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Green technology in green macroalgal biorefineries

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ABSTRACT

Marine biorefineries, based on macroalgal (seaweed) feedstocks, could provide sustainable alternative sources of food, energy, and materials. Green macroalgae, with their unique chemical composition, can contribute to marine biorefinery systems associated with a wide range of potential products. This review discusses the challenge of developing industrially relevant and environmentally-friendly green seaweed biorefineries. First, we review potential products from green seaweeds and their co-production, the key element in an integrated biorefinery. Second, we discuss large-scale cultivation, hydrothermal treatments, fermentation, anaerobic digestion, and emerging green solvents, pulsed electric field, microwave, and ultrasound processing technologies. Finally, we analyse the main polysaccharides in green seaweeds: sulfated polysaccharides, starch, and cellulose, as products of a cascading biorefinery, with emphasis on applications and technological challenges. We provide a comprehensive state-of-the-art review of green seaweed as feedstock for the biorefinery, analysing opportunities and challenges in the field.

INTRODUCTION

Marine macroalgae, seaweeds, are emerging feedstocks for the replacement of unsustainable fossil resources. Seaweeds can supply feedstock for biorefineries for the production of fuels, chemicals, food ingredients, pharmaceuticals, and more; thus, playing a major role in a future bio-economy (Balina et al. 2017).

Seaweeds are classified into red (Rhodophyta), brown (Phaeophyceae) and green (Chlorophyta) algae (Chen et al. 2015). Apart from colour, each group and species has its own habitat requirements, morphology, and chemical composition, leading to different possible applications and cultivation systems. Today, red and brown algae are more widely cultivated, mostly as sources of food, hydrocolloids, fertilisers, and animal feed (Jung et al. 2013). All groups contain varying amounts of ash (18%–55%), carbohydrates (25%–60%), proteins (5%–47%) and lipids (< 5%) (Barbot et al. 2016; Chemodanov et al. 2017a) which differ between species and are greatly influenced by biotic and abiotic habitat growth factors, such as temperature and light (Rodrigues et al. 2015a).

Both red and brown macroalgae are widely utilised commercially, mostly in Asia, as a food source (Radulovich et al. 2015) and for their unique polysaccharides (e.g. agarose and alginate) which serve as raw material for different industries (Mohamed et al. 2012). Similarly, green macroalgae are attractive based on their polysaccharide composition, which in addition to the common cellulose and starch, include large amounts of unique sulfated polysaccharides (SPs) (Barbot et al. 2016; Jung et al. 2013). These include ulvan in Ulva sp., sulfated rhamnan in Monostroma sp. and galactan in Codium sp. (Cho & You 2015). In addition, green algae are distributed globally from polar to tropical regions (Wiencke & Bischof 2012), and have a relative advantage in warmer climates, thus being less sensitive to climate change (Gao et al. 2017a; van Den Burg et al. 2013).

Marine biorefineries have been proposed as a sustainable alternative for fossil resources. Examining this proposition, the sustainability of seaweed biorefineries was assessed in various life cycle assessment (LCA) studies (Aitken et al. 2014; Alvarado-Morales et al. 2013; Czyrnek-Delêtre et al. 2017; Langlois et al. 2012; Seghetti et al. 2017, 2016; van Oirschot et al. 2017). Overall, seaweed cultivation has contributed to environmental restoration and climate mitigation. However, designing an environmentally-benign biorefinery process still requires optimisation of a few parameters: energy investment and materials used for cultivation, seaweed productivity and composition, energy invested in biomass drying, and chemicals used for biomass processing. Economic evaluation of such processes highlight the importance of reducing cultivation costs and integrating high-value co-products into the original bioenergy-oriented seaweed biorefinery concept (Aitken et al. 2014).

This review evaluates the opportunities and challenges of developing industrially relevant and environmentally friendly green seaweed biorefineries. First, we review potential products from green seaweeds and their co-production. Second, we discuss large-scale cultivation and survey traditional processing technologies. These include hydrothermal treatments, fermentation, and anaerobic digestion, along with emerging processing technologies, i.e. green solvents, pulsed electric field (PEF), microwave technologies (MWT), and ultrasound...
technologies (UT). Finally, we discuss the main green seaweed polysaccharides (SPs), starch and cellulose, as products of a cascading biorefinery, with an emphasis on applications and technological challenges.

**BIOREFINERY AND GREEN SEAWEED BIOREFINERY**

Biorefineries are the manufacturing units of bio-economies. In a biorefinery, one or several biomass feedstocks are processed into a wide range of products, including food, biomaterials, and biofuel. For the realization of a truly sustainable circular bio-economy, these manufacturing units must prove economic viability while following zero waste and minimal environmental impact. The integrated macroalgal biorefinery concept can support production of high-value products along with biofuels (Balina et al. 2017).

Seaweeds, including green seaweeds, have higher growth rates than terrestrial crops [e.g. 1460 g C m$^{-2}$ year$^{-1}$ for Ulva compressa Linnaeus and 696–4700 g C m$^{-2}$ year$^{-1}$ for Codium fragile (Suringar) Hariat, compared to 631 g C m$^{-2}$ year$^{-1}$ for rice and 378 g C m$^{-2}$ year$^{-1}$ for wheat; Chemodanov et al. 2017b]. In addition, green seaweed can tolerate wide variation in sea conditions, including salinity, irradiance and temperature (Kim 2015). The chemical composition of green seaweed biomass is interesting due to its unique SPs. This is in addition to its taste and texture attributes (Mohamed et al. 2012; see Shannon & Abu-Ghanam 2019). Due to these advantages, green seaweeds are one of the most promising alternative biorefinery feedstocks. Consequently, there has been a sharp increase in publications in the last decade (Baghel et al. 2015; Bikker et al. 2016; Fernand et al. 2017; Goh & Lee 2010; Ingle et al. 2017; Kerton et al. 2013; Lehahn et al. 2016; Seghetti et al. 2016; Wei et al. 2013).

**Green seaweed-derived products**

A wide range of products can be produced from green seaweeds. For instance, some green seaweeds are considered as a source of sold **foods**. These include proteins such as lectin and taurine; fibres such as the SPs ulvan; vitamins such as tocols; and antioxidants, e.g. carotenoids and chlorophylls, bromophenol and phloroglucinol (Holdt & Kraan 2014) with excellent nutritional properties (essential amino acids, polyunsaturated fatty acids and minerals) (Barrow & Shahidi 2007; Cardoso et al. 2015; Holdt & Kraan 2011; Pangestuti & Kim 2011; Sánchez-Machado et al. 2004; Satpati & Pal 2011). They can also be used as **animal and fish feed** (Abudabos et al. 2013; Ergün et al. 2008; Makkar et al. 2016; Singh et al. 2015, 2016). In addition, green seaweed have been extensively investigated for: (1) **energy and biofuel** production (Bruhn et al. 2011; Chen et al. 2015; Fernand et al. 2017; Hughes et al. 2012; Jiang et al. 2016a; Miledge et al. 2014; Neveux et al. 2015; Nikolaisen et al. 2011; Rocca et al. 2015; Sambusiti et al. 2015; Suutar et al. 2015); and (2) for **bioremediation and water treatment**, including fishpond and industrial effluent, due to their high capacity for uptake and accumulation of nutrients and metals (Kumar et al. 2016; Mata et al. 2016; Mwangi & Ngila 2012; Neori et al. 1996; Tsagkamilis et al. 2010; Zeroual et al. 2003). Moreover, green seaweed polysaccharides are excellent **biomaterials** with numerous applications including tissue engineering and papermaking (Bedoux et al. 2014; Castelló et al. 2016; Chen et al. 2016; Mihran et al. 2011; Toskas et al. 2011). In agriculture, green seaweed extracts have been used as **plant biostimulants** (Craigie 2011; Khan et al. 2009). Lately, their pigments have been investigated for **bio-electronic applications** such as in solar cells (Bella et al., 2015; Kuo & Sheen 2011).

