *L. digitata* + 33.3% dairy slurry)	[77]
Laminaria digitata (Fresh fronds)	West Cork, South of Ireland March–Sept	2 washing pre-treatments at 15 °C and 40 °C 2 mechanical pre-treatments cutting and maceration	Lab-scale reactors processing grass silage, dairy slurry & macroalgae	2:1	Batch, 37 °C, 30 days	282 L CH_4 kg^{-1} VS (Pre-treatments, washing at 40 °C and macerated)	[95]
Laminaria digitata	Denmark August 2013	Stored at − 20 °C	Thermophilic inoculum from biogas plant	n/k	Batch, 55 °C, 30 days	358.9 N mLCH_4 g^{-1} VS	[134]
Laminaria digitata	Cork, Rep. of Ireland Sept. & Oct	Washed under tap water. Minced to 4–5 mm Hydrolysis reactor Methanogenesis reactors	AD plant treating dairy slurry, grease and food waste	n/k	Single-stage (reactor M_3) & Two-stage ($H_1 + M_1 + M_2$) systems 37 °C, 6 weeks	234 L CH_4 kg^{-1} VS at HRT of 14 days	[150]
Laminaria digitata (Frond only)	Northumberland, UK July 2015	Washed to remove marine salts and sediments Roughly chopped to ~ 10 cm, dried at 70 °C for 24 and 48 h, blended to < 1 mm	Sludge obtaining for AD operating on grass silage, pig and cow manure	3:1	Batch at 25 °C, 35 °C, 45 °C & 55 °C for 40 days	352 mL CH4 g^{-1} VS at 55 °C	[111]

Table 2 (continued)

Seaweed species	Location details	Pre-treatments	Inoculum/ Co-digestion	Inoculum to Sludge ratio	Process conditions	Methane yield	References
Laminaria digitata (residue)	East & West coasts of Ireland, seasonal collections	Stored at − 20 °C Chopped < 0.5 cm Bioproducts extracted using ethanol, acetic acid, Na_2CO_3	Sewage sludge (acclimatise and non-acclimatised)	1:0.3	Batch, 38 °C, 21 days	523 mL CH_4 g^{-1} VS	[151]
Laminaria digitata	West Coast Ireland Sept	Washed with tap water, cut into small pieces, oven dried 105 °C, ground to powder, cryo-stored at − 20 °C Hydrothermal Hydrothermal dilute acid Enzymolysis Combination of above	Inoculum from digester treating food waste	2:1	Batch, 35 °C, 21 days	282.2 L CH_4 kg^{-1} VS Hydrothermal pre-treatment (140 °C for 20 min)	[121]
Laminaria digitata *Laminaria digitata* + *Phragmites australis*	West Coast, Sweden July/Aug 2015	Frozen. Cut to 100 mm prior to use	Granular sludge from WWTP	n/k	Two-stage pilot scale process *L. digitata* 70 days *L. digitata* + *P. australis* 100 days	170 L CH_4 kg^{-1} VS yperbo. *L. digitata* only *L. digitata* + *P. australis* no increase in methane yield and showed process instability	[152]
Laminaria hyperborean (Frond only)	Tyne & Wear, UK July 2009	Frond material only, roughly chopped to 10 mm, blended to slurry	Mixed methanogenic sludge Paper:sugar:sewage (2:2:1)	Reactors inoculated to 28% wv	Continuous, 35 °C, 179 days 3 reactors	Average over 3 reactors 236.7 mL CH_4 g^{-1} VS	[103]
Laminaria hyperborea	Firth of Forth, Edinburgh, Scotland	Washed with tap water to remove sand and dirt, minced to ~ 6 mm, stored at − 20 °C. Before use reduced to 2 mm size, blended and autoclaved	Seven different inocula and mix of these (inoculum 8) Sheep rumen contents (× 2), sheep faeces (× 2), mix of leachates, human sewage, marine sediments, mix of all seven	1:1	Semi-continuous single- and two-stage process, 37 °C, 28 days	Inoculum 8 (mix of 7 inocula) produced most methane at single-stage 253 mL CH_4 g^{-1} VS	[38]

Table 2 (continued)

Seaweed species	Location details	Pre-treatments	Inoculum/ Co-digestion	Inoculum to Sludge ratio	Process conditions	Methane yield	References
Laminaria hyperborean (Stipe only)	Culler Coats, Tyne & Wear Dec. 2013	Washed to remove marine salts and sediments. Stipes broken to < 5 mm pieces. Two pre-treatments, fresh and dried Fresh: stipes roughly chopped to approx. 10 mm, macerated to consistency of thick slurry (< 2 mm) Dried: stipes dried in oven at 104 °C for 24 h	Lab scale mesophilic AD	3:1	Batch, 35 °C, 32 days	Fresh—105.06 L CH_4 kg^{-1} VS Dried—113.28 L CH_4 kg^{-1} VS	[76]
Laminaria spp. mix (*L. digitata, S. latissima, L. hyperborea*)	Dublin, Ireland May 2014	Roughly cut, no washing Mechanical pre-treatment—beaten for 5, 10, 15 min. producing pulp of different consistencies VS concentrations 1, 2.5, 4%	Sewage sludge from WWTP	n/k	Batch, 38 °C, 14 days	240 L CH_4 kg^{-1} VS (15 min, 2.5% VS)	[99]
Laminaria sp. (mix of *L. digitata, S. latissima, L. hyperborea*)	Dublin, Ireland November 2013	Beaten—pulped Ball milled—dried for 24 h at 80 °C, milled, sieved to 1 and 2 mm particle size Microwaved—rough cut	Sewage sludge from WWTP	1:1	Batch, 38 °C, 25 days	335 N mL CH_4 g^{-1} VS (with beaten pre-treatment)	[108]
Saccharina latissima	Trondheimsfjord, Norway Spring—March 1982, 1989 Autumn—Sep. & Nov. 1988	Fresh algae milled to < 4 mm, stored at − 25 °C	Batch—inoculum from semi-continuous digestion Semi-continuous- fermented spring kelp	n/k	Batch, 32, 35 days Semi-continuous, 35 °C, 40 days	Spring—25 mL CH_4 g^{-1} VS Autumn—51 mL CH_4 g^{-1} VS Spring—220 mL CH_4 g^{-1} VS Autumn—270 mL CH_4 g^{-1} VS	[153]
Saccharina latissima	Brittany, France July 2010	Dried at 40 °C for 24 h. Roughly chopped to ~ 2 cm	Sludge	2:1	Batch, 35 °C, 60 days SCSTR, 35 °C, 182 days	210 mL CH_4 g^{-1} VS 270 mL CH_4 g^{-1} VS	[154]
Saccharina latissima	Brittany, France May–August 2011	Washed with seawater, dried at 40 °C for 24 h Roughly chopped	Anaerobic sludge	2:1	Batch, 35 °C, 40 days	209 mL CH_4 g^{-1} VS	[96]

