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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.

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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; Seghetta *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

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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) Hariot, 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 nutritive value derived from high soluble dietary fibre (up to 55% in seaweed in general;), high-quality proteins, and remarkably high free-radical scavenging properties than terrestrial crops (Mohamed *et al.* 2012; see Shannon & Abu-Ghannam 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; Seghetta *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 2011) 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; Milledge *et al.* 2014; Neveux *et al.* 2015; Nikolaisen *et al.* 2011; Rocca *et al.* 2015; Sambusiti *et al.* 2015; Suutari *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; Mihranyan 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.

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

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

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, macroalgal feedstock cannot be based on the harvesting of wild stocks or on cultivation in onshore or near shore farms. Wild-stock 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

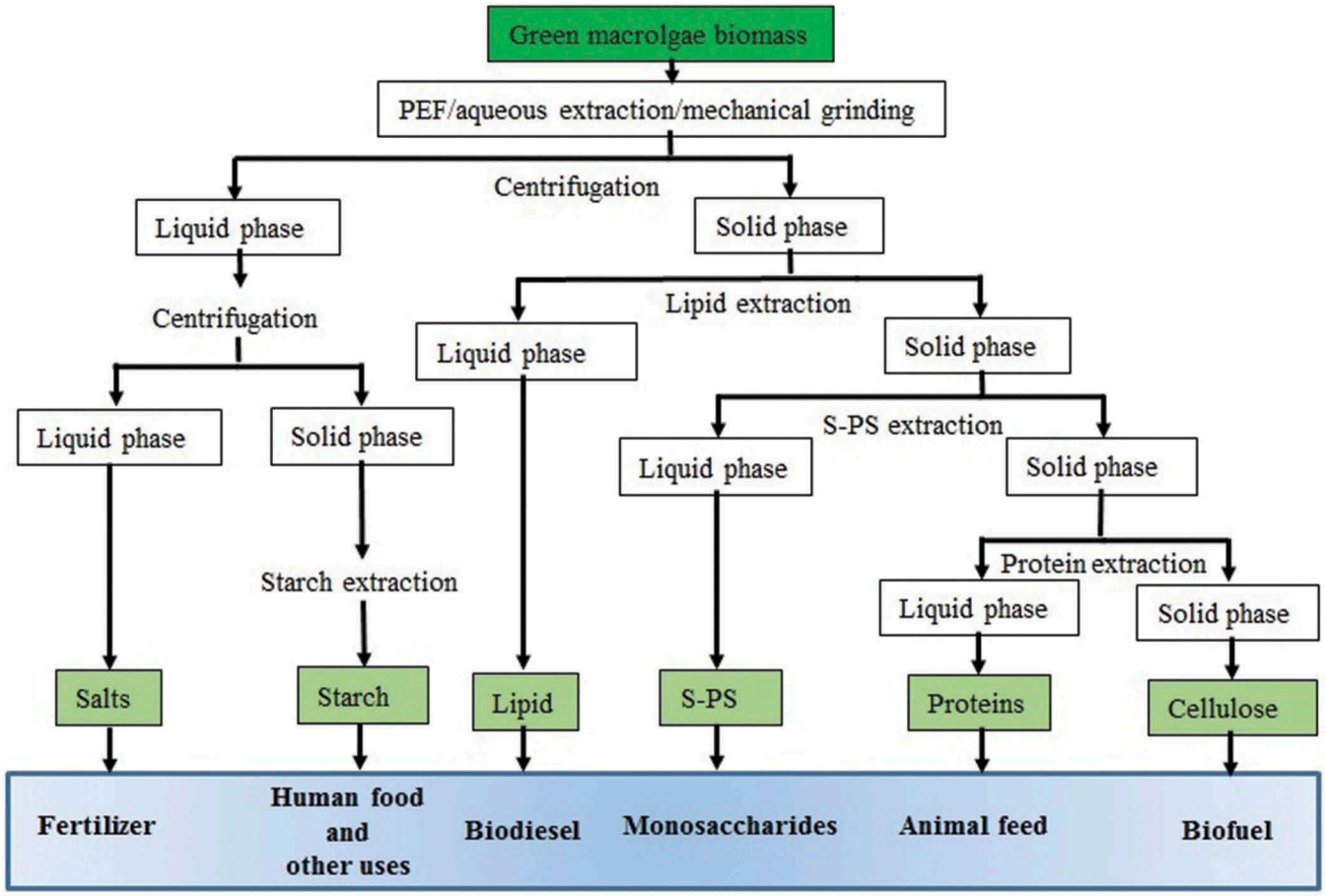

Fig. 1. Green macroalgal biorefinery process for co-production of a wide range of valuable products.

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

Table 2. Near- and offshore *Ulva* sp. cultivation experiments.

