Research review paper

Marine algal carbohydrates as carbon sources for the production of biochemicals and biomaterials

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ABSTRACT

The high content of lipids in microalgae (> 60% w/w in some species) and of carbohydrates in seaweed (up to 75%) have promoted intensive research towards valorisation of algal components for the production of biofuels. However, the exploitation of the carbohydrate fraction to produce a range of chemicals and chemical intermediates with established markets is still limited. These include organic acids (e.g. succinic and lactic acid), alcohols other than bioethanol (e.g. butanol), and biomaterials (e.g. polyhydroxyalkanoates). This review highlights current and potential applications of the marine algal carbohydrate fractions as major C-source for microbial production of biomaterials and building blocks.

1. Introduction

The biorefinery concept is currently under hot debate. The biorefinery that relies on terrestrial crops to obtain liquid biofuels, namely bioethanol and biodiesel, impacts the economy and competes for water and energy. It is thus necessary to develop biorefineries that do not constitute an environmental burden in terms of feedstocks and of components extraction and processing.

For millennia, the sea has been a source of valuable commodities (Chew et al., 2017; Wei et al., 2013). However, intensive harvest and irresponsible human actions can cause environmental unbalances and affect the survival of marine species. Marine resources are worldly exploited for different end uses, ranging from food, food additives and nutritional supplements, to agro fertilizers, cosmetics and pharmaceuticals (Cardoso et al., 2014). A conscious biorefinery concept applied to marine algae effectively improves its commercial feasibility by directing every fraction/stream towards high added value products, hence providing an environmentally sustainable approach to the exploitation of marine algal resources. Indeed, after the extraction of biomolecules (such as bioactive compounds, proteins, gel polymeric materials, pigments), the cellulose-rich fraction remains may be processed towards monomeric sugars, which can then be converted into a wide range of products by fermentation. In the last decades, research efforts have been aimed at decreasing the world's dependency on petrochemicals and petrofuels. As a consequence, many of the bioprocesses developed so far, both from marine and freshwater algae, have focused on the production of biofuels (de Jong and Jungmeier, 2015; Wijffels et al., 2010). Algal biomass is worldwide being considered as a sustainable source of simple sugars for bioethanol fermentation. The present review doesn't intend to detail or highlight ethanol processes from algae biomass, as this has been done extensively elsewhere (Alam et al., 2015; Borines et al., 2013; Choi and Lee, 2016; Goh and Lee, 2010; Harun et al., 2011; Harun et al., 2010; Jambo et al., 2016; Kim et al., 2011; Kraan, 2013; Shukla et al., 2016). In particular, bioethanol production from macroalgae has been recently reviewed by Varejão and Nazaré (2017) (Varejão and Nazaré, 2017). Instead, this review addresses the applications of carbohydrates from both marine micro- and macroalgae directly from biomass, and after biorefinery approach for the production of biochemicals and biomaterials. Although several uses of macroalgae-derived sugars as C-sources in biological processes have been reported in the literature, high-scale marine algal carbohydrates saccharification is still to be explored.

2. Marine microalgae

Microalgae, the major eukaryotes in phytoplankton, are unicellular plants that live individually, aggregated, or in a filamentous form in marine, freshwater and terrestrial environments (Metting, 1996). They perform over 50% of the primary photosynthetic productivity on earth (Chisti, 2007), producing approximately half of the atmospheric oxygen
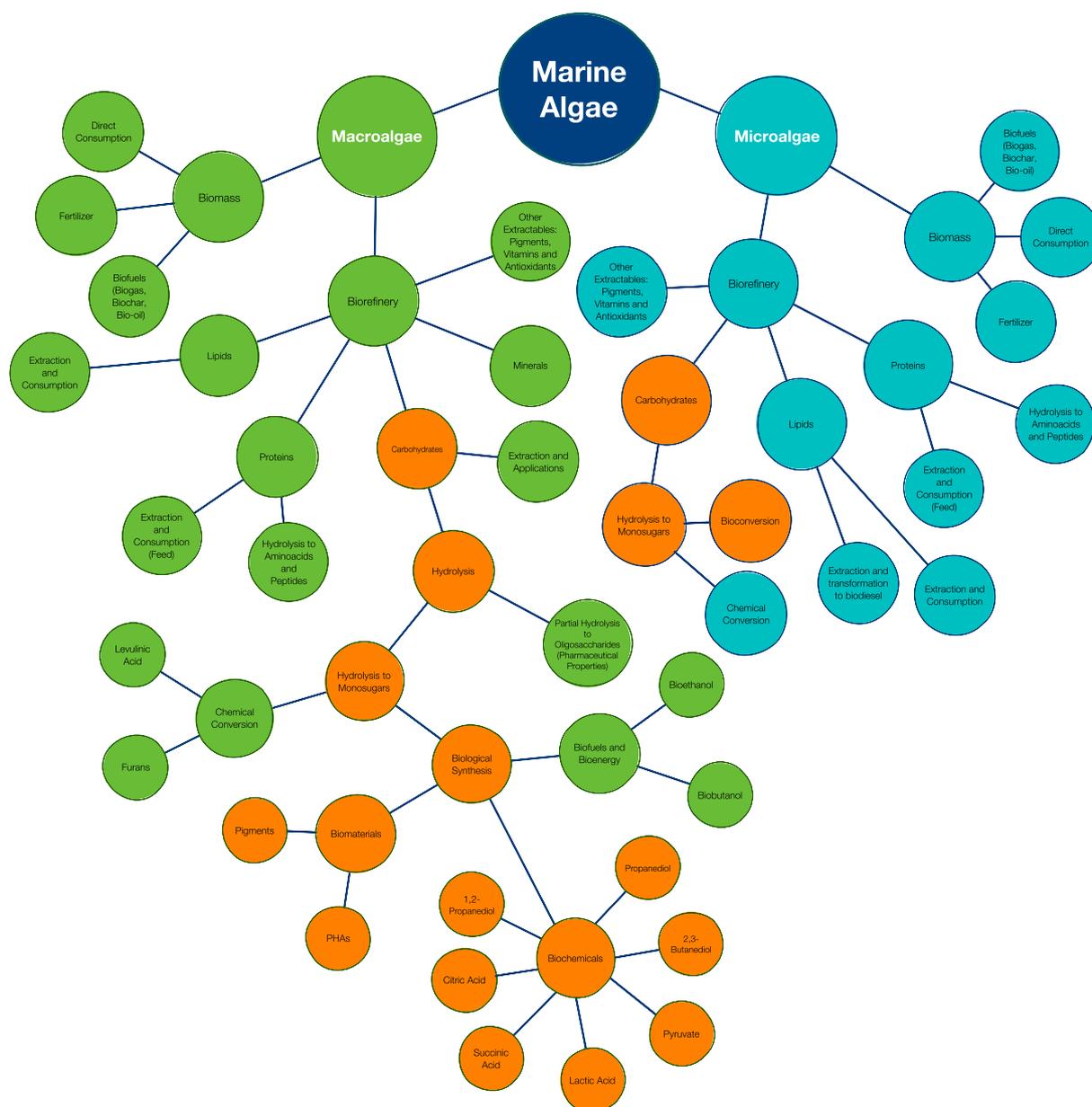
Fig. 1. Marine algae - a renewable feedstock for a myriad of products (in orange, biochemicals and biomaterials from carbohydrates).

Some species are particularly tolerant to high carbon dioxide concentrations and can thus play an important role in the capture of CO_2 from flue gases. The marine green alga *Chlorococcum littorale* is an example of extreme resilience to CO_2 stress since it can grow under 40% CO_2 (Kodama et al., 1993). Microalgae are also invaluable synthesizers of omega-3 and omega-6 fatty acids which climb the food chain and are accumulated higher up, namely by the "fat fish".

There are over 50,000 microalgae species described, but only a few are being cultivated. Other than for biofuel purposes, marine microalgae are currently produced around the planet (Japan, China, India, North America, Europe, Australia) for direct consumption (human and animal nutrition) or use (e.g. as organic fertilizer) and for extraction of proteins, lipids, sugars, antioxidants, pigments and nutraceuticals (Fig. 1). The present review does not include information with regard to cyanobacteria, which are often called "blue-green algae". This is indeed a misleading designation because, even though cyanobacteria are unicellular, aquatic and photosynthetic, they are prokaryotic organisms (*Cyanophyta*).

Microalgae are also a source of unique enzymes and it is likely that a large number of further, still untapped, microalgal compounds will be identified in the near future. Novel products from microalgae have also been announced, like the formulation of sustainable algae inks, a project for the development of biodegradable inks and print products to be carried out jointly in the U.S. by Cellana Inc. (San Diego) and Living Ink Technologies (Colorado) (www.livinginktechnologies.com). Table 1 presents some of the marine microalgal species commercially exploited for purposes other than bioenergy, soil amendment, bioremediation, and wastewater treatment.