Table 2 (continued)

Seaweed species	Location details	Pre-treatments	Inoculum/ Co-digestion	Inoculum to Sludge ratio	Process conditions	Methane yield	References
Saccharina latissima	Cork, South of Ireland July	Macerated to < 4 mm	Combination of lab scale reactors processing grass silage, dairy slurry & macroalgae	2:1	Batch, 37 °C, 30 days	341.7 L CH_4 kg^{-1} VS	[52]
Saccharina latissima (cultivated) + Dairy slurry	West Cork, Ireland	Washed with tap water, macerated to < 4 mm, stored at − 20 °C	Originating from digester treating grease trap waste and slurry	2:1	Batch 37 °C, 30 days	252 L CH_4 kg^{-1} VS (66.6% *S. atissimi* + 33.3% dairy slurry)	[77]
Saccharina latissima	Denmark 2012, 2013	Stored at − 20 °C	Thermophilic inoculum from biogas plant	n/k	Batch, 55 °C, 30 days	285.0 N mL CH_4 g^{-1} VS (August 2013)	[134]
Saccharina latissima (residue)	East & West coasts of Ireland, seasonal collections	Stored at − 20 °C Chopped < 0.5 cm Bioproducts extracted using ethanol, acetic acid, Na_2CO_3	Sewage sludge (acclimatise and non-acclimatised)	1:0.3	Batch, 38 °C, 21 days	535 mL CH_4 g^{-1} VS	[151]
Saccharina latissima	West Cork, Rep. of Ireland June	Washed with tap water, minced to ~ 5 mm, dried at 105 °C, pulverised to 0.02 mm mesh size Hydrothermal pre-treatment from 100, 120,140, 160, 180 °C for 30 min	Lab digester using cellulose as substrate	2:1	Batch, 35 °C, 11 days	281.4 mL CH_4 g^{-1} VS untreated 345.1 mL CH_4 g^{-1} VS treated at 140 °C	[78]
Saccharina latissima	Beadnell Bay, Northumberland, UK June 2017	Freeze-dried, stored under nitrogen gas, ground to < 1 mm Hydrothermal pre-treatment (HTC) at 150, 200, 250 °C for 60 min	Sludge from WWTP	2:1	Batch, 37 °C, 30 days	Untreated—~ 200 mL CH_4 g^{-1} VS Slurry 150 °C–217 mL CH_4 g^{-1} VS Slurry 200 °C–202 mL CH_4 g^{-1} VS Slurry 250 °C–196 mL CH_4 g^{-1} VS	[35]
Saccharina latissima	Dunstaffnage, West Coast of Scotland August 2012	Freeze dried, manually grinded to < 1 mm	Anaerobic digested sludge Anoxic surface sediment (SS)	1:1	Batch, 37 °C, 50 days	230 dm^3 kg^{-1} VS (DG) 175 dm^3 kg^{-1} VS (SS)	[106]
Saccharina latissima	Coast of Frederikshavn, Denmark	Washed Unwashed Chopped to ~ 2 × 2 cm) Macerated to homogenised paste	Cattle manure	n/k	Batch, 53 °C, 34 days	340 mL CH_4 g^{-1} VS (washed, chopped) 333 mL CH_4 g^{-1} VS (washed, macerated)	[81]
Saccharina latissima	Co. Sligo, Ireland Sept. 2011	Washed, dried at room temp. for 48 h. Milled to < 1.0 mm	Co-digested with cattle manure	1:1	Batch, 35 °C, 109 days	335 mL CH_4 g^{-1} VS	[101]

Table 2 (continued)

Seaweed species	Location details	Pre-treatments	Inoculum/ Co-digestion	Inoculum to Sludge ratio	Process conditions	Methane yield	References
Saccharina latissima	Trondheim, Norway Aug/Oct 2014 & Feb/ May 2015	Stored at 10° in sea-water tanks. Epiphytes removed manually. Stored at − 80°. Cut to ≤ 1 cm^2	Wastewater sludge (mixed primary and waste active sludge)	n/k	Batch, 37 °C, 60 days	195 NmL CH_4 g^{-1} VS (Feb. 2015) 352 NmL CH_4 g^{-1} VS (May 2015)	[9]
Saccharina latissima + wheat straw	Trondheimsfjord, Norway August 2010	Frozen, ground to finely shredded slurry Steam explosion: *L. saccharina* 130 & 160 °C, 10 min Wheat straw 210 °C, 10 min	Anaerobic sludge	n/k	Batch, 37 °C, 119 days	223 mL CH_4 g^{-1} VS (untreated) 268 mL CH_4 g^{-1} VS (130 °C, 10 m) 260 mL CH_4 g^{-1} VS (160 °C,10 m) 270 mL CH_4 g^{-1} VS (Co-digested with steam exploded wheat straw)	[114]
Saccharina latissima	Trondheimsfjord, Norway August 2017	Washed with tap water, milled to pulp, dried at 30 °C for 48 h Enzyme hydrolysis	Large-scale continuous mesophilic multifuel anaerobic digester	2:1	Batch, 39 °C, 40 days	With hydrolysis 257 mL CH_4 g^{-1} VS No hydrolysis 208 mL CH_4 g^{-1} VS	[119]
Saccorhiza polyschides	Brittany, France May–August 2011	Washed with seawater, dried at 40 °C for 24 h. Roughly chopped	Anaerobic sludge	2:1	Batch, 35 °C, 40 days	216 mL CH_4 g^{-1} VS	[96]
Saccorhiza polyschides	Cork, South of Ireland July	Macerated to < 4 mm	Lab scale reactors processing grass silage, dairy slurry & macroalgae	2:1	Batch, 37 °C, 30 days	263.3 L CH_4 kg^{-1} VS	[52]
Saccorhiza polyschides	Co. Sligo, Ireland Sept. 2011	Washed, dried at room temp. for 48 h. Milled to < 1.0 mm	Co-digested with cattle manure	1:1	Batch, 35 °C, 109 days	225 mL CH_4 g^{-1} VS	[101]