Species	Cultivation system	Location	Yields	Cultivation period	Reference
U. rigida	Nylon net cages integrated with fish cages	Offshore Mikhmoret, Israel	Maximal daily growth rate (DGR) of 16.8%	2 weeks	Korzen *et al.* (2016)
U. rigida	Flat double-layer net reactors	Reading power station, Tel Aviv, Israel	Mean DGR of 4.5 ± 1.1%, Annual average productivity of 5.8 ± 1.5 g DW m^{-2} day^{-1}	1 year	Chemodanov *et al.* (2017b)
U. prolifera, *U. intestinalis*	Raft	Yellow Sea offshore Jiangsu coastline, China	198.6 and 89.2 kg ww ha^{-1} 5 $months^{-1}$	5 months	Liu *et al.* (2010); Fernand *et al.* (2017)

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 predicted 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 km^2 rafts, floating away from shipping lanes. This concept, even on much smaller scales, is challenging due to the need to design robust and self-sustained cultivation systems that can withstand harsh offshore conditions. 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 bioremediation 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 obstacle 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 conversion (OTEC) technology, which utilises deep seawater for power generation based on temperature difference with surface water (Roels *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 (Roels *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) modelled smart mixing regimes to improve biomass productivity by enhancing light harvesting and carbon fixation. Mixed water cultivation is commonly applied to onshore reactor cultivation 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 uncontrolled macroalgal blooms (Liu *et al.* 2009). Furthermore, the energetic and technical feasibility of mixing seaweed in offshore 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 expensive 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 further 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 treatments such as cleaning, washing, size reduction, preservation, 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 trans-esterification 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 liquefication (HTL), which occurs at water temperatures above 280 °C, biomass is liquified 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.

$$Q = 151.2C + 499.77H + 45.0S - 47.7(O) + 27.0N \quad (1)$$

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, oxygen and nitrogen in DW percent. Neveux *et al.* (2014b) conducted HTL on six green algae (Table 3) and measured HHV of 4 to 20.5 MJ kg^{-1} for the hydrochar, and 32.5 to 33.8 MJ kg^{-1} for the bio-oil. Highest hydrochar HHV was measured for *Chaetomorpha* genus, while bio-oil HHV showed no significant differences between species. Zhou *et al.* (2010) liquified *U. prolifera* and found that the bio-oil yield, calculated with the Dulong formula (2) (Mason & Gandhi 1980), increased with temperature up to a maximum of 20.4% (w/w) and HHV of 28.7 MJ kg^{-1} at 300 °C.

$$Q = 145.44C + 620.28H + 40.5S - 77.54(O) \quad (2)$$

where Q is the gross heating value in BTU lb^{-1} on the dry basis and C, H, S and (O), are the 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,

Table 3. Hydrothermal treatments for the processing of green seaweed biomass.

Species	Treatment	Temperature/ Pressure range	Reaction time	Maximal yield	Optimal conditions	Reference
Ulva pertusa Kjellman	Hydrolysis	100–200 °C	2–12 min	8.5% glucose (w/w)	180 °C, 10.48 bar and 8 min	Choi *et al.* (2013)
Codium fragile	Hydrolysis	100–240 °C	10 min	> 50% soluble carbohydrates (w/w), HHV of 22.6 MJ kg^{-1}	210 °C	Daneshvar *et al.* (2012)
U., Derbesia, Chaetomorpha, Cladophora, Oedogonium	HTC, HTL	330–341 °C/ 140–170 bar	5 min	20.5 MJ kg^{-1} for hydrochar and 33.8 MJ kg^{-1} for bio-oil		Neveux *et al.* (2014b)
U. prolifera	HTL	220–320 °C		20.4% bio-oil (w/w) and HHV of 28.7 MJ kg^{-1}	300 °C	Zhou *et al.* (2010)

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* Delile 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 cellulase 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-Dalatony *et al.* 2016; Trivedi *et al.* 2015), amyloglucosidase 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 suboptimal 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% CO_2 (Hosseini & Wahid 2014), and variable trace amounts of CO, N_2, O_2, H_2 and the undesirable H_2S. H_2S 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 tenuissima* (Moris & De Notaris) P.Crouan & H.Crouan demonstrated high production potentials of 45–56 and 138 tons DW per

Table 4. Comparison of reported ethanol production from *Ulva* sp. using different microorganisms.

Species	Hydrolysis method	Total sugar (mg g^{-1} DW)	Microorganism	Ethanol yield (g/g sugar)	Reference
U. lactuca	Acid + enzyme	343	*Clostridium beijerinckii*	0.4	Bikker *et al.* (2016)
Ulva sp.	Enzyme	200	*E. coli* Ko11[1]	0.4	Kim *et al.* (2011)
U. fasciata	Hot buffer + enzyme	± 112	*S. cerevisiae* MTCC No. 180	0.47	Trivedi *et al.* (2015)
U. lactuca	Acid	113	*S. cerevisiae*	0.55	El-Sayed *et al.* (2016)

[1]*E. coli* Ko11 was modified to allow more efficient sugar utilisation

Table 5. Potential methane yield for green macroalgae.