At large-scale, species of microalgae that have environmental selective advantages are usually cultivated in open raceway ponds (Milledge, 2011). However, due to the variations in yield, to the difficulties of maintaining a monoculture and also to the risk of microbial contamination, a lot of effort has been dedicated to the design of efficient closed bioreactors in recent years. These become also invaluable for the growth of species with selective advantages. A comprehensive review on enclosed vessels for microalgae cultivation was published by

Table 1
Examples of commercially exploited marine microalgal species.

Species	Major products	Reference
Chaetoceros sp.	Feed for aquaculture	(Milledge, 2011)
Crypthecodinium cohnii	DHA (heterotrophic cultivation)	(Milledge, 2011)
Dunaliella salina	Carotenoids	(Oren, 2005)
Isochrysis sp.	Feed for aquaculture	(Fernandes et al., 2016)
Nannochloropsis gaditana	Feed for aquaculture	(Fernandes et al., 2016)
Phaeodactylum tricornutum	Antioxidants, Omega-3, Fatty Acids	(Carvalho et al., 2006)
Porphyridium cruentum	Antioxidants	(Carvalho et al., 2006)
Rhodomonas marina	Feed for aquaculture	(Fernandes et al., 2016)
Schizochytrium limacinum	DHA	(Ethier et al., 2011)
Skeletonema sp	Feed for aquaculture	(Mata et al., 2010)

Carvalho et al., 2006. The novel designs aim at overcoming the major constraints related to the productivity of closed bioreactors, e.g., improvement of the operation conditions related to mixing, illumination, heat dissipation, and escape of oxygen. Hybrid systems, consisting of closed and open vessels in series, are also being implemented (Xianhai et al., 2016).

Harvest of microalgae can be an expensive operation, which is slightly facilitated if the species flocculates. Usual methods, with or without addition of flocculent, include decantation, centrifugation in stack centrifuges, cross-flow filtration and air floatation. Yang and co-workers (Yang et al., 2016) were able to improve the harvest of marine *Chlorella* sp. up to ca. 20-fold by applying the high-pH-induced flocculation method, with a flocculation efficiency of 90%. Recently, a harvester using advanced membranes (Zobi harvester™) became available on the market (http://www.globalgae.com/technology/). The suppliers claim an efficiency of 100%, with a very low energy use at a harvest rate of 20 m³/h, leading to 15–20% algal slurry.

According to Cellana Inc. (San Diego), there is currently a shortage of available microalgal biomass and industrial-scale biomass producers. Cellana has patented a system of photobioreactors coupled with open ponds in series which, according to the claims, enables low-cost and continuous production of diverse strains of microalgae (Huntley et al., 1996; Huntley and Redalje, 2010). A demonstration facility at Kona, Hawaii, installed in 2008, will be followed by a commercial plant to eventually produce over 800 megatons of whole algae by 2018. Very recently, Evonik Industries and Royal DSM have announced the construction of a commercial scale facility in Nebraska, US, for the cultivation of marine microalgae, producers of DHA and EPA, to supply the salmon industry (http://www.oilgae.com/blog/2017/06/dsm-evonik-select-nebraska-for-200m-plant-to-produce-omega-3-oil-from-algae.html).

2.1. Composition and applications of microalgae biomass

The composition of each microalgal species depends on a variety of factors including light exposure, temperature, salinity, C/N and C/P ratios, and harvest time (growth stage and season).

The lipid content of microalgae is usually 30–50% dw (Chew et al., 2017). Moreover, high C/N ratios and photo-oxidative stress favor the accumulation of triglycerides by microalgae. The oil content of some species, namely *Botryococcus braunii*, *Nannochloropsis* sp. and *Schizochytrium sp.*, can surpass 700 kg per ton of dry biomass (Bwapwa et al., 2017). This is the main reason why microalgae are a potential important feedstock for biofuels.

The gross composition of marine microalgae in protein can reach 52% (w/w) (Brown et al., 1997; Knoshaug et al., 2016; Renaud et al., 1999). The formation of foam in aerated natural waters, namely on the wave edges, is mostly ascribed to the enhanced surface tension conferred by the proteins released by wall disrupted cells of cyanobacteria

and microalgae. On their study on the nutritional value of ca. 40 species of microalgae from seven algal classes, Brown et al. (1997) found that all species had similar amino acid composition and contained considerable amounts of the essential amino acids. Additionally, Guil-Guerrero et al. (2004) have claimed that microalgae can provide the essential amino acids to humans and animals. Microalgae are thus an invaluable and unconventional source of protein (Guil-Guerrero et al., 2004).

Microalgae are particularly rich not only in protein, but also in polyunsaturated fatty acids, hence their high value as food and feed supplements (Table 1 and Fig. 1). The direct use of microalgae for mariculture has been addressed by many authors as feed for molluscs and for the larvae of crustaceans, as well as feed for zooplankton which is subsequently fed to fish larvae (Brown et al., 1997; Fernandes et al., 2016; Hemaiswarya et al., 2011). Moreover, the colour enhancement of salmon and salmon trout by whole microalgae has been an important contribution to the aquaculture of these species for many years now (Paniagua-Michel et al., 2015).

The content of microalgae in carbohydrates varies widely from species to species and also varies, for a particular strain, with medium composition. There are many species that secrete exopolysaccharides (EPS), as a result of their own physiology or under stress conditions (Delattre et al., 2016).

The most abundant polysaccharides (PS) in microalgae are cellulose and starch, but several others have been identified which offer important properties (Chew et al., 2017). Cellulose is present in the cell wall, while starch is accumulated intracellularly as reserve material by various species under e.g. nitrogen limitation. Upon saccharification, glucose is thus the predominant monosaccharide in microalgae (21% to 87% w/w of the total carbohydrate content (Brown et al., 1997; Knoshaug et al., 2016; Renaud et al., 1999).

A recent paper has carefully analysed the carbohydrate composition of species of *Nannochloropsis, Rhodomonas* and of the flagellated *Isochrysis,* as a function of nutrient availability (Fernandes et al., 2017). These species are commonly used in aquaculture and are supplied by the Mariculture Center of Calheta (Madeira, Portugal). The cultivation of *Rhodomonas marina* in low nutrient media gave higher contents of monosaccharides as compared to nutrient-richer media. The digestibility of the cell wall is an especially important parameter in species selection for aquaculture (Camacho-Rodríguez et al., 2013).

Besides cellulose and starch, other PS have been identified in some microalge species which offer important properties. Diatoms present leucosin, a β-(1,3)-linked and β-(1,6)-linked glucose polymer, as main reserve materials (de Jesus Raposo et al., 2015b). However, the vast majority of PS in microalgae are heteropolymers in which glucose, galactose and xylose are the predominant units, but mannose, rhamnose and fucose are present in many of them, as well as several methyl sugars (de Jesus Raposo et al., 2015a). According to Brown et al. (1997), the digestibility of a PS by molluscs and crustaceans depends on its mono-sugar composition and on the type of glycosidic bond between monomers, with glucose-rich PSs being more easily digested than mannose-rich PSs.

In contrast to what happens in macroalgae, the PSs from microalgae have not been grouped under common names (de Jesus Raposo et al., 2015b). They offer a vast array of properties and applications ranging from biomedical and nutraceutical to anti-adhesive, bioflocculant and drag-reducing substances in ship engineering (de Jesus Raposo et al., 2013; Yim et al., 2007).

The economic importance of β-glucans is gathering interest since they are being increasingly incorporated in dietary products and health foods. The potential of β-glucans as pharmaceuticals is also being investigated (Vo et al., 2012). β-glucans enhance the host immune system since they bind to β-glucan receptors of cells involved in immune responses (Akramiene et al., 2007). Schulze and coauthors (Schulze et al., 2016) have screened microalgae for primary metabolites including β-glucans and studied the production parameters leading to the

maximization of their production. In fact, they identified two strains, one of *Scenedesmus ovalternus* and another of *Porphyridium purpureum*, as the best β-glucan producers in saline media, attaining over 20% (dw) in β-glucan. Additionally, nitrate starvation enhanced the β-glucan content of *S. ovalternus* from 23.3 to 36.7%. This is a breakthrough result, which highlights the ability of marine microalgae to produce carbohydrates with experimentally proved or potential therapeutic properties.

de Jesus Raposo et al. (2015a) reviewed the biomedical applications of PSs from marine algae and in particular the bioactivity and applications of sulfated PSs from marine microalgae. The later are EPS containing up to ca. 15% of sulfate residues and depicting an acidic character ascribed to the presence of glucuronic acid and half-ester sulfate groups (de Jesus Raposo et al., 2013). According to these authors, much work is required to further unravel the properties of the microalgal sulphated PS. The most studied action is the remarkable antiviral activity of *Porphyridium* sp. sulfated EPSs ((Radonic et al., 2010; Talyshinsky et al., 2002). Also important is the finding by Chen and co-workers regarding the *Rhodella reticulata* crude sulfated PS which offers an anti-oxidant activity twice as strong as α-tocopherol (vitamin E) (Chen et al., 2010). Meanwhile, de Jesus Raposo et al. (2013) have called upon the need for risk assessment of sulfated PSs in human therapy (de Jesus Raposo et al., 2013).