n/k: not known

allowed better methane production rates to be achieved. Ground biomass had a higher methane yield at 177 L CH_4 kg^{-1} VS compared to non-ground samples at 145 L CH_4 kg^{-1} VS. In the red seaweed *Gracilaria vermiculophylla,* pre-treatments of washing and maceration caused significant increase in BMP reaching 481 CH_4 kg^{-1} VS, while thermochemical pre-treatment increased the algae solubilisation but not its BMP [88].

Physical (mechanical) pre-treatment

Mechanical pre-processing to decrease the size of macroalgae biomass and concomitantly increase the surface/volume ratio increases the access for degrading enzymes and enhances the hydrolysis of storage and structural polysaccharides. The result is either an increase in the final methane yield or a more rapid biogas production at the initial stage [94]. Physical pre-treatment has been used in many studies of macroalgae as a biofuel and covers the processes of maceration [52, 95], chopping [76, 96, 97], shredding [98], beating [99, 100], milling [90, 101, 102], blending [103] and grinding [35, 104–106] of macroalgae biomass.

The size reduction of dried macroalgae has been reported as enhancing biogas production from brown macroalgae [107]. A study of *Laminaria* sp. compared the effects of three different pre-treatment methods, beating, ball milling and microwave on methane production. Beating pre-treatment samples yielded the best result by achieving a methane increase of up to 37% with respect to raw seaweed [108]. The authors concluded that the main effect of beating *Laminaria* spp. biomass was to promote the start of anaerobic digestion and a reduction in incubation time. The findings of Montengelli et al. [99] suggested that ball milling pre-treatment hampered anaerobic digestion of macroalgae and that a particle size < 1 mm of dried macroalgae negatively affected methane production. The study showed that unlike lignocellulosic biomass, which must be reduced to 1–2 mm in order to decrease heat and mass transfer limitations during the hydrolysis step, their work showed that for macroalgae a reduction of particle size in this range did not show any improvement in anaerobic digestion performance. In general, the main effect of reducing particle size is to increase the surface area available to anaerobic microorganisms and thereby increasing gas production. The authors suggested that excessive particle reduction can speed up the hydrolysis and acidogenesis phases in anaerobic digestion, increasing production rates of volatile fatty acids and decreasing the pH [99]. A pH that is within the acidic range will hamper methanogenic activity and consequently inhibit biogas production rates.

A study on *Pelvetia canaliculata* with the aim to maximise methane yield while minimising the pre-treatment beating time showed that it was possible to optimise the methane yield of 283 mL CH_4 g^{-1} VS with pre-treatment, which represented an increase of 45% compared to non-pre-treated algae [100]. Mechanical pre-treatment using a Hollander beater was shown to increase biodegradation effectiveness in *P. canaliculata*, *Fucus vesiculosus* and *Fucus serratus*. Results showed that up to 20% extra biogas with a positive net energy gain of 85% was possible [109]. A further study using a Hollander beater on particle size reduction optimisation of *Laminaria* spp. biomass, estimated that when about 80% of particles are sized below 1.6 mm^2, a methane yield improvement of up to 53% can be achieved [110].

Nielsen et al. [81] investigated different pre-treatments, including washing, pre-treatment without washing, chopping, macerating, on four macroalgal species for the suitability of bioconversion to methane. In batch experiments (53 °C), methane yields varied from 132 mL CH_4 g^{-1} VS for *Gracilaria vermiculophylla* (washed, macerated), 152 mL CH_4 g^{-1} VS for *Ulva lactuca* (washed, macerated), 166 mL CH_4 g^{-1} VS for *Chaetomorpha linum* (washed, macerated) and 340 mL CH_4 g^{-1} VS for *Saccharina latissima* (washed, chopped). The authors stated that *S. latissima* appeared very suitable for anaerobic digestion, and that methane yields for *U. lactuca*, *G. vermiculophylla* and *C. linum* could be increased by 68%, 11% and 17%, respectively, by maceration pre-treatment. In all seaweed species, methane yield was enhanced with washing of biomass. Maceration was also noted to increased methane yield of green macroalgae *Ulva lactuca* from 174 to 271 L CH_4 kg^{-1} VS, increasing the BMP yield up to 56% when compared to untreated biomass [82].

Thermal pre-treatment

The rate limiting hydrolysis step can be assisted by thermal pre-treatment to disrupt the cell wall structure and assist the conversion of polymeric carbohydrates into monomeric sugars. In a study by Lin et al. [78], hydrothermal pre-treatments were assessed for hydrolysis and subsequent production of biomethane by the brown macroalgae *Saccharina latissima*. Biomethane yields were found to increase from 281.4 mL CH_4 g^{-1} VS untreated to 345.1 mL CH_4 g^{-1} VS pre-treated at 140 °C, an increase of 22.6%. The authors stated that hydrothermal pre-treatment of *S. latissima* could break down the recalcitrant macro- and micro-structures of macroalgae and enable seaweeds to be effective producers of gaseous biofuels. Thomson et al. [80] found that hydrothermal pre-treatment of pelagic brown macroalgae *Sargassum* spp. increased the degradation and solubilisation of organic components in *Sargassum* spp. for effective and accelerated methane fermentation downstream. Peak methane production of 116.7 mL CH_4 g^{-1}

VS was achieved following pre-treatment at 145–150 °C for 30 min. Hydrothermal pre-treatment also reduced the concentration of H_2S in biogas from 3 to 1%, mitigating the challenges associated with biodigester performance and harmful emissions.