**Co-production as a key element in biorefineries**

Current production processes involving seaweeds are still focused mostly on single products, while the leftover biomass is treated as waste. Similarly, most seaweed research programs focus on a single product, predominantly biofuel (Baghel et al. 2015; Gegg & Wells 2017; Jiang et al. 2016a; Murphy et al. 2013; Reith et al. 2005; van Hal et al. 2014). Recently, co-production of two or more products from green macroalgae as an integrated, cascading biorefinery has been used, thus maximising benefits of the biomass (Ben Yahmed et al. 2016; Bikker et al. 2016; Gajaria et al. 2017; Glasson et al. 2017; Magnusson et al. 2016; Mhatre et al. 2019; Pezoa-Conte et al. 2015; Postma et al. 2017; Trivedi et al. 2016; van der Wal et al. 2013).

A summary of recent green seaweed biorefinery studies is presented in Table 1. Gajaria et al. (2017) stands out in applying a full cascading biorefinery process to Ulva lactuca Linnaeus, and reports the extraction of five chemical products: minerals, lipids, ulvan, protein, and cellulose. However, the extraction of lipids and cellulose was not environmentally benign as it required hazardous organic solvents (chloroform and methanol) and chemicals (sodium chlorite and hydrochloric acid). Of note is that the yield of products extracted in this integrated sequential fashion is similar to those extracted in isolation. Trivedi et al. (2016) achieved in a sequential extraction yields (% of dry weight, DW) of 26% mineral rich liquid extract (MRLE), 2.8% total lipid, 25% ulvan, and 11% cellulose compared to the respective yields of 26%, 3%, 26%, and 12% in direct extraction. Gajaria et al. (2017) carried out a similar sequential extraction study and measured similar yields (19.9% ulvan, 11% protein and 12% cellulose). This suggests that co-extraction of various products in an integrated biorefinery approach does not have a major negative affect on product yield. In addition, chemical consumption in a cascading biorefinery is reduced to around 30–40%, reducing both the economic and environmental costs of biomass processing (Trivedi et al. 2016). Hence, integrated sequential extraction of salt, lipid, ulvan, protein, and cellulose is recommended for utilising the full potential of the biorefinery. Specific design and methods are discussed in the next sections.

**Biorefinery design**

A sustainable biorefinery design should enable industrial production with minimum environmental impact. Therefore, extraction procedures should be chosen and integrated wisely.
The process design itself is important for solving some of these challenges. Suitable protocols should be chosen for extraction so that structural and functional properties of different products are maintained. Thus, specific and non-destructive processes must be applied first for extraction of sensitive products (Balina et al. 2017). Once sensitive molecules are recovered, more severe and destructive methods can be applied on remaining biomass to convert it to monosaccharides that can be later fermented into organic chemicals or biofuels. Sequential reduction of residual biomass after each step reduces energy and reagent demand and improves the yield of downstream extraction.

Based on the various integrated biorefinery concepts mentioned above, a process design was developed for the co-production of maximum products and applications (Fig. 1). An important preliminary step is ash (salt) removal which structural and functional properties of different products are maintained. Thus, specific and non-destructive processes must be applied first for extraction of sensitive products (Balina et al. 2017). Once sensitive molecules are recovered, more severe and destructive methods can be applied on remaining biomass to convert it to monosaccharides that can be later fermented into organic chemicals or biofuels. Sequential reduction of residual biomass after each step reduces energy and reagent demand and improves the yield of downstream extraction.

Table 1. Green seaweed-based biorefinery studies for Ulva sp. carried out for production of various products.

<table>
<thead>
<tr>
<th>Species</th>
<th>Biorefinery products</th>
<th>Technologies/Methods</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulva lactuca</td>
<td>Proteins and carbohydrates</td>
<td>Osmotic shock, enzymatic hydrolysis,</td>
<td>Postma et al. (2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pulsed electric field (PEF) or high shear homogenisation</td>
<td></td>
</tr>
<tr>
<td>U. lactuca</td>
<td>Animal feed, acetone, butanol, ethanol, and 1,2-propanediol</td>
<td>Thermal and enzymatic hydrolysis and fermentation</td>
<td>Bikker et al. (2016)</td>
</tr>
<tr>
<td>Chaetomorpha</td>
<td>Bioethanol and biogas</td>
<td>Thermo-chemical and enzymatic hydrolysis,</td>
<td>Ben Yahmed et al. (2016)</td>
</tr>
<tr>
<td>Linum O.F. Müller</td>
<td></td>
<td>fermentation and anaerobic digestion</td>
<td></td>
</tr>
<tr>
<td>U. fasciata</td>
<td>Mineral rich liquid extract (MRLE), lipid, ulvan, and cellulose</td>
<td>Mechanical grinding, thermal and chemical extraction and water extraction</td>
<td>Trivedi et al. (2016)</td>
</tr>
<tr>
<td>U. ohnoi, U. tepida</td>
<td>Mainly salt (demonstrating the use of leftover biomass for protein, fertiliser, animal feed and fuel)</td>
<td>Aqueous washing and drying</td>
<td>Magnusson et al. (2016)</td>
</tr>
<tr>
<td>Pesoa-Conte et al. (2015)</td>
<td>Liquid stream with carbohydrate and salt; a remaining stream with concentrated protein</td>
<td>Ionic liquid deconstruction</td>
<td></td>
</tr>
<tr>
<td>U. rigida</td>
<td>Acetone, Butanol, and Ethanol (ABE)</td>
<td>Pre-treatment, enzymatic saccharification, and fermentation</td>
<td>van der Wal et al. (2013)</td>
</tr>
<tr>
<td>U. ohnoi</td>
<td>Salt, pigment, ulvan, and protein</td>
<td>Aqueous pre-treatment, thermal and chemical extraction</td>
<td>Glasson et al. (2017)</td>
</tr>
<tr>
<td>U. lactuca</td>
<td>MRLE, ulvan, protein and methane</td>
<td>Aquous thermal and chemical extraction and anaerobic digestion</td>
<td>Mhatre et al. (2019)</td>
</tr>
</tbody>
</table>

The process design itself is important for solving some of these challenges. Suitable protocols should be chosen for extraction so that structural and functional properties of different products are maintained. Thus, specific and non-destructive processes must be applied first for extraction of sensitive products (Balina et al. 2017). Once sensitive molecules are recovered, more severe and destructive methods can be applied on remaining biomass to convert it to monosaccharides that can be later fermented into organic chemicals or biofuels. Sequential reduction of residual biomass after each step reduces energy and reagent demand and improves the yield of downstream extraction.

Based on the various integrated biorefinery concepts mentioned above, a process design was developed for the co-production of maximum products and applications (Fig. 1). An important preliminary step is ash (salt) removal which increases organic biomass content and improves biomass quality before further processing. This also reduces the inhibitory effects of ash on various processes such as hydrothermal treatment, enzymatic hydrolysis, fermentation, and anaerobic digestion. Initial salt removal can be done by washing biomass with distilled water (Glasson et al. 2017; Magnusson et al. 2016). Alternatively, salt can be removed quickly and efficiently by PEF (Robin et al. 2018b). Another option could be the initial grinding of fresh biomass, producing MRLE (Gajaria et al. 2017; Trivedi et al. 2016). Our recent work showed that the liquid fraction obtained from such approaches can be processed to extract starch in its native form (Prabhu et al. 2019). Once the solid fraction is separated from the liquid extract, it can be processed for extraction of pigments and lipids using ethanol (Glasson et al. 2017) or a chloroform-methanol mixture (Gajaria et al. 2017). Next, residual biomass can be processed for SPs extraction using hot (85°C) dilute hydrochloric acid (0.05 M; Glasson et al. 2017), after which protein can be extracted from leftover biomass by an alkaline extraction, using a 1 N sodium hydroxide solution (Gajaria et al. 2017). It should be noted that although alkaline extraction of proteins is quick and widely used, it results in low yields (e.g. 15.3% of total protein, Angell et al. 2017). Higher protein yields (22%) can be extracted following a prolonged aqueous-alkaline method reported by Angell et al. (2017). Finally, the remaining biomass, which is mostly cellulose, can be anaerobically digested to produce biogas, or hydrolysed and subjected to microbial fermentation to produce organic chemicals. Alternatively, cellulose can be extracted and used as raw material for various industrial applications (Ng et al. 2015).

Finally, as demonstrated in various studies, state-of-the-art designs of seaweed biorefineries utilise specific properties of different biomass fractions to allow cascading extraction of multiple products. However, specific extraction technologies, which are discussed below, require further research to achieve maximal yields and minimal environmental impact.