Even though microalgae offer the advantages over their macro-counterparts of an easier control of cultivation parameters, there has been, so far, an absence of studies on the potential use of their sugar fraction as C-source for the production of biochemicals. The sugar residue, being a by-product after extraction of other valuable constituents like proteins, lipids, carotenoids and pigments, is a good candidate for upgrading purposes, namely as carbon source in fermentation. This topic has already deserved attention in the area of biofuels. An example is the study of the utilization of residues of DHA microalgal producers, after enzyme digestion, to obtain bioethanol with *Saccharomyces* sp. (Jayasinghe and Gray, 2014). This highlights the fact that there is indeed a large scope for research leading to a novel integrated microalgal biorefinery concept, which will regard the carbohydrate residues as a feedstock to obtain fine chemicals.

2.2. Microalgal biorefinery

Biorefinery of algae biomass has been under much study and evaluation during the last decades in an attempt to achieve integrated approaches for full exploitation of this resource, to obtain a wide range of compounds, spanning from energy products to platform chemicals and high added value products (Chew et al., 2017; Wijffels et al., 2010).

The first step in a microalgal biorefinery approach usually involves the disruption of the cell wall. A wide range of methods, singularly or combined, have been attempted, from physical (high-pressure, micro-waving, heat), chemical (acid, alkaline, use of organic solvents) to enzymatic. Marine *Chlorella* species have been successfully pre-treated for the production of lipids and reducing sugars by combining a high-pressure homogenization process with a microwave process (Lee et al., 2013). Choi and Lee (2016) achieved a lipid extraction of over 20% (w/w), also from marine *Chlorella sp.,* by applying two pressure cycles of ca. 207 MPa (Choi and Lee, 2016). Simultaneous extraction of sugars and lipids from the green alga *Chlorococcum infusionum* was recently achieved with high yields by bead-beating followed by acid treatment (Karemore and Sen, 2016). The optimization of cell disruption and saccharification has been addressed in two marine and one freshwater species by Hernández et al., 2015. For *Chlorella sorokiniana* and *Nannochloropsis gaditana* 128 and 129 mg of monosaccharides per gram dry weight could be obtained, respectively, by applying a H_2SO_4 treatment followed by enzymatic hydrolysis (Hernández et al., 2015).

In the context of a biorefinery, the valorization of the various microalgal fractions imposes the use of mild methods and should exclude those that damage one or more of the potentially useful components (Vanthoor-Koopmans et al., 2013).

3. Marine macroalgae

Macroalgae, also called seaweed, are multicellular, aquatic photosynthetic organisms. They are abundantly present in oceans, particularly in coastal areas, where they may attach to rocks and other solid surfaces or exist as free-living forms.

Macroalgae are classified as green, brown and red algae and their colours are derived from natural pigments and chlorophylls. Some pigments reflect or absorb specific light wavelengths giving the characteristic colours: green (*Chlorophyta*), brown (*Phaeophyta*) and red algae (*Rhodophyta*). This feature allows a differential distribution of seaweed at different depths in marine ecosystems. While most macroalgae live near the coastal line, the red algae *Gelidium* sp. inhabits the deep sea (over 25 m below the surface) where sunlight availability is limited.

The chemical composition of macroalgae is significantly different from terrestrial plants. Particularly concerning the carbohydrate fraction, seaweed includes glucose polysaccharides such as cellulose and starch (25–50% dw and 30–60% dw in green and red, respectively), and cellulose and laminarin in brown (30–50%) (Husemann, 1968), but also other complex polysaccharides such as ulvan in green (Husemann, 1968), alginate and fucoidan in brown (Husemann, 1968), and agar or carrageenan in red (Jol et al., 1999) (Table 2). Macroalgal polysaccharides are classified according to their biological function in two groups: energy storage and structural polysaccharides. While starch and laminarin are examples of reserve polysaccharides of green and brown algae, respectively; structural polysaccharides like cellulose and agar (red algae) belong to the cell wall of macroalgae.

Macroalgae have higher water contents (70–90% fresh wt.) compared to terrestrial biomass (sugarcane approximately 75%, grain maize 14%–31%) (Milledge et al., 2014) and also higher amount of minerals as alkali metals (10–50% dw). In contrast, they have relatively low protein (7–15% dw) and lipid contents (1–5% dw) compared to microalgae; the latter with 40–60% dw of proteins and 30–50% dw of lipids (Chew et al., 2017; Jung et al., 2013).

Differently from terrestrial plants, macroalgae lack lignin because in marine environments they do not need the rigidity conferred by this polymer. This constitutes a major advantage for biorefinery purposes because it simplifies carbohydrate extraction and saccharification. Hence, macroalgae have an enormous potential as carbon source for biotechnology processes from biofuels to biochemicals, building blocks and biomaterials.

Besides the lack of lignin and a high content of easily-degradable carbohydrates (25–60% dw), marine plants are attractive renewable feedstocks as they do not use arable land and grow in seawater, thus not competing with terrestrial food crops. Moreover the production yields of algae/unit area are higher as they are highly photosynthetic (Jung et al., 2013). In short, the ability of seaweeds to absorb CO_2, their rich carbohydrate content and lack of lignin increase their potential for biofuel, biochemicals and bioproducts production.

3.1. Green seaweed

Green seaweeds live mostly in shallow waters and are common in bays and estuaries. They are sometimes considered opportunistic seaweeds.

Examples of green algae are *Ulva* spp. (e.g. *Ulva latuca, Ulva rigida, Ulva pertusa*), *Cladophora rupestris*, *Monostroma kuroshiense*, among others (Husemann, 1968; Percival, 1979). Green algae have reserve polysaccharides in the form of starch (1–4%) and as structural polysaccharides: water-soluble ulvan and insoluble cellulose (38–52% dw) (Table 2). Ulvans are acidic water-soluble sulphated

Table 2
Polysaccharides in the three macroalgae groups and monosugars resulting from their hydrolysis.

Seaweed group	Examples	Polysaccharides		Major monosaccharides
		Storage	Structural	
Red	*Gelidium sesquipedale, Gracilaria sp.*	Floridean starch (glucose units)	Cellulose	Glucose
			Agar	Galactose
	Chondrus crispus		Cellulose	Glucose
	Gigartina papillata		Carrageenan	D-galactose
				Anhydrogalactose
Green	*Ulva lactuca, Ulva pertusa*	Starch (glucose units)	Ulvan	Glucose
				Xylose
				L-Rhamnose
				Glucuronic acid
				Iduronic acid
			Cellulose	Glucose
Brown	*Laminaria hyperborea, Fucus vesiculosus, Macrocystis pyrifera*	Laminarin (glucose + mannitol)	Alginate	Mannuronic acid
				Guluronic acid
			Fucoidan	Fucose
				D-xylose
				D-galactose
				D-mannose
				Glucuronic acid
			Cellulose	Glucose

heteropolysaccharides that contribute to the strength of the cell wall and give flexibility to the plant. Furthermore, they may have a role in preventing the desiccation of the biomass exposed to the tides (Lahaye and Robic, 2007; Percival, 1979). The content in ulvans ranges from 8 to 29% of the algal dry weight. Ulvan is composed of uronic acids, namely glucuronic acid and iduronic acid, and of sulphated L-rhamnose, xylose and glucose.

Ulvans are thermoreversible gels, with potential industrial applications in the chemical, pharmaceutical, biomedical and agricultural areas (Cardoso et al., 2014). There are however fewer commercial applications of these gels compared to other algal hydrocolloids.

Ulvan is rich in L-rhamnose, a rare sugar that has several market applications: as a synthetic spice (e.g. synthetic aroma Furaneol), as food additives and as biochemical reagents (https://www.watson-int.com/l-rhamnose-unique-rare-sugar/28.08.2017). Moreover, L-rhamnose is also an essential component of the surface antigens of many microorganisms. Apart from rhamnose, ulvan is composed of iduronic acid, which is another rare sugar important in the synthesis of heparin analogues with antithrombotic activities.

Cardoso et al. (2014) refer that ulvan and its oligosaccharides offer antiviral, antitumor, anticoagulant, antihyperlipidemic, hepatoprotection, protection of the colonic mucosa and immuno-stimulating activities (Cardoso et al., 2014).

For all these reasons, applications of ulvan in the pharmaceutical and in the food area are expected to increase in the next years (Lahaye and Robic, 2007).

3.2. Brown seaweed

Brown macroalgae have a characteristic olive-green to dark brown colour due to the presence of fucoxanthin, a yellow-brown pigment that covers the green colour of chlorophyll. This group includes the largest and most complex macroalga: the kelp (*Laminaria*), which reaches lengths of 100 m. Besides the genus *Laminaria* other examples of brown seaweeds are *Saccharina latissima, Alaria sp.*, *Fucus sp., Macrocystis* sp. and *Sargassum sp.* (Jung et al., 2013).