The effect of temperature on biogas and methane yield from *Laminaria digitata* was investigated at 25, 35, 45, 55 °C [111]. Results showed that cumulative biogas production was best at 35 °C, while overall methane yield was best at 55 °C giving 352 mL CH4 g^{-1} VS. The performance of mesophilic and thermophilic co-digestion of *L. digitata* with cattle manure operating at various feeding ratios of macroalgae (15, 24, 41%) was examined by [112]. The results showed specific methane yields of 165 L CH_4 kg^{-1} VS and 185.7 L CH_4 kg^{-1} VS at feeding rates of 24% and 41%, respectively, from thermophilic digestion. The study revealed that variation in *L. digitata* feeding did not largely contribute to the specific methane yield for mesophilic co-digestion which was similar to control levels. The methane yield of green macroalgae *Ulva lactuca* was shown to increase from 174 to 187 L CH_4 kg^{-1} VS when subject to thermal pre-treatment at 130 °C for 20 min [82]. Thermal pre-treatment has also been applied at low temperatures ranging from 50 to 70 °C, where biological mechanisms may be involved. The duration is longer, from about 10 h to a few days. This pre-treatment can take advantage of sludge endogenous enzymes (temperature phased anaerobic digestion (TPAD), which uses first-stage AD [85]. Steam explosion is a thermal pre-treatment where biomass is placed in a vessel and steam is applied at high temperatures (~160 °C) and pressure (~6 bars) for a few minutes (10–30 min) after which the steam is flashed and the biomass is quickly cooled. The sudden pressure drop leads to cell wall rupture and biomass disintegration [113]. Vivekanand et al. [114] noted a marginal improvement in methane yield when a steam explosion pre-treatment at 130 °C was applied to the brown macroalgae *Saccharina latissima*.

Thermo-chemical pre-treatment (60–220 °C, combined with acidic or alkali reagents)

Acid hydrolysis uses acids such as sulphuric acid (H_2SO_4) to hydrolyse glycosdic bonds. For effective acid hydrolysis of macroalgal biomass, solid/liquid (S/L) ratio, acid type, acid concentration, reaction time and reaction temperature are important parameters that need to be optimised [10]. Acid hydrolysis can either serve as a pre-treatment step before enzymatic hydrolysis or as the chemical method to hydrolyse biomass to produce reducing sugars. Barbot et al. [104] used thermo-acidic pre-treatment to enhance hydrolysis of polymeric molecules in biomass and increase methane production from the brown macroalgae *F. vesiculosus* [104]. The author stated that 0.2 HCl at 80 °C for 2 h can boost methane formation by 140% (113 mL CH_4) compared to untreated biomass (47 mL CH_4), and a lower pre-treatment temperature of 50 °C improves methane recovery by 83% (86 mL CH_4). It was noted that thermochemical pre-treatment of red seaweed *Gracilaria vermiculophylla* increased the algae solubilisation but not it's BMP, possibly due to the release of polyphenols and other toxic compounds on heating the substrate [88]. Lymperatou et al. [115] found that thermos-acidic pre-treatment improved methane yields by 78% compared with untreated biomass though a five-fold increase in cost from 0.7 to 3.5 USD/m^3. The authors stated that alkaline pre-treatment of room-temperature mixing with 15 w/w% was the most cost-effective process with a 33% decrease in cost per unit methane.

Microwave and ultrasonic pre-treatment

Ultrasound technology is an effective method to enhance biogas production [116]. Ultrasound promotes cell disintegration releasing intracellular soluble organic matter and can enhance the efficiency of the anaerobic digestion process. Microwave pre-treatment may also enhance biogas production. The alternating electric field caused by microwave irradiation can rapidly change the dipole orientation of polar molecules and generate heat evenly across the particles. Advantages over water-bathing and autoclaving are that it has a shorter reaction time and can maintain the temperature of each part relatively equal across the reaction vessel. It can also be well controlled and stopped immediately [28].

Romagnoli et al. [105] applied microwave pre-treatment to *Fucus vesiculosus* to increase methane yield. Microwave pre-treatment at 700 w for 90 s improved biogas production in the range of 7.8–43.7% and a range of 37.2–45.2% when applied for 3 min. The authors stated that the effects of microwaves were generally positive for biogas production. A study by Montingelli et al. [108] on *Laminaria* spp. showed that microwave pre-treatment resulted in a 27% reduction of biogas production compared to untreated macroalgae. The authors stated that in general microwave pre-treatment increases biomass solubilisation which should accelerate and/or increase anaerobic biodegradability; however, their data showed that the use of microwave pre-treatment at 100 °C impacted negatively and that methane production from macroalgal biomass does not benefit from pre-treatments involving the use of high temperatures. The negative effects of microwave pre-treatment are most likely due to an increase in rapidly digestible materials and inhibitors that causes microbial imbalances during batch digestion.

Karay et al. [87] demonstrated that 2.53 g/L of reducing sugars in green macroalgae *Ulva rigida* was obtained after ultrasonic pre-treatment compared to 0.6 g/L from

the crude macroalgae, suggesting that ultrasonic pre-treatment could promote hydrolysis of carbohydrate polymers to reducing sugars. Kumar et al. [117] stated that higher energy input with longer duration increased organic release; however, better solubilisation may not directly contribute to biogas production as the formation of some by-products can have a toxic effect on the microbial activities that will affect anaerobic digestion. A study by Wu et al. [118] on the effect of ultrasonic and microwave pre-treatments on a mixture of *F. vesiculosus* and *F. serratus* stated that a combined microwave and ultrasonic pre-treatment obtained the highest cumulative methane yield of 260 mL CH_4 g^{-1} VS, which is two-fold higher than for mechanical (chopped and ground) pre-treatment with methane yield of 122 mL CH_4 g^{-1} VS [118].