CULTIVATION

Looking towards sustainable large-scale biorefineries, macro-algal feedstock cannot be based on the harvesting of wild stocks or on cultivation in onshore or near shore farms. Wildstock harvesting leads inevitably to over-exploitation, while on- and near shore farming competes with food crops and coastal uses (Buschmann et al. 2017; Espi et al. 2019) and is limited by decreasing available areas (Möller et al. 2012). The main solution to withstand these challenges and to obtain global implementation, is offshore cultivation (e.g. Azevedo et al. 2019).

With increasing awareness of environmental effects of the industrial era (Suutari et al. 2015), scientific study of with offshore biomass cultivation, which developed during the 1960’s through to the 1980’s (Roesijadi et al. 2008, 2010), has become significant again (Buck et al. 2004; Buck & Buchholz 2005, 2004; Hughes et al. 2012; Korzen et al. 2016; Reith et al. 2005; Roesijadi et al. 2008, 2010; Suutari et al. 2015; van Den Burg et al. 2013). Although previous techno-economic assessments were not favourable for offshore algal cultivation (Golberg & Liberzon 2015; Roesijadi et al. 2008, 2010), four decades of technological evolution and the current political-environmental context, have led to a re-examination
of this approach. This technological evolution includes experience gained through oil and gas exploration, advancements in oceanographic and atmospheric science, major improvements in both tensile strength and weight of materials that can be used at sea, and improved understanding of seaweed life cycles (Roesijadi et al. 2008; Santelices 1999). One example is the development of flexible and submersible offshore aquaculture structures, such as the SUBFLEX, which has been used in offshore Israel since 2006 for fish cultivation (Drimer 2016). Simultaneously, the establishment of offshore wind farms (Reith et al. 2005) and the inevitable distancing of aquaculture facilities from the coast (Troell et al. 2009) facilitated potential reduction in cultivation costs via integration of infrastructure and operations (Buck & Buchholz 2004; Reith et al. 2005).

Improved knowledge of the life cycle of different species enables a better design of a complete cultivation cycle. Therefore, macroalgae cultivation systems may include multiple cultivation steps, combining intensive on-land tanks or ponds and extensive open-sea systems (Buschmann et al. 2017; Santelices 1999). In addition, a hatchery/nursery may be used for a preliminary stage before sea cultivation, enabling continuous cultivation with lower dependence on seasonality effects and lower susceptibility to biomass degradation, diseases, and pests (Gupta et al. 2018). For example, by controlling the timing of germination of Ulva sp. or by preserving sporelings in a hatchery (Gao et al. 2017b), cases of sudden sporulation of adult thalli, which are common for this species (Niesenbaum 1988), may be decreased.

Traditional offshore algal cultivation systems include ropes, lines, nets, rafts, and cages, all of which are popular due to inexpensive installation and maintenance (Fernand et al. 2017). Table 2 presents results from near- and offshore Ulva sp. cultivation experiments in net and raft systems. Whereas Korzen et al. (2016) and Chemodanov et al. (2017b) assessed the productivity potential of Ulva sp. near- and offshore in the Eastern Mediterranean Sea, the goal of Liu et al. (2010) was to examine the potential for opportunistic Ulva prolifera O.F. Müller and Ulva intestinalis Linnaeus to exploit aquaculture rafts and cause green-tide events. More advanced systems that may be adjusted for green seaweeds are the offshore-ring (Buck et al. 2004; Buck & Buchholz 2005), an easy-to-handle base for rope cultivation, and the moored, multi-body seaweed farm (Olanrewaju et al. 2017), both of which were designed to withstand rough offshore conditions.

Different approaches have been suggested for future design of offshore cultivation systems. The commonly used extensive approach allows the algae to grow without adding nutrients or applying external mixing. The main advantage of this approach is a decrease in labour, technology, and energy inputs, thus

![Fig. 1. Green macroalgal biorefinery process for co-production of a wide range of valuable products.](image)
improving energy balance; the main disadvantage is decreased 
biomass yields, leading to a large area-demand (Buck et al. 2008). 
Extensive cultivation can be performed on anchored platforms or 
on free-floating enclosures (Roesijadi et al. 2008).

Free-floating enclosures can be released in areas with pre-
dicted currents, or alternatively followed with tracking 
devices, and harvested when time and location are suitable 
and biomass is satisfactory. Notoya (2010) proposed growing 
seaweed beds on 100 km² rafts, floating away from shipping 
lanes. This concept, even on much smaller scales, is challenge-
ging due to the need to design robust and self-sustained 
cultivation systems that can withstand harsh offshore condi-
tions. Furthermore, this concept may be suitable only to 
particular species, such as Sargassum, which are stiff and 
have internal floating mechanisms (Radulovich et al. 2015).

 Anchored platforms can be sited in areas that are favourable 
for cultivation, aiming for optimal temperature and sunlight as 
well as water motion sufficient to break down diffusion barriers 
and natural supply of nutrients, e.g. in natural upwelling zones 
(Roesijadi et al. 2008). Furthermore, cultivation platforms can 
be located in eutrophic regions, combining environmental biore-
mediation with biomass production (Cui et al. 2019; Fei 2004; 
Xu et al. 2011). When environmental concentrations of nutrients 
are low, nutrients may be provided by artificial upwelling of deep 
nutrient-rich water as suggested in the 1970s in the Marine 
Biomass Program (Roesijadi et al. 2008, 2010). The main obsta-
cle for applying this concept is the high energy requirement of 
pumping large volumes of water from depths of hundreds of 
meters. Therefore, artificial upwelling may become feasible only 
when combined with offshore, self-sustained power sources. An 
interesting venture can be the integration of deep seawater 
pumping for nutrient supply with ocean thermal energy conver-
sion (OTEC) technology, which utilises deep seawater for power 
generation based on temperature difference with surface water 
(Roëls et al. 1979). This technology, now in early stages of 
implementation, is relevant only for regions where temperature 
difference between surface and deep water is high enough (i.e. 
above 10 °C), such as in tropical regions (Roëls et al. 1979). 
Another solution for supplying nutrients offshore is multi-
trophic aquaculture, also known as Integrated Multi-trophic 
Aquaculture (IMTA; Ashkenazi et al. 2018; Fernández et al. 
2019; Neori et al. 2004). This approach, used in large scale 
near- and onshore seaweed cultivation facilities, can significantly 
increase system sustainability. The underlying theory of IMTA is 
that waste nutrients from higher-trophic-level species can be 
recycled into the production of lower trophic-level crops of 
commercial value, such as macroalgae (Troell et al. 2009).

Theoretically, co-cultivation of different seaweed species can 
increase productivity by increasing light harvesting efficiency. 
This can be done, for example, in a layered seaweed cultivation 
system, employing typical light absorption characteristics of 
green, brown and red macroalgae, thus improving light use 
(Fernández et al. 2019; Reith et al. 2005).

In contrast to the extensive approach, the intensive 
approach emphasises maximal biomass yields, even at the 
expense of energy costs. Golberg & Liberzon (2015) modell-
ed smart mixing regimes to improve biomass productivity by 
enhancing light harvesting and carbon fixation. Mixed water 
cultivation is commonly applied to onshore reactor cultiva-
tion of free-floating green algae (Chemodanov et al. 2017a). 
However, whether free-floating algal cultivation offshore, 
mixed or non-mixed, is challenging due to strong ocean 
currents and increased loss risks, which may lead to uncon-
trolled macroalgal blooms (Liu et al. 2009). Furthermore, the 
energetic and technical feasibility of mixing seaweed in off-
shore cultivation farms is yet to be assessed.

Lehahn et al. (2016) analysed the global potential of offshore 
Ulva biorefineries to provide food, chemicals, and energy. In 
addition, this analysis located suitable cultivation areas, defined 
distance and depth limitations, and analysed environmental risks 
and benefits of large-scale offshore macroalgal cultivation. Finally, 
although offshore cultivation of macroalgae is regarded as 
a sustainable alternative biorefinery biomass source, it faces 
major challenges before it can meet the requirement for 
a consistent supply of high-volume feedstock. These challenges 
include rough offshore conditions that increase construction and 
maintenance costs (Azevedo et al. 2019; van Den Burg et al. 2013), 
a need for mechanised harvesting solutions for non-linear algal 
morphologies (Roesijadi et al. 2010), scarce nutrients and expen-
sive fertilization (Reith et al. 2005; Roesijadi et al. 2008), losses 
and pests that may decrease productivity (Ingle et al. 2018; Rocca 
et al. 2015), incomplete understanding of life cycle of some species 
(Gupta et al. 2018), and potential ecological effects that require 
进一步 research (Lehahn et al. 2016; Roesijadi et al. 2010).