In brown macroalgae, the main storage polysaccharide is laminarin (Table 2), a water-soluble polysaccharide consisting of 20–25 glucose units. Mannitol, a sugar alcohol is linked to the reducing end of the glucose unit in laminarin (Percival, 1979). Laminarin accounts for up to 35% dw. in brown algae (Mautner, 1954). Laminarin is presently

attracting attention because of some of the potential biological activities: antioxidant, antitumor, antimicrobial, immune modulation, drug delivery and anticoagulant properties (Cardoso et al., 2014). Furthermore, laminarin is not digested by the human digestive system, i.e. it is a natural fiber, thus stimulating the growth of favourable intestinal microbiota (prebiotic).

The major structural polysaccharide of brown seaweeds is alginate and it accounts for up to 40% dry wt. (Draget et al., 2005). Alginate is composed of two different types of uronic acids: mannuronic (M) and guluronic (G) acids. These form chains of contiguous blocks of only mannunoric acid or only guluronic acid or chains of alternate mannuronic and guluronic units. Guluronic-richer alginate has different properties than mannuronic- richer alginic acid (Percival, 1979). The way these M and G units are arranged in the chain and the overall ratio M/G can vary from one species of seaweed to another. Some seaweeds species produce an alginate that gives a high viscosity when dissolved in water, others yield a low viscosity alginate. Alginate is present as salts of different metals, mainly sodium and calcium.

Alginates have been used mostly in textile and food industries to increase the viscosity of aqueous solutions, to form gels and jellies, and to stabilize products like ice-cream, as they impair the formation of large ice crystals and are responsible for a smooth texture (McHugh, 2003).

Brown seaweeds possess also a sulphated polysaccharide named fucoidan. Fucoidan is a heterogeneous highly branched polysaccharide consisting of 1,2-linked α-L-fucose-4-sulfate units with very small amounts of D-xylose, D-galactose, D-mannose, and glucuronic acid (Percival, 1979). The structure of fucoidans has been described to vary significantly among macroalgal species and even within species. Cardoso and co-authors (2014) present an extensive review of the composition of some fucoidans and/or water extracts from brown seaweed (Cardoso et al., 2014). Like alginates, these polysaccharides are included in the cell wall and intercellular region. Apart from other functions, they protect plants from desiccation.

Fucoidan has been extensively studied due to its potential therapeutic properties, including anti-inflammatory and anti-coagulant activities, as well as anti-proliferative effects on cancer cells (Ale and Meyer, 2013). A review by Fitton (2011) discusses the role for fucoidan in the control of acute and chronic inflammation (Fitton, 2011). *Fucus vesiculosus* was shown to be the species most enriched in fucoidan (up to 20% on a dry weight basis) (Cardoso et al., 2014; Duarte et al., 2001).

3.3. Red algae

Red macroalgae have a characteristic red or pink colour from the pigments phycocyanin and phycoerythrin that allow growth in deep waters. Examples of red seaweed are: *Chondrus crispus, Gracilaria* sp. *Porphyra sp. and Gelidium sesquipedale,* among others (Jung et al., 2013).

In red algae, the typical reserve carbohydrates are floridean starch and floridoside, with a structure similar to common starch (Table 2). The cell wall of red seaweed contains cellulose and one of two types of long-chain structural polysaccharides: agar (up to 52% dw) or carrageenan (up to 75%) that are commercially valued for their gel-forming abilities. Both agar and carragenaan are water-soluble. Carrageenan consists of repeating D-galactose units and anhydrogalactose, which may be sulphated. Commercial carrageenans have been extracted from *Chondrus, Gigartina,* and *Eucheuma sp.* (McHugh, 2003). Carrageenans are used as food thickeners in yogurt, ice cream and pudding. In the production of agar and carrageenan, floridean starch must be removed by using a thermophilic α-amylase treatment, since its presence weakens gel strength.

Agar, the structural polysaccharide of other red seaweed, namely *Gelidium* sp. and *Gracilaria sp.,* is made up of alternating β-D-galactose and α-L-galactose with few sulphate groups. Agar has application as algal hydrocolloids in food, pharmaceutical, and biological industries. Agars are not digested by humans and therefore can be regarded as dietary fibers (Cardoso et al., 2014). The functions of these sulphated polysaccharides in the red seaweed are probably similar to those in green and brown macroalgae (Percival, 1979).

Agar oligosaccharides obtained by partial hydrolysis of this hydrocolloid have been found to present important medical applications (Cardoso et al., 2014). For instance, the oral administration of agaro-oligosaccharides could potentially be a therapeutic strategy for the treatment of the inflammatory bowel disease (Higashimura et al., 2013).

It is thus clear that macroalgae accumulate various types of polysaccharides with therapeutic properties. According to Patel (2012) especially the sulfated polysaccharides, namely fucans, carrageenans, and ulvans, have been shown to have antioxidant, antitumor, immunostimulatory, anti-inflammatory, anticoagulant/antithrombotic, antiviral, antibacterial, and antiprotozoan properties (Patel, 2012),. They have been used in hyperplasia prevention, gastrointestinal, regenerative, as well as nano medicine applications. These PS are constituents of cell walls and are most commonly obtained by aqueous (Ghosh et al., 2009) and acetone extraction (Marques et al., 2012). Patel (2012) discusses in detail the mechanisms that explain the aforementioned effects.

3.4. Utilization of macroalgae biomass

Marine macroalgae are composed of many constituents that may be consumed as a whole or upgraded separately. Fig. 1 depicts the many components and applications of marine algae.

3.4.1. Whole algal biomass

Macroalgae have been used for generations as food and in soil conditioning. Currently, seaweed is mostly consumed for human food, fertilizers, phycocolloids and cosmetic ingredients. Asian countries, in particular Japan, China and Corea, are the main seaweed consumers and the main application is for human food. According to FAO (FAO, 2014), the annual world production of seaweeds has been estimated at about 26 million t (fresh), with China being the largest producer (13.5 million t). Due to their high organic matter content, another major utilization of algae is for energy production. This can be attained either by thermolytic or biological methods. A good review on the various methods of energy production from macroalgae is given by Milledge and co-authors (Milledge et al., 2014). These authors divide the various methods in two categories: those that need prior drying of the biomass

such as direct combustion, pyrolysis, gasification; and those where energy can be extracted directly from the wet macroalgae as hydrothermal treatments and anaerobic digestion. When comparing various types of biomass, this division is meaningful because drying costs of macroalgae have a significant impact on the final energy return on investment (EROI) as the water content of seaweed is very high (80%–90%) compared to that of many terrestrial crops (sugarcane approximately 75%, grain maize 14%–31%). It has been suggested that only wet processes can produce net energy due to the high energy requirement to dry the algae. From the thermolytic techniques different types of biofuels can be produced, namely a solid fraction (biochar), an oil fraction (bio-oil) and a gaseous fraction (syngas), while from the anaerobic digestion, biogas (approx. 60% methane) is produced.

From the thermolytic techniques, different types of biofuels can be produced, namely a solid fraction (biochar), an oil fraction (bio-oil) and a gaseous fraction (syngas), while from the anaerobic digestion, biogas (approx. 60% methane) is produced. The oils resulting from pyrolysis of biomass are typically complex mixtures of highly oxygenated organic compounds like phenols, pyrroles and furanes (Ferrera-Lorenzo et al., 2014; Milledge et al., 2014). The composition of bio-oils derived from macroalgae is very different from those of land biomass, due to the composition of seaweeds and the high N, S and ash contents. The presence of nitrogen compounds in bio-oils generates harmful NO_x gases upon combustion and must thus be removed, encompassing so additional fuel refining costs. The utilization of bio-oil as a fuel product still needs further assessment (Bae et al., 2011). Syngas is a combustible gas mixture composed of hydrogen (30%–40%), carbon monoxide (20%–30%), methane (10%–15%), ethylene (1%), nitrogen, carbon dioxide and water vapour. The gas can be burnt to produce heat or converted to electricity and heat in combined gas turbine systems (Milledge et al., 2014). Biochar can be used as a solid fuel and as a precursor of activated carbon (Ferrera-Lorenzo et al., 2014).

Hydrothermal liquefaction requires no feedstock drying and is particularly suited for high moisture raw materials like macroalgae. During this process, seaweed macromolecules such as lipids, proteins, fibers and carbohydrates, break down at high pressures (5–20 MPa), low temperatures (250–350 °C) and in the presence of a catalyst to partially oxygenated hydrocarbons (bio-oil) as well as gaseous, aqueous and solid by-products. The bio-oil produced by liquefaction is lower in oxygen and moisture content (and is thus more stable) than the one derived from pyrolysis (Neveux et al., 2014). It can be used for direct combustion or refined for transportation grade fuels (Toor et al., 2011). The aqueous solution is rich in sugars (Anastasakis and Ross, 2015) that can, in turn, be used to produce bio-ethanol or other chemical commodities through biochemical processes as will be described below. Microalgae have higher yields of bio-oil due to their higher lipid content, which is easily converted under hydrothermal conditions, while the carbohydrates, the dominant fraction of macroalgae, are converted to a lesser extent (Biller and Ross, 2011).