Biological (microbial, enzymatic digestion) pre-treatment

Macroalgae possess an array of polysaccharides such as cellulose, alginate and laminarin which have the potential for production of alternative biofuels. These polysaccharides are not easily accessible for biological digestion; however, pre-treatment of macroalgae with enzymes may make these polysaccharides easier to access by microbes and thereby allowing effective utilisation in anaerobic digestion. The brown macroalgae *S. latissima* contains high levels of carbohydrates and was assessed by Lamb et al. [119] for the production of biogas through anaerobic digestion following enzymatic pre-treatment. Analysis of harvested *S. latissima* was shown to contain 30.11 g of reducing sugars per 100g of dry sample upon enzymatic hydrolysis yielding 459 mL per gVS of biogas through anaerobic digestion with methane content of 56% and biomethane production of 257 mL CH_4 g^{-1} VS and 208 mL CH_4 g^{-1} VS with and without enzyme hydrolysis, respectively. These results suggest a biomethane potential of 1760 m^3 per ha of productive sea floor growing *S. latissima*.

Macroalgae consortium composed of wild brown and green seaweeds found in the Mexican Caribbean were investigated for biogas production using fungal and enzymatic pre-treatment [120]. Two biological pre-treatments were carried out, macroalgae + fungi (Bm-2 strain) and macroalgae + enzymatic broth. Enhancement of biogas production was obtained with the macroalgae + fungi pre-treatment and was statistically higher than values obtained with enzymatic pre-treatment at 86 L CH_4 kg^{-1} VS and untreated macroalgae at 81 L CH_4 kg^{-1} VS.

Combined processes

A study by Jard et al. [96] looked at the efficiency of the anaerobic digestion of red macroalgae *Palmaria palmata* which was subject to a range of thermal (between 20 and 200 °C) and chemical (addition of NaOH and HCl) pre-treatments. Results showed that at 20, 70, 85 and 120 °C and soda and acid pre-treatments at 160 °C there was no significant effect on methane potential. After high temperature pre-treatment of 180–200 °C, BMP decreased due to the formation of refractory compounds in the liquid fraction. However, the addition of NaOH at 20 °C led to a release of proteins and induced an increase in BMP from 308 mL CH_4 g^{-1} VS (untreated) to 365 mL CH_4 g^{-1} VS. Gruduls et al. [97] combined CO_2 and thermal pre-treatments of boiling, microwaving and autoclaving on the BMP of Baltic Sea macroalgae biomass. The values of BMP were assessed for untreated biomass of four species, brown algae *F. vesiculosus* (97.9 mL CH_4 g^{-1} VS), red algae *Furcellaria lumbricalis* (173.5 mL CH_4 g^{-1} VS), green algae *Cladophora* sp. (377.1 mL CH_4 g^{-1} VS) and *U. intestinalis* (364.8 mL CH_4 g^{-1} VS). The best results were obtained by combining CO_2 treatment with subsequent autoclaving with an increase of BMP for *F. vesiculosus* of 132.5% (227.7 mL CH_4 g^{-1} VS) and for *F. lumbricalis* of 116.4% (375.5 CH_4 g^{-1} VS). An increase of BMP of around 12–14% was also observed for the other two species *Cladophora* sp. 14% (429 mL CH_4 g^{-1} VS) and *U. intestinalis* 12.5% (410 mL CH_4 g^{-1} VS).

Ding et al. [121] deployed the pre-treatments of hydrothermal, dilute hydrothermal acid hydrolysis, enzymatic hydrolysis and a combination of above to facilitate degradation of macroalgae *Laminaria digitata* and to improve the two-stage dark-fermentation biohydrogen and biomethane co-production. Hydrothermal (140 °C for 20 min) pre-treatment was considered the optimum pre-treatment securing biohydrogen and biomethane yields of 44.8 and 282.2 L CH_4 kg^{-1} VS, respectively. The addition of dilute H_2SO_4 during hydrothermal pre-treatment contributed to a higher yield of carbohydrate monomers but generated more inhibitive products [121]. In order to improve product yields achieved by the brown macroalgae *Nizimuddinia zanardini*, the biomass was pre-treated with dilute sulphuric acid (7.0% w/w) and hot water (121 °C, 30 min in autoclave). Both pre-treated and untreated biomasses were subject to enzymatic hydrolysis by cellulose and B-glycosidase. The results showed that biogas was increased from 170 to 200 m^3 per ton of dried algae biomass and the ultimate methane yield for untreated and hot water pre-treated macroalgae biomass was 117 mL CH_4 g^{-1} VS and 143 mL CH_4 g^{-1}, VS, respectively [122]. The authors reported that hot water pre-treatments were promising processes to improve the digestibility of the initial biomass and increase the yield of ethanol and biogas.

An integrated biorefinery approach using the green macroalgae *Chaetomorpha linum* was investigated for the co-production of bioethanol and biogas by Yahmed

et al. [123]. Among acidic, neutral and alkali pre-treatments of *C. linum* biomass, 3% NaOH gave the best results in terms of thallus disintegration, biomass recovery and enzymatic digestibility. Anaerobic digestion for biogas production included all effluents and co-products issued from various stages of saccharification and fermentation aimed at ethanol production, cell wall degrading enzyme production and intermediate operations. Hydrolysis with a crude specific enzyme preparation, fermented from *Aspergillus awamori* at 45 °C, pH 5 for 30 h, gave the maximum yield of fermentable sugar of 0.22 g/g dry substrate, which corresponded to a biomethane yield of 260 mL CH_4 g^{-1} VS. The authors stated that this method presents an eco-friendly biorefinery process co-producing bioethanol and biomethane with almost complete conversion of macroalgae biomass.