### PROCESSING

#### Bio- and thermochemical conversion

Cultivation and subsequent harvesting of seaweeds are only 
the first steps of ‘resource to product’ processing, supplying 
the feedstock for the biorefinery. Next, post-harvest treat-
ments such as cleaning, washing, size reduction, preserva-
tion, drying, storage, and energy extraction are applied
(Milledge et al. 2014). Biorefinery conversion technologies can be divided into those requiring a drying step, and those that do not. Conversion methods such as direct combustion, pyrolysis, gasification, and transesterification to biodiesel, require dry input (Milledge et al. 2014). Although drying algae can extend storage time and decrease feedstock transport cost (Lehahn et al. 2016), it requires high energy inputs (Milledge et al. 2014); this is problematic if the energy source is expensive or non-renewable. In addition, these methods are less suitable for seaweeds due to their high ash and alkali contents (Na and K; Bruhn et al. 2011). For this reason, we next focus on conversion methods that do not require drying and can utilise wet algae, such as hydrothermal treatments, fermentation and anaerobic digestion (Milledge et al. 2014).

HYDROTHERMAL TREATMENTS: Hydrothermal subcritical water technologies utilise liquid state high-pressure–high-temperature (100 to 374 °C) water to process biomass into a variety of products (Cocero et al. 2018). These can be used as, or further processed to, different types of biofuel and other products such as artificial soil, fertilisers, activated carbon, and more (Libra et al. 2011). Hydrothermal hydrolysis, which occurs in water heated to 100 to 240 °C, is the break down of polymers into monomers such as simple sugars, which can be fermented into organic chemicals such as ethanol, butanol, and acetone (Roesijadi et al. 2008). In hydrothermal carbonisation (HTC), which occurs in water heated to 180 to 250 °C, the carbon fraction in the solid residue (hydrochar) (Kambo & Dutta 2015) is enhanced, thus providing carbon-based products (Libra et al. 2011) and increasing the residue’s caloric value. In hydrothermal liquefaction (HTL), which occurs at water temperatures above 280 °C, biomass is liquefied to a high-energy liquid bio-oil (Toor et al. 2011). This bio-oil can be upgraded using different techniques (solvent addition, emulsification, esterification, hydro or zeolite cracking and others) and refined for different fuel applications (Saber et al. 2016).

Results from hydrothermal treatments performed on green seaweed biomass are presented in Table 3. Daneshvar et al. (2012), for example, reported that soluble sugar production in C. fragile began at 170 °C and reached a maximum at 210 °C where more than 50% of dry algal biomass was converted to soluble carbohydrates. The higher heating value (HHV), calculated by the Boie equation (1) (Mason & Gandhi 1980) increased constantly from 140 to 230 °C, until a maximum of 22.6 MJ kg⁻¹, an increase of 6 MJ kg⁻¹ compared to initial algal HHV.

\[ Q = 151.2C + 499.77H + 45.05S - 47.7(O) + 27.0N \]  

where \( Q \) is the gross heating value in Btu lb⁻¹ on a dry basis and \( C, H, S, (O) \), and \( N \) are respective contents of carbon, hydrogen, sulphur and oxygen in DW percent.

Hydrothermal hydrolysis of green seaweeds, which does not involve hazardous chemicals, can potentially serve as a preliminary, green fermentation step. However, this technology is not yet efficient enough, and faces optimisation challenges due to temperature overlaps with monosaccharide deconstruction (Toor et al. 2011) and carbonisation processes. In this instance, HTC and HTL have the advantage of achieving possible full conversions into hydrochar or bio-oil. However, beyond the high energy consumption disadvantage, large-scale implementation of HTC and HTL on seaweeds is challenging because of high ash content, which causes fouling issues in large-scale continuous flow reactors due to presence of alkali metals, earth alkaline metals or halides (Neveux et al. 2014a). This problem may be solved by pre-treatment ash removal, for example by PEF (Robin et al. 2018b) or rinsing (Neveux et al. 2014a).

FERMENTATION FOR ETHANOL PRODUCTION: Ethanol is a common fermentation product and is commonly blended into transportation fuels (Mussatto et al. 2010; Wang et al. 2011b). Ethanol production from seaweeds was examined by the American government as an economic bio-alternative to fossil fuels in the 1980’s (Mcintosh 1985; Wagener 1981).

Prior to fermentation, polysaccharides in seaweeds must be hydrolysed into monosaccharides. Green macroalgae contain several distinctive monosaccharides, including rhamnose, xylose, gluconic acid and more (Kim et al. 2011; Robin et al. 2017). Acid hydrolysis is widely used in biomass degradation. However,
a detailed parameter optimisation of thermochemical hydrolysis of Ulva was performed only recently (Jiang et al. 2016b). Although common, acid hydrolysis produces non-sugar by-products, causing environmental hazards and slowing subsequent fermentation (Palmqvist et al. 1999). These by-products include formic acid, levulinic acid, acetic acid, 5-hydroxymethylfurfural (HMF), phenols and heavy metals (Trivedi et al. 2015; Wu et al. 2014). An alternative which is considered environmentally friendlier, but slower (more than a day compared to less than an hour) and more expensive, is enzymatic hydrolysis (Trivedi et al. 2015). Effective enzymatic hydrolysis requires a pre-treatment which enables the enzymes better accessibility to the cellulose by increasing surface area and removing structural interference (for example hemicellulose; Alvira et al. 2010). This energy and/or chemically demanding pre-treatment can be eliminated if hydrolysis is performed after prior extraction of other biomass fractions, in a biorefinery cascading extraction process. An optimisation study by Trivedi et al. (2013) for Ulva fasciata Delille achieved a maximal sugar yield of 206.82 ± 14.96 mg g⁻¹ after pre-heat treatment in aqueous medium at 120 °C for 1 h, followed by incubation in 2% (v/v) of cellulose 22119 for 36 h at 45 °C. This study also confirmed that enzymes can be used twice without compromising saccharification efficiency, which is an industrial and environmental advantage. Suitable enzymes include cellulase for cellulose (El-Dalaty et al. 2016; Trivedi et al. 2015), amylglucosidase and α-amylase for starch (Korzen et al. 2015a), and advanced enzymatic complexes such as Viscozyme, combining cellulase, β-glucanase, hemicellulase and xylanase (Kim et al. 2014). Another hydrolysis method is hydrothermal hydrolysis (discussed below).

The most effective fermenting microorganism known today is Saccharomyces cerevisiae. This yeast has high fermentation rates for glucose, fructose, and mannose (van Maris et al. 2006), and in anaerobic conditions achieves high yields of ethanol (Wang et al. 2004). However, saline environments are toxic to S. cerevisiae (Tekarslan-Sahin et al. 2018), and therefore algal pre-washing is required (Roesijadi et al. 2010). Pre-wash may be avoided in the future by utilising newly developed salt-resistant strains (Tekarslan-Sahin et al. 2018). In addition, this yeast has limited fermentation of non-glucose sugars. Therefore, Escherichia coli was genetically engineered to produce ethanol from pentose and hexose sugars, offering a seaweed fermentation alternative (Asghari et al. 1996). Thus, E. coli can be used to ferment monosaccharide sugars such as rhamnose and glucuronic acid, which occur in large quantities in green algae (Kim et al. 2011), but with lower ethanol yields (Kim et al. 2011; Saha et al. 2003). Other alternative microorganisms are Clostridium species that produce acetone, butanol, and ethanol by anaerobic fermentation from a variety of sugars (van der Wal et al. 2013).

Fermentation can be performed subsequent to hydrolysis, in the separate hydrolysis fermentation (SHF) method, or simultaneously with hydrolysis, in the Simultaneous Saccharification and Fermentation (SSF) method (Golberg et al. 2014). In SHF, both hydrolysis and fermentation steps can be optimised separately, enabling higher sugar and ethanol yields. However, this comes at a cost of time and capital (Olofsson et al. 2008). In comparison, SSF saves time and reduces steps and capital costs. However, the prominent downside of SSF is operating the enzymes in sub-optimal temperature conditions, leading to lower yields of sugar and ethanol (Olofsson et al. 2008). For future optimisation, Vitkin et al. (2015) have built BIO-LEGO, a web-based application for biorefinery design and evaluation of serial biomass fermentation. Results from ethanol production experiments performed on Ulva sp. are shown in Table 4.