Another alternative process for producing energy directly from wet algae is through anaerobic digestion (AD) to biogas. Methane can be used to produce heat and electricity or compressed for use as transport fuel methane. The suitability of seaweed biomass for the production of methane by anaerobic digestion has been investigated by various groups since the 70s (Forro, 1987). Those studies indicate that seaweeds are in general a suitable biomass for AD and that methane yields ranging from $0.14 \, m^3 \, kg^{-1}$ to $0.40 \, m^3 \, kg^{-1}$ volatile solids (Murphy et al., 2013) can be achieved. Some aspects that may compromise digester performance are related to the macroalgal polysaccharide composition, the synthesis of antimicrobial or toxic substances by algal cells (e.g. polyphenols with antioxidant activity), unfavourable C/N ratios in the substrate biomass (Yen and Brune, 2007) or the presence of a high saline, sulphur or heavy metals content in marine algal species. The hydrolysis of complex seaweed polysaccharides, like alginates, is the rate-limiting step in AD processes. The addition to the anaerobic digesters of special innocula capable of

hydrolysing marine phycocolloids together with some methanogenic archae was found to increase considerably methane production (Sutherland and Varela, 2014). Generally, the use of an inoculum isolated from seaweed sediments with identical origin can enhance microbial tolerance to high concentrations of heavy metals, salts or sulphur (Barbot et al., 2016). In particular, the addition of bacteria isolated from the rumen of Ronaldsay sheep from the Orkney Islands, Scotland, which had a diet almost entirely of seaweed, increased substantially the efficiency the anaerobic digestion of *Laminaria hyperborean* (Sutherland and Varela, 2014; Williams et al., 2012).

Ross and co-authors in their study on the combustion and pyrolysis of macroalgae, concluded that the most suitable technologies for exploitation of seaweeds for fuels are hydrothermal or digestion methods because those conversion processes are not as influenced to the ash components in seaweeds (Ross et al., 2008).

Biofuels from algae are considered "third generation biofuels" (Lee and Lavoie, 2013), however, their production should focus on the use of residual and waste biomass to avoid competition with the seaweed biomass for food industry, as has occurred in the case of terrestrial biomass (Barbot et al., 2016). Seaweed industries such as the phycolloid extraction industries as well as the pharmaceutic and cosmetic sectors generate a considerable amount of biomass waste that can be used for energy generation in particular biogas production. Furthermore, overabundant seaweeds that appear in shallow water, beach and coastal areas, causing eutrophication of marine ecosystems, also offer an abundant biomass supply for biogas plants ((Allen et al., 2013; Bucholc et al., 2014). An estimation of the quantity and origin of industrial and eutrophic macroalgal waste products available if given by Barbot et al. (2016) The possibility to simultaneously produce combustibles and avoid the disposal of undesirable biomass in a synergistic waste management system is a concept with environmental and resource-conserving advantages (Barbot et al., 2016).

3.4.2. Macroalgae biorefinery

Besides being consumed/processed as a whole, algae biomass can be fractionated in different constituents and each upgraded separately: carbohydrates, proteins, lipids, minerals (ash) and other extractables namely: pigments, vitamins and antioxidants (Fig. 1). The fractionation is facilitated compared to lignocellulosic biomass because seaweed lacks lignin. This implies fewer costs during the physical-chemical pretreatment (milder conditions) as well as no need for detoxification of the hydrolysate produced, thus saving considerable costs. The following description is not exhaustive but intends to highlight the composition of each algae component and some of their most recent applications. For each fraction relevant references from the literature are given.

3.4.2.1. Lipid fraction

Despite the low lipid content in seaweed, the percentage of ω-3 and ω-6 polyunsaturated fatty acids (PUFAs) in the lipid fraction is higher than that in terrestrial vegetables (Darcy-Vrillon, 1993). The red seaweed *Gracilaria corticata* contains a low lipid content, nevertheless it is rich in nutritionally important PUFAs (65.6 ± 2.5% of total fatty acids) (Kumari et al., 2013). Lipids recovered from seaweed in integrated processes could thus be used as nutraceuticals in the functional food industry or in the pharmaceutic industry, as PUFAs are known to exhibit anti-hypercholesterolemic, antioxidant, anticancer, antidiabetic, antihypertensive and anti-inflammatory activities (Kumari et al., 2013).

3.4.2.2. Protein fraction

Another important fraction of seaweeds is proteins. In particular, red seaweeds are known to contain protein levels similar to those of traditional protein sources, such as meat, egg, soybean, and milk (Harnedy and FitzGerald, 2011). The protein content of marine algae varies greatly with species and seasons, the same happening with the individual amino acid profile. Reports have shown that, in general, red seaweeds contain high protein levels (max. 47% (w/w) dw), green seaweeds contain moderate amounts [9%–26% (w/

w) dw], while brown algae contain much lower protein contents [3%–15% (w/w) dw] (Fleurence, 2004). In general, most seaweeds contain all the essential amino acids and are a rich source of the acidic residues aspartic and glutamic acid which contribute to the umami taste associated with seaweed (Bleakley and Hayes, 2017; Fleurence, 2004).

Algae can be a valuable source of protein for athletes requiring high levels of protein and in particular vegan athletes for who eggs and dairy whey protein are not suitable. The high protein content of algae can also be used as animal feed, including in aquaculture, farm animals, and pets Different protein applications as feed additives are extensively reported in Bleakley and Hayes (2017). Moreover, macroalgae proteins are a source of bioactive compounds such as linear peptides, cyclic peptides and depsipeptides, peptide derivatives, amino acids, and amino acid–like components. Macroalgal proteins are thus good candidate raw materials for biofunctional peptide mining ((Harnedy and FitzGerald, 2011). Besides acting as sources of nitrogen and amino acids, bioactive peptides have numerous potential physiological functions such as opioid, immunomodulatory, antibacterial, antithrombotic, and antihypertensive activity (Murray and FitzGerald, 2007).

Conventional and recent protein extraction methods have been lengthily described in a recent review by Bleakley S. and Hayes M, 2017.

3.4.2.3. Pigments

Seaweeds have also been investigated for natural pigments. The pigments recovered from plant sources are excellent substitutes for synthetic pigments and can be used in the biomedical field, as food colorants in food industry, cosmetics and pharmaceutical applications. Examples are R-phycoerythrin and R-phycocyanin found in different species of red algae (Baghel et al., 2014; Beer and Eshel, 1985).

3.4.2.4. Minerals

Seaweed mineral content is generally high, 8–40 g/100 g dw, and the essential minerals and trace elements needed for human nutrition are present, while the ash content of terrestrial plants ranges from 5 to 10 g/100 g dw (Rupérez, 2002). Mineral content was determined in several brown (*Fucus vesiculosus*, *Laminaria digitata*, *Undaria pinnatifida*) and red (*Chondrus crispus*, *Porphyra tenera*) seaweed species. Seaweeds contained high proportions of ash (21.1–39.3%) and sulphate (1.3–5.9%). In brown algae, ash content (30.1–39.3%) was higher than in red algae (20.6–21.1%). Brown and red seaweeds can be used as a food supplement to help meet the recommended daily intake of some essential minerals and trace elements (Rupérez, 2002).

The high ash content of seaweed also suggests their use as fertilizer in agriculture. Species of *Ascophyllum*, *Ecklonia* and *Fucus* are sold as soil additives and function as both fertilizer and soil conditioner. They have a suitable content of nitrogen and potassium, but are much lower in phosphorus than animal manures or chemical fertilizers. However for those that prefer an "organic" or "natural" fertilizer, especially in horticulture, they partially replace chemical fertilizers. Insoluble carbohydrates in brown seaweeds act as soil conditioners (they improve aeration and soil structure, especially in clay soils) and have good moisture retention properties. Their effectiveness as fertilizers is also sometimes attributed to the trace elements they contain, but the actual contribution they make is very small compared to normal plant requirements (McHugh, 2003). Blunden (1991) refers that "there is a sufficient body of information available to show that the use of seaweed extracts is beneficial in certain cases, even though the reasons for the benefits are not fully understood" (Blunden, 1991).

3.4.2.5. Carbohydrate fraction

Carbohydrates constitute the largest fraction in macroalgae. Alginate, carrageenan and agar, typical polysaccharides extracted from brown and red algae, besides the already mentioned applications in the fields of food technology, biotechnology, microbiology and medicine have received much attention as a film-forming material (Gade et al., 2013). Edible films

made from seaweeds are nontoxic, degradable and biocompatible and they demonstrate high rigidity and low deformability. However, they have poor water vapour barrier properties due to seaweed's hydrophilic nature (Tavassoli-Kafrani et al., 2016). A combination of two polymer components can improve the desired characteristics and widen the applications. Among the seaweed derivatives, alginate is the most investigated film-making material. κ-carrageenan and alginate resulted in an interesting blend film, in which κ-carrageenan improved the moisture barrier and tensile properties of the alginate film (Paula et al., 2015). The composite formed by processing seaweed and cellulose constitutes also a potential film-forming polymeric material (Khalil, 2017).