Further work by Yahmed et al. [124] on *Ulva* sp. investigated the effect of fungal (*Aspergillus fumigatus* SL1) pre-treatment on biogas production compared to conventional acid (4% HCL) and alkali (4% NaOH) pre-treatments. Acid pre-treatment had a significant negative impact on BMP at mL 77 CH_4 g^{-1} VS (132 mL CH_4 g^{-1} VS untreated). This was suggested as resulting from hemicelluloses and cellulose removal. Alkali pre-treatment increased BMP from 132 mL CH_4 g^{-1} VS (untreated) to 148 mL CH_4 g^{-1} VS (pre-treated), explained by the biochemical composition changes, particularly solubilisation of cell wall sugars, cellulose and hemicellulose. Biogas production was enhanced with the fungal pre-treatment reaching 153 mL CH_4 g^{-1} VS, statistically higher than values obtained for the chemical pre-treatments.

Freshwater (washed) and thalassic (non-washed) anaerobic digestion of *Ulva* sp. were compared to determine the most suitable conditions for biogas production [125]. Biological hydrolysis (hydrolytic bacteria) pre-treatment was used to improve methane yield and a 1% NaOH pre-treatment was employed to minimise any limitation of biological hydrolysis. The low improvement of the methane yield from both pre-treatments under freshwater conditions, 77.66 mL CH_4 g^{-1} VS (hydrolysis) and 61.67 mL CH_4 g^{-1} VS (NaOH), significantly improved under thalassic conditions. Here, methane yield after biological hydrolysis pre-treatment was 180.9 mL CH_4 g^{-1} VS, higher than using the NaOH pre-treatment of 158.19 mL CH_4 g^{-1} VS. Duration time was less for the 1% NaOH pre-treatment at 27 days compared to 62 days for biological hydrolysis pre-treatment. Experiments were applied to evaluate different strategies to enhance methane yield of the red macroalgae *Gracilaria vermiculophylla*. The BMP of *G. vermiculophylla* after physical pre-treatments of washing and maceration reached 481 L CH_4 kg^{-1} VS, corresponding to a methane yield of 79%. No significant effects were achieved in BMP after thermochemical pre-treatment; however, algal solubilisation increased up to 44% [88]. Investigation into the use of organic acid (6% oxalic acid at 120 °C for 1 h) and enzymatic (cellulose) pre-treatments to enhance the recovery of reducing sugars from *L. digitata* and *S. latissima* and to improve biogas production from *L. digitata*, found that despite the enhancing effect of the pre-treatments no considerable improvement in biogas production was observed [107].

Factors that can influence anaerobic digestion of macroalgae feedstocks

The performance of anaerobic digestion in biogas production depends mainly on the nature and type of biomass being digested as the composition substrate provides both raw materials for conversion and the necessary nutritional materials for microorganisms involved in the process [126]. Many factors including feedstock characteristics, reactor design, and operational conditions may affect the performance of anaerobic digestion processes, either by process enhancement or inhibition [4]. Several studies have shown that the hydraulic retention time—the average time interval when the substrate is kept inside the digester and organic loading rate—how much organic dry matter can be fed into digester, per volume and time unit, are key parameters that can affect gas yield. Temperature, pH-value, nutrient supply, stirring intensity, and amount of inhibitors can all affect gas production significantly. The differences between practical and potential biogas yields are often ascribed to the presence of inhibitors [127]. Material may be judged inhibitory when it causes an adverse shift in the microbial population or inhibition of bacterial growth, leading to lower or no methane production. A variety of compounds are known to inhibit anaerobic digestion, these include NH_3 (ammonia), H_2S (hydrogen sulphide), heavy metals, salts and polyphenols [40] (Table 3).

Temperature

Biogas formation can be achieved over a wide range of temperatures from: psychrophilic at < 20 °C, minimum retention time 70–80 days; mesophilic at 20–40 °C, minimum retention time 30–40 days; thermophilic at > 40 °C, and a minimum retention time 15–20 days [43]. Temperature is one of the most critical parameters in influencing microbial activity across all the major stages of anaerobic digestion, with a direct correlation between increased temperature and rates of reaction. Thermophilic conditions exceeding 40 °C lead to process intensification by reduced reactor size, shorter retention times and higher methane yields for terrestrial substrates [128]. However, the thermodynamic effects of higher temperature on

Table 3 Advantages and disadvantages of macroalgae as a feedstock for anaerobic digestion (modified from [4, 19])

Advantages	Disadvantages
• Anaerobic digestion can be performed on wet algal biomass negating the need for energy using dry methods [155]. Drying of seaweed is not required for anaerobic digestion • High polysaccharide content in seaweeds is favourable for anaerobic digestion [96] • Low or negligible amounts of recalcitrant lignin, low cellulose content and easily biodegradable sugars making algal biomass to methane by anaerobic digestion easier than lignocellulosic substrate [92, 101] • No competing with agricultural crops for land or freshwater [11] • Space efficient in terms of energy yield per unit area • Wastewater, brackish water and even seawater can be used for algal cultivation; therefore, water quality is less critical • Carbon dioxide sequestration as seaweeds convert carbon dioxide into biomass and exports significant quantities of detritus • Socio-economic benefits particularly in rural and coastal areas • Integration with other technologies. The anaerobic digestion process can be used as a co-technology for algal residues utilisation after biodiesel [4]	• In general, seaweeds contain significantly higher levels of ash, mainly chlorine and sulphur salts, than terrestrial biomass [82] • Methane production can be inhibited by high content of alkali earth metals, sodium concentrations above 10 g Na^+/L can strongly inhibit methanogenesis [125] • The combination of high sulphur along with nitrogen content, particularly in green seaweeds, can be problematic in biogas production due to NH_3 toxicity [92, 127, 156] • During anaerobic digestion seaweeds can produce high levels of hydrogen sulphide (H_2S), up to 3.5% making it unsuitable for energy recovery without specific treatment [140] • High fibre content can lower methane production as insoluble fibres can be difficult to degrade [96] • Polyphenols and tannins present in seaweed are potential inhibitors in anaerobic digestion [96, 127] • Lack of knowledge of the characterisation and biomethane potential, particularly seasonal variation, of selected seaweeds [4]

reduced CO_2 solubility and increased ammonia ionisation constant significantly reduces the reactor buffering capacity and lead to process instability that can cause reactor failure. A study by Sarker et al. [112] found that that by using acclimatised inocula the methane yields by *Laminaria digitata* were improved by 13% operating at 50 °C compared to 35 °C before a process instability caused an uncontrolled acidification and a reactor failure after 81 days. On a batch scale of 500 ml over 40 days, Membere and Sallis [111] observed that the relative performance of operating temperatures for the anaerobic digestion of Laminaria digitata has to be 55 °C > 25 °C > 35 °C > 45 °C. Though, a study by Vanegas et al. [101] examining the utilisation of five seaweed species to produce biogas and methane showed that digestion temperature influenced biogas production where reactors operating at 35 °C produced higher quantities than at 20 °C.