Fermentation is a long known and well-industrialised process. However, fermentation of marine biomass still needs to be optimized. Carbohydrates from green seaweed include very low lignin ratios compared to terrestrial crops (Dave et al. 2013); this is an advantage due to structural interference of lignin with cellulose extraction (Cheng 2017). However, a major challenge is maximising the conversion ratios of monosaccharides to ethanol, which is challenging due to limitations of the fermenting organisms to ferment non-glucose sugars. In addition, this partial conversion leads to high organic matter content in process effluent, which requires additional treatment before discharge (Pimentel 2003). Other challenges relate to the hydrolysis step that is subject to toxicity or efficiency problems, depending on the method used. Finally, despite its potential, sustainable ethanol production from algae requires more research to increase yields prior to future implementation.

ANAEROBIC DIGESTION: Algae are potential sources of anaerobic digestion (AD). The AD product is biogas, a mixture containing about 60–70% methane, 30–40% CO₂ (Hosseini & Wahid 2014), and variable trace amounts of CO, N₂, O₂, H₂ and the undesirable H₂S. H₂S must be removed prior to downstream conversion (Roesijadi et al. 2010). Biogas can be exploited directly as fuel or used as raw material for production of synthetic gas or hydrogen (Hosseini & Wahid 2014; Song et al. 2015).

Early attempts to cultivate algae as AD feedstock were performed in the United States during the 1970’s and 1980’s (Roesijadi et al. 2010). Later, green algal genera such as Ulva were examined (Habig et al. 1984). A major advantage of green algae is their low lignin content, which is beneficial for methane generation (Dave et al. 2013). For instance, U. lactuca contains 1.56 ± 0.08 lignin (% w/w on dry basis; Yaich et al. 2011). In addition, Ulva and Derbesia tenuwissima (Moris & De Notaris) P.Crouan & H.Crouan demonstrated high production potentials of 45–56 and 138 tons DW per

<table>
<thead>
<tr>
<th>Species</th>
<th>Hydrolysis method</th>
<th>Total sugar (mg g⁻¹ DW)</th>
<th>Microorganism</th>
<th>Ethanol yield (g/g sugar)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>U. lactuca</td>
<td>Acid + enzyme</td>
<td>343</td>
<td>Clostridium beijerinckii</td>
<td>0.4</td>
<td>Bikker et al. (2016)</td>
</tr>
<tr>
<td>Ulva sp.</td>
<td>Enzyme</td>
<td>200</td>
<td>E. coli Ko11¹</td>
<td>0.4</td>
<td>Kim et al. (2011)</td>
</tr>
<tr>
<td>U. fasciata</td>
<td>Hot buffer + enzyme</td>
<td>± 112</td>
<td>S. cerevisiae MTCC No. 180</td>
<td>0.47</td>
<td>Trivedi et al. (2015)</td>
</tr>
<tr>
<td>U. lactuca</td>
<td>Acid</td>
<td>113</td>
<td>S. cerevisiae</td>
<td>0.55</td>
<td>El-Sayed et al. (2016)</td>
</tr>
</tbody>
</table>

¹E. coli Ko11 was modified to allow more efficient sugar utilisation
hectare per year, respectively (Bruhn et al. 2011; Mata et al. 2016; Prabhu et al. 2019), and high carbohydrate content of up to 58% of DW (Rasyid 2017). This combination of rapid growth, high carbohydrate, and low lignin levels makes Ulva an appropriate biomass for biogas production (Dave et al. 2013; Table 5). However, pre-processing such as washing is necessary (Roesijadi et al. 2010) to prevent salt inhibition. Inhibition can be caused also by increased H_2S content. This is because sulphur, which appears in green algae mainly as SPs, dimethyl sulfiniopropionate (DMSP) and sulphur-containing amino acids (Stefels 2000; Wang et al. 2014) is only partly removed during washing (Bruhn et al. 2011). Additional pre-treatment procedures such as mechanical maceration, drying, thermal treatment or solid/liquid separation, can further increase methane yields, but again, these come with an energetic cost (Nikolaisen et al. 2011). Methane production is also affected by feedstock C:N ratio, as high nitrogen may be harmful to methanogenic microorganisms. Compared to the optimal range of 15.5–19 C:N (Sievers & Brune 1978), Ulva can be found in a wide C:N range of 7.9 up to 24.4 (Bruhn et al. 2011), depending on culture conditions.

Finally, although green algae have high potential for methane production, they are still not widely implemented as an AD source because of high cultivation and pre-treatment costs, and the difficulty of ensuring reliable and constant feedstock supply. However, a major opportunity may be co-utilisation of seaweeds for bioremediation and bio-energy needs. Another issue is high biomass water content, which, until cost-efficient concentrating methods are developed, leads to AD digesters too big to be economically feasible (Bruhn et al. 2011).

### Green solvents for clean processing

Most biomass processes take place in liquid media. Thus, solvents become a major process input. Their cost, availability, and recyclability are crucial. Moreover, with the strengthening of environmental and safety regulations, solvents that are hazardous, polluting and/or non-renewable (such as petroleum-based solvents) are becoming less relevant (Chemat et al. 2012). The quest for green solvents has therefore been a key step toward green processing and green chemistry. This section presents some major green solvents, i.e. water, bio-based solvents, supercritical fluids, and ionic liquids, and their application for algal processing.

Water is by far the most used green solvent, and is the solvent of choice for any green process, notably because it is also a major component of fresh biomass (Chemat et al. 2012; Herrero & Ibáñez 2015; Rombaut et al. 2014). Since ‘classic’ uses of water, e.g. in acidic, enzymatic, and hydrothermal treatments, are discussed above, here we present a new, water-based, catalyst-free process: pressurized liquid extraction (PLE). PLE is the reduction of high water polarity by changing temperature or pressure, leading to extraction of low-polarity components, that are usually poorly water soluble (Herrero et al. 2006). Classic solid–liquid extraction methods are sometimes enough to extract compounds with low polarity from seaweed (Tierney et al. 2013). However, PLE potential was demonstrated by Fayad et al. (2017) for Padina pavonica (Linnaeus) Thivy. Here, PLE (two 60-second cycles at 60 °C and 150 bar) was compared to other green extraction technologies (i.e. supercritical fluid extraction, electroporation extraction and microwave-assisted extraction). Both PLE and microwave-assisted extraction achieved the most efficient extraction of the cosmetically valuable anti-hyaluronidase. The potential of PLE for green seaweeds has yet to be shown.

Bio-based solvents are renewable and usually less toxic and more environmentally friendly than their petroleum counterparts including ethanol, acetone, butanol, glycerol, methanol and limonene, which can be used to extract low polarity components from various biomass, including green seaweeds. Simple solid–liquid extraction, or enhanced processes can be used (e.g. ultrasound technologies (UT), microwave technologies (MWT) or pulsed electric field (PEF) (Herrero & Ibáñez 2015). These solvents can be used alone, blended with other solvents (usually water) or as a diphasic solvent system. They are effective in extracting phenolic compounds, lipids or polysaccharides from green seaweed (Cho et al. 2010; Ray & Lahaye 1995; Wang et al. 2009).

Supercritical fluids (SF) are green solvents in temperature and pressure conditions above their critical point, which gives them a unique set of properties of liquid and gas (Herrero et al. 2006; Turner 2015). Those properties can enhance performance during extraction of reaction catalysis, but they can also be tuned according to process temperature and pressure, making it a versatile and robust method (Herrero & Ibáñez 2015; Turner 2015). The most used SF is CO_2, due to its moderate critical state condition, non-toxicity, and high recyclability. However, CO_2 applications are limited due to its very low polarity, unless mixed with a co-solvent, such as bio-based green solvent or water, thus improving its efficiency for extraction of polar compounds (Herrero et al. 2006; Turner 2015). Today, applications on green seaweeds focus on the extraction of lipids, phenolic compounds, pigments, fibres and other bioactive compounds (Fabrowska et al. 2017; Messyasze et al. 2017; Michalak et al. 2017, 2016).