3.4.3. Seaweed pre-treatment and hydrolysis of the carbohydrate fraction

Marine macroalgae are attractive renewable feedstocks with a high content of total polysaccharides (60–70% dw) (Gorham and Lewey, 1984) of which 25–60% dw may comprise easily degradable carbohydrates. Due to the growing interest on finding sugar sources for third generation ethanol, an effort is being made on improving the carbohydrates extraction yields from marine macroalgae, as well as PS saccharification efficiency (Lee and Lavoie, 2013; Matsumoto et al., 2003; Shokrkar et al., 2017).

Complete carbohydrate saccharification in macroalgae is feasible by applying a physico-chemical pre-treatment followed by enzymatic hydrolysis as in the saccharification of lignocellulosic biomass. Some studies suggest however that enzymatic hydrolysis can be avoided and a simple hydrolysis with sulphuric acid is efficient. Khambhaty and co-authors used 2.5% H_2SO_4 at 100 °C to saccharify the red seaweed *Kappaphycus alvarezii* and produce a seaweed hydrolysate for ethanol fermentation (Khambhaty et al., 2012). Dilute-acid hydrolysis is a common physicochemical method to treat macroalgal biomass. For each case, optimum conditions need to be determined to maximize the concentrations of monosugars such as the acid concentration, the biomass load, temperature and duration of the hydrolysis.

Using H_2SO_4 hydrolysis, Jang et al. (2012a, 2012b), optimized the process conditions for the saccharification of *Ulva pertusa, Gelidium amansii* and *Laminaria japonica* (59.1%, 71.4% and 54.4% dw total carbohydrate content, respectively). Saccharification of dried *Ulva pertusa* yielded rhamnose (37.9% w/w) and glucose (16.1% w/w), while galactose (49.3% w/w) and glucose (12.6% w/w) were obtained from dried *G. amansii*. Mannitol (31.5% w/w) was produced from L. *japonica* (Jang et al., 2012b). Recently, citric acid-catalyzed pre-treatment was also proven efficient and has the advantage of being cheap and environmentally friendly (Kwon et al., 2016).

Besides acid pre-treatment, other pre-treatment techniques include size reduction (Manns et al., 2016) hydrothermal treatment (Rodríguez-Jasso et al., 2013; Ruiz et al., 2013) and ultrasonic treatment (Karray et al., 2015). An alternative or a complement to the physical-chemical techniques is enzymatic saccharification.

If enzymatic hydrolysis is carried out, the enzymes should be chosen based on the composition of the algal polysaccharides. Because a single species of macroalgae consists of more than one type of polysaccharide, a multi-enzyme complex is thus needed. In the last years, several reports have been published on the use of enzyme cocktails for a complete hydrolysis of several algae species (Sharma and Horn, 2016). In biorefining processes, enzyme recovery (He et al., 2012) and re-use is an economically relevant issue. Membrane separation has been demonstrated as an effective way for enzymes recovery.

The full potential of brown macroalgae as a C-source in biological processes is difficult to achieve because industrial microbes are not able to metabolize the alginate component (Wei et al., 2013). In brown algae, alginate is generally the most abundant polysaccharide followed by laminarin and fucoidan. The depolymerization of alginate under acid or hydrothermal conditions predominantly produces mannuronic and guluronic acid. According to some authors (Wang et al., 2016a, 2016b), these products are poor substrates to most industrial microbes. A

feasible alternative is the use of alginate lyases. When alginate undergoes enzymatic hydrolysis by alginate lyases (Wang et al., 2016a, 2016b), DEH (4-deoxy-L-erythro-5-hexoseulose) is formed being easily further metabolized to 2-keto-3-deoxy-D-gluconate (KDG) and and finally to pyruvate.

Laminarin can reach up to 35% (w/w) of total dw of brown macroalgae, which is the highest level reported in Laminariaceae (Kadam et al., 2015). Unlike alginate, which monomeric sugars are not easily fermented, the final hydrolysis product of laminarin is glucose. Laminarin can thus be fully hydrolysed by a mild acid pre-treatment and/or followed by enzymatic hydrolysis with laminarinase.

From the viewpoint of substrate specificity, fucoidanases are apparently more complex enzymes than alginate lyases or laminarinase and are potentially inhibited by sulfate groups (Berteau et al., 2002). Therefore, the enzymatic saccharification of fucoidan remains an unexplored research area. Furthermore, it is worth noting that, rather than fermenting fucose into other commodity products, it could be commercially more interesting to develop an effective protocol for the enzymatic release of fucose from fucoidan due to the high value of this rare sugar (100 g of L-fucose 99% purity; 2342 €) (www.sigmaaldrich.com).

4. Marine algal carbohydrate bioconversion

Following saccharification, reducing sugars can be used as carbonsource for the biological production of several biochemicals and biomaterials (Fig. 2).

4.1. Biochemicals and bioproducts

Bioconversion of algal carbohydrates could be a significant contribution to the production of organic chemicals as algae-derived sugars can be fermented to commodity chemicals by the appropriate microbes. In 2004, the US Department of Energy (DOE) released a list of building block chemicals that could be produced from biorefinery carbohydrates by chemical or biological conversion (Werpy and Petersen, 2004). The screening criteria included: raw material and estimated processing costs, estimated selling price and market potential. In the next six years this list was changed based on additional criteria for prioritizing opportunities (Bozell and Petersen, 2010). This new list includes two of the biochemicals that have been produced from macroalgae and are reported in the present review, namely succinic acid and lactic acid. Some of the other chemicals are considered new opportunities in biofuels, although they are also important chemicals and building blocks, namely biobutanol and isobutanol. Some others constitute a significant replacement for fossil-derived chemicals (2,3-Butanediol and 1,2-Propanediol) and products (natural pigments), or biologically produced chemicals (citric acid, pyruvate, polyhydroxyalkanoates), which are currently being produced from edible feedstocks (sugars and oils). Table 3, summarizes the estimated prices and market volumes for some of the here reported bio-based products. Although ethanol and levulinic acid are not the focus of the present review they are referred to in this table as a means of comparison. As can be seen in Table 3, these chemicals already have established markets, which are dominated by bioethanol at 58 billion $ a year, n-butanol (from ABE process) at 1 billion $ a year and lactic acid at around 700 m$ a year. Regarding the 8 selected products, bio-based levulinic acid and PHA (prices include homo and *co*-polymers) have the highest current prices. The target indicative future bio-based production cost is around 1000 $/t, and many biobased products are expected to meet this value in the coming years, provided the conversion technology is successfully commercialized (E4tech et al., 2015). Many bio-based products are likely to soon overcome the petro-based ones. However, the price of crude oil is a critical factor for their competitiveness. Moreover, according to the same report ("From the Sugar Platform to Biofuels and Biochemicals", 2015), bio-based succinic acid market is currently increasing at the
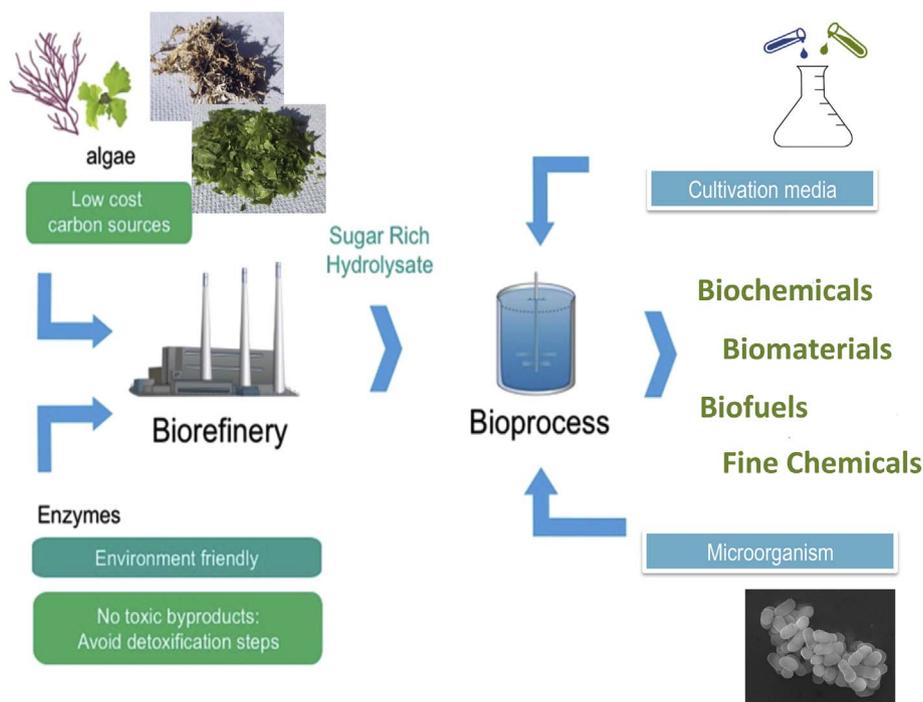
Fig. 2. Algal biomass as carbon source for the biological production of biofuels, bioproducts and biomaterials.