Inhibitory substances

Feedstocks can contain substances that can inhibit anaerobic digestion, and therefore, the levels of these inhibitory substances must be managed. A material may be judged inhibitory when it causes an adverse shift in the microbial population or inhibition of bacterial growth. Common inhibitors are ammonia, hydrogen sulphide, light metal ions, heavy metals and polyphenols. Hydrophobic free ammonia (NH_3) is the most common form of inhibition since it is membrane-permeable. Methanogens have the least tolerance to NH_3 inhibition amongst all the microbes in anaerobic digesters [129]. An investigation by Akunna et al. [126] looked at factors that could affect anaerobic degradation of *L. digitata* used as a co-substrate with terrestrial plant biomass. The authors speculated that *L. digitata* may contain certain compounds that even at trace concentrations appear to inhibit anaerobic microbes and these unidentified compounds appear to have a more adverse effect on methanogens than on other anaerobic digestion microbial groups. The authors recommended that an appropriate adaptation strategy involving an initial low proportion of seaweed relative to the total organic loading rate is necessary to ensure effective adaptation of microorganisms to inhibitory constituents of seaweed. The study also suggested that where the availability of seaweed was seasonal then a fresh adaptation or start-up procedure may be necessary during each cycle of seaweed availability to ensure sustainable process stability.

Sulphur

Macroalgae contain significantly higher amounts of ash consisting mainly of chlorine and sulphur salts than terrestrial biomass [59]. The green algae *Ulva lactuca* can have a sulphur content of up to 5 d.wt.% which leads to significant levels of hydrogen sulphide (H_2S) in anaerobic digestion [17]. In *Ulva* sp., the cell wall consists of up to 29% of ulvan, a sulphated polysaccharide [92]. While the sulphated polysaccharides fucoidan, ulvan and carrageenan contain up to 25%, 8% and 11% sulphur, respectively, on a dry basis depending on their degree of sulphation. Peu et al. stated that biogas from *U. lactuca* contained high levels of hydrogen sulphide up to 3.5% which resulted in a biogas being unsuitable for energy recovery without specific treatment. Hydrogen sulphide can be removed from biogas by using chemical absorbers (Fe^{3+} or NaOH) or adsorption on activated carbon and zeolite structures. One effective treatment widely used in biogas plants is biological desulphurisation [130]. This treatment is based on the introduction of a limited

volume of air into a gasometer. Hydrogen sulphide in the biogas is then oxidised into soluble sulphate by the oxygen contained in the air tanks by specific sulpho-oxidising microorganisms, such as *Thiomicrospira* sp. or *Thiobacillus* sp.

Phenolic compounds

Phenolic compounds are secondary metabolites not directly involved in algal primary processes that are characterised as stress compounds involved in chemical protective mechanisms against grazing, bacterial settlement and other fouling organisms, UV-radiation and metal contamination. In brown algae, phenolic compounds such as phlorotannins exhibit primary functions such as growth and development of cell walls with adhesive functionality. Polyphenols (such as phlorotannins) and tannins found in seaweeds are potential inhibitors in anaerobic digestion [131]. Polyphenols have been found to specifically inhibit alginate lyase, an enzyme that breaks down alginic acid [132]. Phlorotannins inhibit enzyme activities of various microbes, including methanogens, in the anaerobic digestion process. Polyphenol content of seaweed is dependent on harvesting time, location, light intensity, salinity, temperature and ambient nutrients [132].

Fucus serratus, found across the Atlantic coast of Europe, seems generally to be poorly biodegradable under anaerobic conditions, possibly due to a relatively high content of recalcitrant and inhibitory compounds such as polyphenols. Although inhibitory polyphenols are present in most brown seaweeds, their concentration in *Fucus* sp. has been reported to be up to 14% of the total solids, which is significantly higher than the 2% in *Laminaria* and *Saccharina* spp. [133, 134]. The brown seaweed *Ascophyllum nodosum* contains phlorotannins which inhibit the anaerobic digestion process. Seasonal variation in polyphenol content of *A. nodosum* has previously been described by Parys et al. with high polyphenol levels in the summer months having an adverse effect on biogas production, with suggested harvesting dates of *A. nodosum* in March and October in Ireland. Specific gas production from *A. nodosum* was reported as 50% less than with brown seaweed *Laminaria* spp. [131].

Halogenated hydrocarbons

Seaweeds produce a range of halogenated secondary metabolites, especially chlorinated, brominated and iodinated compounds, as part of their defence systems. The brown alga *Laminaria digitata* shows the strongest accumulation of iodine among all living systems, with more than 30,000 times the concentration found in seawater, or more specifically up to 1.2% dry weight [135]. Halogenated hydrocarbons have moderate to high toxicity, particularly for methanogenic archaea. Brominated halogens such as 2-bromoethanesulfonic acid (BESA) have been shown to competitively inhibit the methyl transfer reaction at the terminal reductive step during methane formation using H_2 and CO_2 [136]. As such, several brown seaweeds, including *Aschophyllum nodosum* and *Laminaria japonica,* have been found to reduce methane production from rumen fermentation [137]. In addition to enzyme inhibition, halogenated compounds have been found to disrupt membrane structures which, is further exacerbated by the limited anaerobic dehalogenation pathways [41]. Furthermore, halogenated organic compounds constitute a large group of environmental chemicals due to their use in industry and agriculture. Concerns over the environmental fate and releases of these halogenated organic compounds have resulted in research into their biodegradation which shows that many of these compounds are more easily degraded under anaerobic conditions. Biosorption via seaweed has become an alternative to the existing technologies in removing these pollutants [41].