Ionic liquids (ILs) are a relatively new type of green solvent ILs are made of organic ionic compounds that are liquid and stable at room temperature (Irik et al. 2014). Although there is some controversy regarding the environmental toxicity of some ILs (Cvjetko Bubalo et al. 2014), they have proven excellent at dissolving what other green solvents could not, such as cellulose (Irik et al. 2014). Therefore, ILs could play a role in the extraction, recovery and hydrolysis of cellulose (Pezoa-Conde et al. 2015). However, the efficiency needs to be compared to other processes (Jmel et al. 2017). Additionally, ILs can help in hydrolyse various non-green seaweeds and a

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**Table 5. Potential methane yield for green macroalgae.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Reported potential methane yield</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulva sp.</td>
<td>0.22–0.33 m^3 kg^-1 volatile solids (VS)</td>
<td>Roesijadi et al. (2010)</td>
</tr>
<tr>
<td>U. lactuca</td>
<td>4000–7000 m^3 Ch4 hectare^-1</td>
<td>Bruhn et al. (2011)</td>
</tr>
<tr>
<td>Cladophora</td>
<td>0.48 m^3 kg^-1 VS</td>
<td>Gunaseelan (1997)</td>
</tr>
</tbody>
</table>
extracting seaweed polysaccharides or proteins (Gerenuk et al. 2017; Malihan et al. 2014; Martins et al. 2016; Uju et al. 2015).

Finally, solvent choice is important for solubilising the target compound(s). Achieving enhanced efficiency will usually depend on increased mass transfer, solvent diffusion, and tissue damage, that can be promoted by stirring or combining other extraction technologies such as UT, MWT or PEF. This was reviewed recently by Cikoš et al. (2018), who focused on extraction of bioactive molecules from seaweed, utilizing technologies such as UT, MWT, PLE and SF.

Emerging ‘smart’ technologies

Increased interest in biomass processing has pushed investigations of new smart technologies. Here, we refer to smart processing technology as a technology with some of the following attributes: energy-efficient, quick, non-chemical (not requiring addition of chemicals except water), zero-waste, non-hazardous, environmentally friendly, scalable, low cost, applicable to untreated biomass, versatile, and combinable with other green processes. Such technologies lead to enhanced efficiency. PEF, MWT and UT are examples for such technologies, and application to seaweed processing are presented below.

PULSED ELECTRIC FIELD: Pulsed electric field (PEF) is a versatile technology that combines an environmentally friendly process with a unique output — increased permeability of cell membranes. This phenomenon, called electro-permeabilisation or electroporation, is created by applying pulses of the electric field. PEF has been used to introduce molecules (i.e. drugs, DNA and dyes) into cells, to extract intracellular components (i.e. water, ions, sugars, proteins and secondary metabolites), and to induce lethal or non-lethal stress. Besides versatility of applications and targets (bacteria, yeast, microalgae, mammalian cells, plant tissues, and seaweeds), PEF treatment does not require chemical additions, and has low energy (from few 100 J kg\(^{-1}\) down to 1 J kg\(^{-1}\)), and even lower in certain applications, and lower water consumption than other cell permeabilization processes (Golberg et al. 2016). PEF also has several industrially relevant advantages as it is quick (few seconds to minutes), applicable to fresh biomass, food grade, scalable and mild-thermal, thus preserving most thermosensitive components (Golberg et al. 2016).

Although PEF has been applied to many different tissues, applications of PEF to seaweeds are scarce. However, potential PEF applications for integrated macroalgal biorefinery were suggested by Robin & Golberg (2016) and considered as ‘feedstock improvement by genetic engineering, dehydration, valuable chemical extraction, pre-treatment to enhance hydrolysis or biochemical reactions, and process waste treatments’. Nevertheless, Polikovsky et al. (2016) showed that PEF could be used for specific protein extraction from Ulva sp. Postma et al. (2017) showed that PEF improved protein extraction from Ulva sp. and was more energy-efficient (6.6 kW kg\(^{-1}\) protein) than protein extraction by high shear homogenisation (> 300 kW kg\(^{-1}\) protein). Robin et al. (2018a) found that PEF could also improve protein extraction, producing an extract with high antioxidant activity. However, those studies reported a rather low protein yield (< 15% of initial protein content), which is lower than homogenisation (39%), enzymatic-assisted extraction (26.1%) and 24 h osmotic shock extractions (19.5%; Postma et al., 2017), and similar to values (10–11%) obtained by aqueous and chemical extractions with or without ultrasonic treatment (Kazir et al. 2019). Robin et al. (2018a, b) reported that protein content in residue increased due to removal of a significant fraction of the salt, and suggested applying PEF for de-ashing green seaweed biomass. Using PEF treatments (20–50 kV cm\(^{-1}\), 20–50 pulses of 5 µs) coupled with hydraulic pressing, they obtained up to 45% removal of initial ash content from fresh biomass of Ulva sp., compared to 18% removal by pressing (Robin et al. 2018b), 7–83% by washing (Magnusson et al. 2016; Neveux et al. 2014a), and above 80% by extensive acid washing (Hu et al. 2017). Compared to ash removal from seaweed by water rinsing (Magnusson et al. 2016; Neveux et al. 2014a) and extensive acid washing (Hu et al. 2017), PEF treatment was among the quickest (few minutes compared to hours), the least water intensive (i.e. 100 ml for 140 g of fresh biomass compared to 1 litre per 100 g in a washing treatment), and the simplest (no pre-treatment, two steps), while using no chemicals (Robin et al. 2018b). Process conditions of recent PEF studies on green seaweed are detailed in Table 6.

Finally, although current signs are promising, the effect and applications of PEF on green seaweed are still in their infancy.

MICROWAVE AND ULTRASOUND TECHNOLOGIES: Microwave and ultrasound are promising examples of ‘smart’ and ‘green’ technologies (Chatel et al. 2014; Li et al. 2016; Mason et al. 2011; Tiwari 2015). Microwave technologies (MWT) are based on non-ionising electromagnetic waves in the frequency band of 300 MHz to 300 GHz (Routray & Orsat 2012). MWT is rapid (a few minutes), and improves mass transfer, solvent diffusion, and tissue disruption. Thus, it improves common bioprocesses such as extraction of molecules from tissues, chemical or

Table 6. Pulsed electric field treatment for the processing of green seaweed (Ulva sp.)

<table>
<thead>
<tr>
<th>Extracted product</th>
<th>Solvent</th>
<th>PEF treatment conditions*</th>
<th>Other treatment conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>(\text{H}_2\text{O})</td>
<td>75 pulses/5.7 µs/2.96 kV/cm/0.5 Hz/30.81 kJ/kg fresh algae</td>
<td>Coupled with hydraulic pressing (5 min, 45 daN/ cm(^2))</td>
<td>Polikovsky et al. (2016)</td>
</tr>
<tr>
<td>Protein and carbohydrate</td>
<td>(\text{H}_2\text{O})</td>
<td>2 pulses/0.05 to 5 ms/3 to 7.5 kV/cm</td>
<td>1 h diffusion time</td>
<td>Postma et al. (2017)</td>
</tr>
<tr>
<td>Ash</td>
<td>(\text{H}_2\text{O})</td>
<td>10 to 50 pulses/4 to 6 µs/2 to 6 kV/cm/0.5 Hz</td>
<td>Coupled with hydraulic pressing (5 min, 45 daN/ cm(^2))</td>
<td>Robin et al. (2018b)</td>
</tr>
<tr>
<td>Protein</td>
<td>(\text{H}_2\text{O})</td>
<td>10 to 50 pulses/4 to 6 µs/2 to 6 kV/cm/0.5 Hz</td>
<td>Coupled with hydraulic pressing (5 min, 45 daN/ cm(^2))</td>
<td>Robin et al. (2018a)</td>
</tr>
</tbody>
</table>

*PEF conditions: number of pulses/pulse duration/field strength/pulse frequency/specific energy consumption
enzymatic hydrolysis, and chemical reactions. It is energy-efficient and fast, with uniform volumetric heating and energy consumption of 1800 kJ kg\(^{-1}\) of fresh materials at pilot scale (Périno et al. 2016). In addition, it is a non-chemical treatment that can be applied directly to fresh biomass and is available on an industrial scale (Leonelli & Mason 2010; Li et al. 2016; Mason et al. 2011).