Table 3
Estimated prices and volumes for some bio-based product markets – prices 2013/2014. (Adapted from E4tech, et al., 2015).

Bioproduct	Price ($/t)	Volume (kt/a)	Sales (m$/a)	% of Total market (%)
Ethanol	815	71310	58141	93
Isobutanol	1721	105	181	21
Lactic acid	1450	472	684	100
Levulinic acid	6500	3.0	20	100[a]
n-Butanol	1890	590	1115	20
PHA	6500	17	111	100[a]
Succinic acid	2940	38	111	49

[a] Assumed.

fastest pace.

A summary of the bioprocesses developed up to this date based on algal biomass as feedstock for biochemical and biomaterials is given in Table 4.

4.1.1. Biobutanol

In the last decade, several reports have been published on the use of macroalgae for the production of n-butanol in a mixture of acetone, butanol and ethanol (ABE) (Huesemann et al., 2012; Potts et al., 2012; van der Wal et al., 2013).

Butanol, as well as ethanol, can be used as a gasoline or diesel additive. Butanol constitutes however an attractive alternative to ethanol due to its higher energy content (butanol 29.2 MJ/L; ethanol 19.6 MJ/L), low vapour pressure, lower corrosiveness and the possibility to be mixed in higher concentrations compared to ethanol. Moreover, it can be used in existing combustible engines and shipped using existing pipelines (Fortman et al., 2008). Butanol is also used as solvent in paints, inks, adhesives and coatings, as pharmaceutical and food ingredient, and as a building block, which is converted into chemicals with a wide range of applications. Indeed, *n*-butanol's value as a chemical is over 3 times higher that its value as fuel (http://www.greenbiologics.com/blog/what-is-n-butanol/).

Butanol can be produced through bacterial fermentation using *Clostridium* strains such as *Clostridium acetobutylicum* or *C. beijerinckii* in

a process traditionally known as ABE fermentation. A representative fermentation with *C. acetobutylicum* can produce ABE in the ratio of 3:6:1 acetone: butanol: etanol (Potts et al., 2012). Although commercial butanol production through fermentation using agricultural feedstocks (i.e. corn, molasses, and whey permeate) was very important during WW I and II, it ceased between the 1950s and 1960s when butanol from inexpensive petroleum sources became available (Zverlov et al., 2006). Due to current concerns about economic stability, energy independence, and global climate change and the need to develop new energy sources from sustainable, renewable resources, biobutanol production is again of interest (Zverlov et al., 2006). To make butanol production economically attractive, inexpensive biomass feedstocks were sought as well as high-productivity reactor designs and energy-efficient butanol recovery techniques (butanol is inhibitory to the fermentation limiting yield and productivity). Unlike ethanol-producing cultures, *Clostridia* can utilize both hexoses and pentoses present in lignocellulosic hydrolysates. Agricultural residues such as corn fiber (Qureshi et al., 2008), wheat straw (Qureshi et al., 2007), corn cobs (Marchal et al., 1992), and distillers' dry grain solubles (DDGS) (Ezeji and Blaschek, 2008) have been evaluated as feedstocks.

Macroalgae, particularly green and brown seaweeds, have also been investigated for the production of butanol by *Clostridium* spp. The green algae *Ulva lactuca* harvested in Jamaica Bay, New York City was tested as a potential feedstock for the production of biobutanol through an ABE fermentation (Potts et al., 2012). Extraction and hydrolysis of the algal polysaccharides was achieved after acid hydrolysis (1% acid hydrolysis at 125 °C for 30 min) yielding approximately 15 g/L reducing sugars. This hydrolysate was used, after solids removal, as substrate for biobutanol production using *Clostridium beijerinckii* and *C. saccharoperbutylacetonicum*. The attained yield of butanol during the pilot study was 0.29 g butanol/g consumed sugars.

In another work, *Ulva lactuca* pre-treated by hot-water followed by enzymatic hydrolysis using commercial cellulases was used as feedstock for fermentation by *Clostridium beijerinckii* for the production of acetone, butanol and ethanol (ABE) with a yield of 0.35 gABE/gsugar, demonstrating thus the great potential of *U. lactuca* as feedstock for fermentation (van der Wal et al., 2013) when compared to the ABE yield on glucose (0.4 gABE/gglucose) (Qureshi and Blaschek, 2010). *C.*

Table 4
Microbial production of biochemicals and biomaterials from algal biomass.

Biochemical/ biomaterial	Algal feedstock	Algae hydrolysis conditions	Hydrolysate composition (sugars)	Microorganism	Product yield and/or concentration	Volumetric productivity	Scale and operation mode	Reference
Organic acids								
Citric Acid	Gelidiella acerosa	No hydrolysis - crude seaweed powder	–	Aspergillus niger	30 g/L	n.a.	Batch Shake Flask (volume n.a.)	Ramesh and Kalaiselvam, 2011
Succinic acid	Laminaria digitata hydrolysate	Enzymatic hydrolysis pH 4.8 Celluclast 1.5 L (cellulase), Novozyme 188 (β-glucosidase) alginate lyase	19.4 g/L glucose and 18.8 g/L mannitol	Actinobacillus succinogenes 130Z	0.86 g/g 24.4 g/L	0.5 g/(L h)	Batch Anaerobic Bottles (200 mL)	Alvarado Morales et al., 2015
		Biomass load 100 g /L 0.1 N HCl; 121 °C;15 min. Enzymatic hydrolysis at pH 5.0. Crude cellulase (Ningxia Xiasheng) including endo/exo-glucanase and β-glucanase 50 °C; 150 rpm for 24 h	10 g/L glucose and 10 g/L mannitol	Escherichia coli BS002	0.63 g/g 33.8 g/L 1.2 mol/mol 17.4 g/L	0.7 g/(L h) 0.2 g/(L h)	Batch reactor (3.0 L) Batch reactor (5.0 L)	Alvarado Morales et al., 2015 Bai et al., 2015
	Saccharina latissima hydrolysate	Biomass load 25% (w/w) Enzymatic hydrolysis pH 4.8 Cellulase, β-glucosidase, alginatelyase. 50 °C; 150 rpm; 48 h.	37 g/L of glucose and 16 g/L of mannitol	Actinobacillus succinogenes 130Z	0.92 g/g 36.8 g/L	3.9 g/(L h)	Batch reactor (3.0 L)	Marinho et al., 2016
	Palmaria palmate hydrolysate	Biomass load 100 g/L 0.1 N HCl; 121 °C; 15 min. Enzymatic hydrolysis at pH 5.0. crude cellulase (Ningxia Xiasheng Industry Ltd., China) including endo/exo-glucanase and β-glucanase added at the ratio of 0.1 g/gDW 50 °C; 150 rpm; 60 h	12.6 g/L glucose and 18.0 g/L galactose	Escherichia coli KLPPP	0.74 g/g 22.4 g/L	0.3 g/(L h)	Batch reactor (5.0 L)	Olajuyin et al., 2016
Lactic acid	Enteromorpha prolifera hydrolysate	Biomass load: 100g$_{DW}$/1 L 0.5 M H$_2$SO$_4$, 2 h, 120 °C	10.3 g/L rhamnose, 5.8 g/L D-xylose, 4.9 g/L D-glucose, 1.6 g/L D-glucuronic acid, 0.8 g/L D-glucuronic acid lactone	Lactobacillus salivarius KCTC 3237	3.7 g/L 0.69 g/g	0.41 g/(L h)	Shake flasks (200 mL)	Hwang et al., 2012
	Gelidium amansii hydrolysate	Biomass load: 3–5% (w/v) 3% (v/v) H$_2$SO$_4$, 5 min, 140 °C	19.6 g/L galactose 10.2 g/L glucose	Lactobacillus rhamnosus KY-3	12.5 g/L 0.42 g/g	0.09 g/(L h)	Batch reactor (1.0 L)	Jang, 2013
	Laminaria japonica hydrolysate	Biomass load: 5% (w/v) 0.5 v/v % H$_2$SO$_4$ or 1 v/v % NH$_4$OH (the two hydrolysates were mixed together)	15.2 g/L mannitol	Lactobacillus rhamnosus	14.4 g/L 0.95 g/g	0.30 g/(L h)	Batch reactor (1.0 L)	Jang et al., 2011
		Biomass load: 30g$_{DW}$/0.3 L 0.1 N HCl, 20 min, 121 °C; pH 5.5—Viscozyme®L, Celluclast® 1.5 L, and AMG®300 L (Sigma-Aldrich, St. Louis, MO) 1% (w/w)	31.5 g/L of total sugars, 20 g/L glucose 10 g/L mannitol 1.1 g/L (galactose, mannose and xylose)	Escherichia coli DSM05-L	0.77 g/g* 23.5 g/L * 0.80 g/g * 37.7 g/L *	0.33 g/(L h)* 0.52 g/(L h) *	Shake Flasks Batch (100 mL) Shake flasks Fed-batch (100 mL)	Mazumdar et al., 2014
Alcohols								