Species	Reported potential methane yield	Reference
Ulva sp.	0.22–0.33 m^3 kg^{-1} volatile solids (VS)	Roesijadi *et al.* (2010)
U. lactuca	4000–7000 m^3 CH_4 $hectare^{-1}$	Bruhn *et al.* (2011)
Cladophora, Chaetomorpha	0.48 m^3 kg^{-1} VS	Gunaseelan (1997)

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 sulfoniopropionate (DMSP) and sulphur-containing amino acids (Stefels 2000; Wang *et al.* 2014) is only party 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, 2016; Herrero *et al.* 2006; Messyasz *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 (Isik *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 (Isik *et al.* 2014). Therefore, ILs could play a role in the extraction, recovery and hydrolysis of cellulose (Pezoa-Conte *et al.* 2015). However, the efficiency needs to be compared to other processes (Jmel *et al.* 2017). Additionally, ILs can help in hydrolise various non-green seaweeds and a

extracting seaweed polysaccharides or proteins (Gereniu *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 kJ kg^{-1} down to 1 kJ 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.).

Extracted product	Solvent	PEF treatment conditions*	Other treatment conditions	Reference
Protein	H_20	75 pulses/5.7 µs/2.96 kV/cm/0.5 Hz/30.81 kJ/kg fresh algae	Coupled with hydraulic pressing (5 min, 45 daN/cm^2)	Polikovsky *et al.* (2016)
Protein and carbohydrate	H_20	2 pulses/0.05 to 5 ms/3 to 7.5 kV/cm	1 h diffusion time	Postma *et al.* (2017)
Ash	H_20	10 to 50 pulses/4 to 6 µs/2 to 6 kV/cm/0.5 Hz	Coupled with hydraulic pressing (5 min, 45 daN/cm^2)	Robin *et al.* (2018b)
Protein	H_20	10 to 50 pulses/4 to 6 µs/2 to 6 kV/cm/0.5 Hz	Coupled with hydraulic pressing (5 min, 45 daN/cm^2)	Robin *et al.* (2018a)

*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

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

Species	Product	Solvent	Treatment conditions*	Reference
Ulva sp. (as Enteromorpha sp.)	Sulfated polysaccharide	H_20	MWT/700 W/70 °C/ 25 min/1:40	Wang *et al.* (2011a)
U. meridionalis, U. ohnoi and *M. latissimum*	Sulfated polysaccharide	H_20	1 kW/2.45 GHz/ 100–180 °C/14 min/ 1:20	Tsubaki *et al.* (2016)
Ulva sp.	Hydrolysate	H_20, various acids and 2–50 mM POM	MWT/1 kW/2.45 GHz/ 140 °C/14 min/1:20	Tsubaki *et al.* (2014)
Ulva sp. (as *Enteromorpha* sp.)	Essential oil	H_20	MWT/40 W/15 GHz/ 240 min/1:10	Patra *et al.* (2015)
Ulva sp., Cladophora sp., red seaweed	Plant biostimulants	H_20	MWT/1000 W/25 to 60 ° C/30 min/1:3	Michalak *et al.* (2015)
Cladophora glomerata (Linnaeus) Kützing, *Cladophora rivularis* (Linnaeus) Kuntze, *Ulva flexuosa* (Collins & Hervey) M.J.Wynne	Chlorophyll and carotenoids	ethanol water 7:3	MWT/800 W/40 °C/ 60 min/1:25	Fabrowska *et al.* (2017)
C. tomentosum	Saccharification	H_20	UT/400 W/50–60 kHz/ 50 °C/60 min/1:25	Rodrigues *et al.* (2015b)
U. rigida	Pre-treatment for anaerobic digestion	H_20	UT/120 W/40 kHz/5 min	Karray *et al.* (2015)
U. rigida	Saccharification and ethanol production	H_20	UT/120 W/40 kHz/ 180 min/37 °C/1:48	Korzen *et al.* (2015b)
C. glomerata, C. rivularis, U. flexuosa	Chlorophyll and carotenoids	ethanol water 7:3	UT/60 min/40 °C/1:25	Fabrowska *et al.* (2017)

*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, Gracilariopsis*, 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 (Løvlie & 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 & Ramus 1982).

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

Table 8. SPs extracted from green seaweeds using different methods, and their applications.

Species	Extraction method	Application	Reference
Codium, Caulerpa, Bryopsis, Ulva, and *Enteromorpha* (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 Børgesen, *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 (HIV-1)	
Gayralia oxysperma (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	Nanofibress in biomedical engineering	Manivasagan & Oh (2016); Toskas *et al.* (2011)

Table 9. Comparison of different starch extraction methods for green seaweeds.

Species	Extraction method	Reference
Ulva sp.	Boiling in DMSO and concentrated HCl at 60 °C	Bikker *et al.* (2016)
Ulva	Boiling in 200 mM sodium acetate (pH 4.8)	Korzen *et al.* (2016)
Ulva	Heating at 97 °C in 0.05 M phosphate buffer	Rosenberg & Ramus (1982)
U. ohnoi	Mechanical grinding and filtration at 25 °C	Prabhu *et al.* (2019)

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 *U. 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 pulverised 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 amylase, 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).

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