MWT was successfully applied to green seaweeds to enhance SPs extraction from Ulva sp. (10.79% yield; Wang et al. 2011a, as Enteromorpha), Ulva meridionalis R.Horimoto & S.Shimada (40.4% yield), Ulva ohnoi M.Hiraoka & S. Shimada (36.5% yield) and Monostroma latissimum Wittrock (53.1% yield; Tsubaki et al. 2016), surpassing the yields of hot water extraction, hot water reflux extraction and ultrasound-assisted extraction (Tsubaki et al. 2014, 2016; Wang et al. 2011a). The combined use of MWT and poly-oxometalate (POM) for hydrolysis of polysaccharides from Ulva sp. achieved a saccharification yield of 35–44%, which is 1.7–6.3 times higher than combined MWT and acid hydrolysis, and 5–33% higher than combined POM and conduction heating hydrolysis (Tsubaki et al. 2014). MWT also improved extraction of essential oil from Ulva sp. (Patra et al. 2015, as Enteromorpha), the extraction of plant biostimulants (Michalak et al. 2015), and the extraction of pigments from various green algae. This achieved higher yields than Soxhlet apparatus extraction and supercritical CO\(_2\) extraction, and higher or similar yields than ultrasound extraction (Fabrowska et al. 2017). MWT was also used for pyrolysis of seaweed biomass (Budarin et al. 2011). Yuan & Macquarrie (2015) demonstrated the full potential of MWT using a step-by-step microwave treatment to obtain various products (fucoidan, alginate and biochar) at different microwave-assisted extraction conditions in a biorefinery approach.

Ultrasound technologies (UT) are based on the application of ultrasonic waves in the range of 20 kHz to 1 MHz. The wave creates cavitation microbubbles that burst and deliver high-energy mechanical shockwaves as well as heat, that enhance mass-transfer and disrupt cell walls (Mason et al. 2011). UT treatments are therefore similar to MWT in terms of output (enhanced mass transfer, solvent diffusion, and cell wall disruption), but apply different mechanisms and may achieve different results by causing less biomass heating. The advantages of UT include speed (few minutes) and low-energy consumption (around 50 kJ l\(^{-1}\) for a pilot continuous system; Alexandru et al. 2013). Furthermore, it is an environment-friendly, non-chemical, mildly thermal, versatile method, applicable to fresh biomass and available on an industrial scale (Leonelli & Mason 2010; Mason et al. 2011; Tiwari 2015). UT was successfully applied to seaweed processing to enhance saccharification of Codium tomentosum Stackhouse, but achieved similar or lower results than those obtained by hot water extraction (24 h, 50 °C) or enzymatic treatment (Rodrigues et al. 2015b). This apparent inferiority was confirmed in another study on Ulva rigida C.Agardh (Karray et al. 2015) where a UT treatment improved only slightly the biodegradability of seaweed (57.1%) compared to untreated biomass (53.5%). At the same time, hot acid and hot alkali treatments proved less effective (16.12% and 35.24%, respectively), and enzymatic treatments achieved better results (62.7% and 57.7%). Nevertheless, using Ulva sp., Korzen et al. (2015b) combined UT, enzymatic treatment and ethanol fermentation in one step, obtaining almost 20% (w/w) glucose, and a glucose to ethanol ratio of 0.33. UT was also listed as one of the most promising green technologies to enhance polysaccharide extraction from seaweed (Wu 2017). Process conditions of recent MWT and UT studies on green seaweed are detailed in Table 7.

In conclusion, although interesting, MWT and UT are usually more expensive than traditional processes and therefore their industrial use is still limited. Yet, their capacity to be combined (Mason et al. 2011) or coupled with other green processes such as

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**Table 7. Microwave and ultrasound treatments for green seaweed processing.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Product</th>
<th>Solvent</th>
<th>Treatment conditions*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulva sp. (as Enteromorpha sp.)</td>
<td>Sulfated polysaccharide</td>
<td>H(_2)O</td>
<td>MWT/700 W/70 °C/25 min/1:40</td>
<td>Wang et al. (2011a)</td>
</tr>
<tr>
<td>Ulva meridionalis, Ulva ohnoi and Monostroma latissimum</td>
<td>Sulfated polysaccharide</td>
<td>H(_2)O</td>
<td>1 kW/2.45 GHz/140 °C/14 min/1:20</td>
<td>Tsubaki et al. (2016)</td>
</tr>
<tr>
<td>Ulva sp.</td>
<td>Hydrolysate</td>
<td>H(_2)O, various acids and 2–50 mM POM</td>
<td>MWT/1 kW/2.45 GHz/140 °C/14 min/1:20</td>
<td>Tsubaki et al. (2014)</td>
</tr>
<tr>
<td>Ulva sp. (as Enteromorpha sp.)</td>
<td>Essential oil</td>
<td>H(_2)O</td>
<td>MWT/40 W/15 GHz/240 min/1:10</td>
<td>Patra et al. (2015)</td>
</tr>
<tr>
<td>Ulva sp., Cladophora sp., red seaweed</td>
<td>Plant biostimulants</td>
<td>H(_2)O</td>
<td>MWT/1000 W/25 to 60 °C/30 min/1:3</td>
<td>Michalak et al. (2015)</td>
</tr>
<tr>
<td>Cladophora glomerata (Linnaeus) Kützing, Cladophora rivularis (Linnaeus) Kuntze, Ulva flexuosa (Collins &amp; Hervey) M.J.Wynne</td>
<td>Chlorophyll and carotenoids</td>
<td>ethanol water 7:3</td>
<td>MWT/800 W/40 °C/60 min/1:25</td>
<td>Fabrowska et al. (2017)</td>
</tr>
<tr>
<td>C. tomentosum</td>
<td>Saccharification</td>
<td>H(_2)O</td>
<td>UT/400 W/50–60 kHz/50 °C/60 min/1:25</td>
<td>Rodrigues et al. (2015b)</td>
</tr>
<tr>
<td>U. rigida</td>
<td>Pre-treatment for anaerobic digestion</td>
<td>H(_2)O</td>
<td>UT/120 W/40 kHz/5 min</td>
<td>Karray et al. (2015)</td>
</tr>
<tr>
<td>U. rigida</td>
<td>Saccharification and ethanol production</td>
<td>H(_2)O</td>
<td>UT/120 W/40 kHz/180 min/37 °C/1:48</td>
<td>Korzen et al. (2015b)</td>
</tr>
<tr>
<td>C. glomerata, C. rivularis, U. flexuosa</td>
<td>Chlorophyll and carotenoids</td>
<td>ethanol water 7:3</td>
<td>UT/60 min/40 °C/1:25</td>
<td>Fabrowska et al. (2017)</td>
</tr>
</tbody>
</table>

*Treatment conditions: Technology/power/frequency/temperature/duration/biomass to solvent ratio*
enzymatic treatments (Korzen et al. 2015a) or green solvent extractions (Chemat et al. 2012; Mason et al. 2011), make them versatile process-enhancers for seaweed processing.

**Biorefinery example – step-by-step polysaccharide extraction**

An integrated cascading extraction requires implementation of different technologies that recognise the specific properties of each component. Here, we discuss extraction of the main polysaccharides (SPs, starch, and cellulose) from green algae. We examine each component in terms of its specific applications, extraction methods and challenges regarding implementation.

**SULFATED POLYSACCHARIDES**

*Monostroma* and *Ulva* are major sources of SPs. Several researchers have extracted sulfated polymers from different genera and species of green algae, but most literature focuses on ulvan (Cunha & Grenha 2016). Ulvan is the main SP present in cell walls in ulvalean genera and is composed mainly of D-glucuronic acid, D-xylose, L-rhamnose, and sulphate (Jung et al. 2013). Several potential applications were investigated for such SPs: animal feed, antioxidant, antitumour, anticoagulant, immune modulator, and biomedical applications such as drug delivery and tissue engineering (Cardoso et al. 2014; Lahaye & Robic 2007; Manivasagan & Oh 2016).