Salts

Unwashed seaweed can contain significant quantities of non-volatile ash primarily consisting of water-soluble salts such as NaCl and KCl up to 20 wt% of the dry solids. During AD, the high salt content manifests as a microbial stressor due to the effect on microbial osmotic pressure [138]. This results in microbes expending energy removing excess ions, thereby reducing methane yields or at high concentrations, completely inhibiting microbial degradation. Patel et al. reported that sodium is essential for AD processes for cell growth and metabolism with an optimum concentration of 350 mg L^{-1} [139] above which, high concentrations of NaCl can affect methane production when marine algae are used for anaerobic digestion, with inhibitory effects at high levels (5–8 g L^{-1}) [139]. Acclimatised inoculum or inoculum sourced from the halophilic marine environment can stabilise the process. Tabassum et al. [74] noted that high salinities could be tolerated at low organic loading rates below 4 gVS L^{-1} d^{-1}. Alternatively, the use of a suitable pre-treatment to remove the salts from the substrate can prevent accumulation of salt in the reactor. However, desalination of macroalgae by heat or pressure may result in lower methane yields compared to untreated algae, possibly due to the loss of easily digested organic matter [140].

Heavy metals

Seaweeds accumulate heavy metals such as lead, cadmium, copper, zinc, nickel and chromium, which are well known toxicants for bacteria. Heavy metals can inhibit anaerobic digestion as low as 32 ppm with inhibition

patterns of (most toxic) Cu > Ni ~ Zn > Cr > Cd > Pb (least toxic) [141]. Several heavy metals such as Co, Ni and Se are essential trace elements for anaerobic digestion but at higher concentrations can cause substitutive ligand binding, inhibition of membrane-transport processes and electron siphoning [142, 143]. The negative effects can be decreased by precipitation with sulphide compounds. In anaerobic digestion, heavy metal contaminants from solid seaweed can be removed using a two-stage anaerobic system by mobilisation into a liquid phase and subsequent metal ions absorption [144, 145]. In addition, these heavy metals can accumulate in the digestate and may affect the value of the digestate as a fertiliser or soil conditioner [9, 146].

Future aspects and challenges

The use of macroalgae for biofuel production is still at an extensive research and development stage, e.g., on a laboratory and pilot-scale and there are very few macroalgae digesters on a commercial scale [20]. Each pilot project has focused predominantly on the technical aspects of biofuel production from macroalgae [147]. Among the various pathways for producing biofuel from seaweed anaerobic digestion is perhaps the closest to industrial exploitation [34]. A prime candidate for European kelp cultivation, *Saccharina latissima*, was estimated by Allen et al. [52] to achieve gross energy yields from anaerobic digestion at 365 GJ ha^{-1} a^{-1}, which was greater than those based on the current liquid biofuel systems such as ethanol from sugarcane (135 GJ ha^{-1} a^{-1}) and biodiesel from palm oil (120 GJ ha^{-1} a^{-1}). Allen et al. [46] based their estimates on a seaweed production of 300 t wwt ha^{-1} a^{-1}, which is at Northern Europe's very top range of productivity [7]. Achieving these high yields is essential for lowering the seaweed production cost and improving the commercial viability of anaerobic digestion. Further research is necessary to validate that high-yielding seaweed can produce similar methane yields to previous studies with wild or low-yield farmed seaweed.

Furthermore, practical yields of biogas from anaerobic digestion of seaweed can be considerably below the theoretical maximum [91]. To achieve high conversion rates several approaches should be considered.

- Pre-treatment of feedstock to aid hydrolysis of biomass can reduce digestion time
- Washing of biomass to remove impurities and reduce salinity
- Characterisation of seaweed biomass to determine chemical composition specifically with regards to structural polysaccharides
- Co-digestion of seaweed with carbon-rich biomass to balance the C/N ratio
- Optimisation of the microbial community by the selection of a suitable inoculum for marine biomass
- Optimisation of operational parameters such as organic loading rate (OLR), hydraulic retention time (HRT) and solids retention time (SRT)

The optimum approach will vary depending on the individual seaweed genera or species due to the variation between species in both recalcitrance and inhibitor profile. The characterisation and reporting of anaerobic digestion inhibitors in published literature is not widespread. Reporting on seaweed characterisation needs further improvement, specifically with regards to salts, phenolics and halogenated compounds. As the effects of each inhibitor have been investigated individually but the interaction between multiple inhibitors has not been investigated. Similarly, the positive effects of pre-treatments on methane yields have been investigated, though the mechanisms by which this occurs are not clearly defined due to the lack of consistent reporting of the pre-treatment process on inhibitor concentrations.

Finally, it is well-known that the initial microbial inoculum has a significant effect on the methane yields. However, due to a lack of commercial anaerobic seaweed digesters with acclimatised sludge, a mixture of terrestrial inocula have been utilised in the literature that may lack required functionality for marine polysaccharide hydrolysis and inhibitor tolerance. The development of marine inspired inocula may be necessary to find the required diversity for complete seaweed degradation. Studies with marine sediments have shown improved methane yields compared to wastewater sludge [70, 106, 148]. These studies indicate a potential reservoir of pre-acclimatised microbes with the required functionalities for the anaerobic digestion of seaweed may be available. The understanding of acclimatised sludge would potentially significantly increase biomethane yields at minimal extra expense that could improve the commercial viability of the bioenergy process.

Conclusions

Anaerobic digestion is largely the method of choice for biomass to energy conversion for feedstocks with high water content [104], such as seaweed as it readily tolerates biomass with high moisture content without the energy drawbacks from dewatering and drying as well as from an infrastructure and engineering perspective. There is a great potential for the substitution of conventional substrates with cultured seaweed biomass [97]. The majority of researchers evaluating the suitability of seaweed for anaerobic digestion agree it is generally appropriate [38]. Further research is required to improve the absolute methane yields from seaweed in a cost-effective

manner before wider adoption can be viable. However, methane production from seaweed is currently unprofitable primarily due to low methane yields.

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