Several authors have reported the extraction of SPs from green algae using chemical methods involving oxalate salt or hydrochloric acid (Cardoso et al. 2014; Glasson et al. 2017; Jiao et al. 2012; Thanh et al. 2016; Tian et al. 2015). However, a green protocol for ulvan extraction using an autoclave (Dumas et al. 2010) and/or hot water (around 80–90 °C) was given by Costa et al. (2012), Barros et al. (2013), and Trivedi et al. (2016). Recently, Tsubaki et al. (2016) reported a green protocol for extraction of ulvan using MWT. The MWT is quick (less than 15 min) compared to thermal or chemical extraction methods, and extracted 37%–40% of ulvan from *Ulva* sp. Various applications of SPs and their extraction methods were summarised by Wang et al. (2014, Table 8).

**STARCH**

Starch is a polymer of glucose monomer units joined by α (1 → 4) glycosidic bonds. Starches, including modified starches, are important for multiple applications, including food, fermentation, textile, cosmetics, pharmaceutical, packaging, synthetic polymer industries and in biotechnological applications (Santana & Meireles 2014). Use of starch for bioenergy production was reported too (Jiang et al. 2016a; Milledge et al. 2014; Potts et al. 2012). Starch is a major storage carbohydrate in various algae such as Cladophora, Gracilaria, and Rhodophyta (Baweja et al. 2016; Farias et al. 2017; Yu et al. 2002). In green algae, starch plates are found around pyrenoids and starch granules among thylakoid membranes (Lovlie & Bråten 1968). Starch content fluctuates seasonally and can reach up to 32% of DW in *U. rigida* (Korzen et al. 2015b), which makes green algae attractive as potential for new sources of raw material for starch industries. Moreover, starch concentration depends on nutrient availability and increases significantly under nutrient stress (Andrade et al. 2004; Korzen et al. 2015a, 2016; Rosenberg & Ramsu 1982).

Several different extraction methods from green seaweeds have been described (Table 9). For extraction of pigments, Rosenberg & Ramsu (1982) used acetone, which can be replaced with non-toxic solvents such as ethanol.

| Species extracted from green seaweeds using different methods, and their applications. |
|----------------------------------------|-------------------------------------------------|-----|-----|
| **Species**                          | **Extraction method**                           | **Application** | **Reference** |
| *Codium, Caulerpa, Bryopsis, Ulva, and Enteronomorpha* (as Ulva) | Hot water extraction                           | Antioxidant     | Wang et al. 2014 |
| Caulerpa cupressoides                 | Extraction by proteolytic digestion             | Antioxidant     |                   |
| *Codium* sp.                         | Hot water extraction                           | Anticoagulant   |                   |
| *Monostroma*                         | Hot water extraction                           | Anticoagulant   |                   |
| *Codium dwarkense Bergesen, C. tomentosum* | Cold water extraction                        | Anticoagulant   |                   |
| *C. cupressoides*                    | Extraction by proteolytic digestion             | Anticoagulant, antinociceptive, anti-herpetic activity |                   |
| *Monostroma nitidum* Wittrock        | Hot water extraction                           | Immunomodulatory|                   |
| *U. intestinalis*                    | Alkali extraction                              | Antitumor       |                   |
| *U. rigida*                          | Acidic extraction                              | Immunomodulatory|                   |
| *U. fasciata*                        | Ultrasonic extraction                          | Antioxidant and moderate antitumor activities   |                   |
| *U. lactuca*                         | Hot water extraction                           | Antiviral, anti-inflammatory          |                   |
| Caulerpa lentillifera J.Agardh        | Aqueous extraction                             | Immunostimulatory |                   |
| *M. latissimum*                      | Cold water extraction                          | Antiviral type 1 (HV-1)                 |                   |
| Gayralia oxyserma (Kützing) K.L.Vinogradova ex Scagel et al. | Aqueous extraction                             | Antiviral herpes simplex virus          |                   |
| *Ulva, Cladophora*                   | Microwave assisted hot water extraction        | plant growth biostimulant              | Michalak et al. 2015 |
| *U. lactuca*                         | Hot water extraction                           | Anticancer                                  | Thanh et al. 2016  |
| *U. rigida*                          | Hot water extraction                           | Nanofibres in biomedical engineering     | Manivasagan & Oh 2016; Toskas et al. 2011 |

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**Table 8.** SPs extracted from green seaweeds using different methods, and their applications.
The thermochemical methods, mentioned in Table 9, do not extract pure starch but rather starch contaminated with ulvan.

A completely green method for pure starch extraction from green algae was reported by Prabhu et al. (2019) who showed the extraction of starch grains in their native form from Ulva ohnoi. Prabhu et al. (2019) applied mechanical grinding followed by filtration, thus isolating starch grains from Ulva biomass. Next, ethanol was used for further starch purification, achieving an extraction yield of 50% of total starch biomass. The Yu et al. (2002) protocol for isolating floridean starch grains from red algae may also be adopted for extracting native starch. The method applies 50 mM citrate buffer (pH 6.5) to pulsed biomass to obtain starch granules. Next, proteins and polysaccharide slurries are separated from the grains by gradient centrifugation, and grains are further purified by sedimentation in distilled water. Generally, extracting starch in its native form is challenging due to small grain size (2–5 μm) (Andrade et al. 2004) and low-temperature stability. Furthermore, the potential of mild-thermal processes, such as PEF, could be investigated for extraction of granular starch.

**CELLULOSE**: Like starch, cellulose is a polymer of D-glucopyranose but joined together by β(1→4) glycoside bonds. It is the most abundant natural polymer on Earth and is used in industrial applications, including paper, reinforcing material, bioplastic and more (Klemm et al. 2005). Cellulose extractions have traditionally used alkali, bleaching and acid treatments (Mihranyan et al. 2004). For example, a cellulose extraction method from Cladophora sp. by Mihranyan et al. (2004) has several drawbacks: large chemical consumption, a need to de-fat the biomass prior to cellulose extraction, and its prolonged duration.

However, simple methods involving 0.5% sodium dodecyl sulfate (SDS) for the extraction of cellulose are encouraging (Modulevsky et al. 2014). The use of an enzyme cocktail containing amylose, xylanase, and hemicellulose and ulvan-degrading enzymes can be studied as a green protocol for extraction of cellulose. Since green algae contain negligible amounts of lignin (John et al. 2011), lignin-degrading enzymes are not required. As cellulose is a strong algal polymer (Abdul Khalil et al. 2017), these enzymes can be used on the biomass fraction remaining after extracting other products. Furthermore, Trivedi et al. (2016) performed a sequential extraction of salt, starch, pigment, SPs and protein from Ulva, obtaining a cellulose-rich residue. They used the method of Mihranyan et al. (2004) for cellulose extraction but increased bleaching time to from 3 h to 6-8 h. This process requires further study; however, it is likely that such solid residue would need much lower concentrations of chemicals or enzymes and a shorter extraction time. Finally, integrated extraction of SPs, starch, and cellulose from green algae-based biorefineries in coastal areas can thus be cost-effective and provide significant added value compared to terrestrial-based production of these important polymers.

### CONCLUSIONS AND PROSPECTS

The unique potential of marine, green algal biorefineries derives from the wide variety of potential products (e.g. food, animal feed, energy, biofuels, biomaterials) combined with the substantial opportunity of using the open ocean for cultivation. Currently, commercially viable exploitation of green seaweeds is limited mostly to food, and multiple product biorefineries remain to be established. Our approach to biorefinery uses green macroalgae, along with technologies for cultivation, and emphasises the importance of co-production and sustainable biorefinery design. Both cultivation and processing still face technological and know-how challenges. These include resilient cultivation techniques suitable for offshore environments and adapting current processing methods to seaweed challenges (i.e. high moisture and ash content and unique polysaccharides). The fulfillment of the potential for green seaweed biorefineries depends on both the ability to integrate efficient processing units and the quality of seaweeds as a sustainable and consistent offshore-grown feedstock.

We suggest that additional hurdles to seaweed biorefineries need to be overcome. These include seasonal feedstock availability, unsustainability of monoculture and high capital costs. These could be overcome by integrating different feedstock from marine (i.e. various types of seaweed and fishery waste) or land-based sources of agricultural waste) in the same ‘flexible’ biorefinery. This approach is process-dependent and feedstock-dependent, but has already been reported for bioenergy production, as those processes are usually feedstock-flexible. Meanwhile, in Europe, efforts have been directed towards developing and implementing seaweed aquaculture guidelines which should be the basis for future green marine biorefineries (Barbier et al. 2019).

### REFERENCES