Table 4 (*continued*)

Biochemical/ biomaterial	Algal feedstock	Algae hydrolysis conditions	Hydrolysate composition (sugars)	Microorganism	Product yield and/or concentration	Volumetric productivity	Scale and operation mode	Reference
n-Butanol (ABE process)	*Ulva lactuca* hydrolysate	Biomass load: 10% (w/w), 1% H_2SO_4 125 °C; 30 min, Biomass load: 10% (w/w) H_2O; 150 °C; 10 min enzymatic hydrolysis by a cellulase cocktail (GC220, Genencor)	15.2 g/L of reducing sugars	*Clostridium beijerinckii* ATTC 35702	4 g/L 0.29 g/g	n.a.	Batch (2 L)	Potts et al., 2012
			7.8 g/L glucose 2.4 g/L xylose 5.5 g/L rhamnose	*C. beijerinckii* NCIMB 8052	3 g/L 0.23 g/g	0.02 g/(L h)	n.a.	van der Wal et al., 2013
	Saccharina spp.	No hydrolysis – extraction at 65 °C; 1 h in 20 volumes H_2O; pH 2.0	–	*Clostridium acetobutylicum* ATCC 824	5.2 g/L** 0.12 g/g **	0.02 g/(L h) **	Shake flasks Batch (250 mL)	Huesemann et al., 2012
2,3-Butanediol	*Laminaria japonica* hydrolysate	Biomass load: 30g$_{DW}$/0.3L 0.1 N HCl, 20 min, 121 °C; pH5.5—Viscozyme®L, Celluclast® 1.5 L, and AMG®300 L (Sigma-Aldrich, St. Louis, MO) 1% (w/w)	31.5 g/L of total sugars, 20 g/L glucose 10 g/L mannitol 1.1 g/L (galactose, mannose and xylose)	*Escherichia coli* DSM02-B	14.1 g/L (Meso and L-(+) isomers of 2,3 BDO)	0.14 g/(L h)	Shake flasks Fed-batch (100 mL)	Mazumdar et al. (2013)
1,2-Propanediol	*Ulva lactuca* hydrolysate	Biomass load: 20% (w/v) H_2O; 150 °C; 10 min enzymatic hydrolysis with a commercial cellulase cocktail (GC220 Dupont Industrial) 24 h 50 °C	38.8 g/L sugars containing glucose, rhamnose and xylose	*Clostridium beijerinckii* NCIMB 8052	1 g/L # 0.3 g/g# $_{rham}$	0.007 g/(L h) #	Anaerobic serum flasks (30 mL)	Bikker et al., 2016
Biomaterials								
P3HB	*Gelidium amansii* hydrolysate	Biomass load: 10% (w/v) 94 mM H_2SO_4 at 121 °C; 60 min	25.5 g/L galactose 3.6 g/L glucose 6 g/L 5-HMF 1.05 g/L levulinic acid	*Bacillus megaterium* KCTC 2194	5.4 g/L ##	0.09 g/(L h) ##	Fed-batch (2.5 L)	Alkotaini et al., 2016
	Sargassum sp. hydrolysate	10% (w/v) 0.15 N H_2SO_4;121 °C, 30 min followed by enzymatic hydrolysis 15 FPU cellulase/gDW, 15 IU cellobiase/g biomass, 50 °C, 100 rpm, pH 5, 48 h	20 g/L reducing sugars	*Cupriavidus necator* PTCC 1615	0.74 g/g; 3.93 g/L	0.07 g/(L h)	Shake flasks (250 mL)	Azizi et al., 2017
	Gelidium amansii	No hydrolysis - crude seaweed powder	–	*Saccharophagus degradans 2–40*	0.25 g/L	0.002 g/(L h)	Batch reactor (5 L)	Sawant et al., 2017
	Gelidium sesquipedale residues hydroysate	Biomass load: Cellulose β-glucosidase pH 5; 50C; 24 h	36 g/L glucose 11 g/L galactose,	*Halomonas boliviensis*	3.0 g/L 0.27 g/g "	0.05 g/(L h)"	Shake flask 500 mL	Cesário et al., 2017
	Ulva lactuca hydrolysate	Biomass load: 6.2% (w/v) 1% H_2SO_4;121C; 30 min Enzymatic hydrolysis Cellulose β-glucosidade glucoamylase; xylanase pH 5; 50C; 24 h	23 g/L glucose 4 g/L rhamnose 3 g/L and xylose	*Halomonas elongata*	0.8 g/L """ 0.04 g/g """	0.013 g/(L h)"""	Shake flask (500 mL)	Cesário et al., 2017
Red pigment	*Laminaria japonica*	No hydrolysis - crude seaweed powder	–	*Talaromyces amestolkiae* GT11	447.62 UA $_{510nm}$/g	6.759 UA $_{510nm}$/(L h)	Shake flask (500 mL)	General et al., 2014b
Yellow pigment					807.03 UA $_{410nm}$/g	24.937 UA $_{410nm}$/(L h)		

n.a. – Not available; DW- dry algal biomass; UA -Units of Absorbance; * - results obtained after a 4-fold concentration of the hydrolysate; ** - initial sugar concentrations: 4.7 g/L glucose, 26.1 g/L mannitol; # - initial sugar concentrations:15.4 g$_{glucose}$/L, 11.5 g$_{rhamnose}$/L and 1.8 g$_{xylose}$/L; ## - feed corresponds to a 10-fold concentration of the hydrolysate; initial sugar concentrations: 9.7 g/L of total sugar (8.5 g/L galactose and 1.2 g/L glucose); " - initial sugar concentrations: 13 g/L of glucose, 3 g/L galactose; """ - initial sugar concentrations: 12 g/L of glucose, 6 g/L rhamnose, 1 g/L xylose.

beijerinckii utilized all sugars in the hydrolysate for ABE production, while *C. acetobutylicum* produced mostly organic acids (acetic and butyric acids). In a later work, the same research group proposed the valorisation of *U. lactuca* biomass as feedstock for several products using a cascading biorefinery approach (Bikker et al., 2016). The sugar-rich *U. lactuca* hydrolysate (38.8 g/L sugars containing glucose, rhamnose and xylose) supported the fermentative production of ABE and 1,2-PD (1,2-propanediol) by *C. beijerinckii*, and the residual algal biomass fraction showed improved value as animal feed ingredient because of the increased amino acid content, ileal digestibility and rumen fermentation compared to intact *U. lactuca*. *C. beijerinckii* produced 1,2-PD from rhamnose with a yield of 0.3 g/1,2-PD grhamnose while the yield of ABE on total sugars was 0.4 gABE/gtotal sugars consumed. The production of 1,2- propanediol from rhamnose by *C. beijerinckii* had been mentioned earlier by the same authors (van der Wal et al., 2013).

Aqueous extracts of the brown algae *Saccharina* spp., containing mannitol and laminarin were subject to fermentation by *C. acetobutylicum* ATCC 824 (Huesemann et al., 2012). Fermentation of the kelp extract exhibited triauxic growth with free glucose being first depleted followed by mannitol and finally laminarin, a glucose polysaccharide. The lag in laminarin utilization reflected the need for a prior enzymatic hydrolysis step into fermentable sugars. The butanol and total solvent (ABE) yields were low at 0.12 g/g and 0.16 g/g, respectively showing that improvements are still needed to make industrial-scale ABE fermentations of brown seaweed economically feasible. Product yields could be further improved if alginate, an abundant complex but recalcitrant carbohydrate in kelp, could also be utilized by clostridia.

4.1.2. Isobutanol

In 2010, the U.S. Advanced Research Projects Agency (ARPA-E) announced funding to support a DuPont and Bio Architecture Lab, Inc. (BAL) project aimed at exploring the commercial viability of producing fuel-grade isobutanol from macroalgae to be marketed by Butamax, the BP-DuPont JV. Isobutanol is another alternative biofuel with significant advantages over ethanol, including higher energy content, lower greenhouse gas emissions, and the ability to be blended in gasoline at higher levels than ethanol without changes to existing automobiles or the fuel industry infrastructure https://www.osti.gov/scitech/servlets/purl/1046709.

4.1.3. Citric acid

Currently one of the most important industrially produced biochemicals, citric acid (CA) has a wide range of applications, e.g. on the food and beverage, pharmaceutical and metal industries. Its worldwide sale was 2756.5 million $ in 2015 and CA market is still expected to increase in the next decade (http://www.futuremarketinsights.com/reports/citric-acid-market). The preferred industrial process for citric acid production is based on *Aspergillus niger* cultivation on carbohydrate rich substrates. Ramesh and Kalaiselvam (2011) studied the feasibility of using the red macroalgae *Gelidiella acerosa,* with a high carbohydrate content (ca. 60% w/w), as carbon source for CA production. The authors describe in detail the process for CA fungal production and performed 3 different sets of assays. The control medium contained sucrose as C-source, and was compared to the one with crude seaweed powder and to a third one with crude seaweed supplemented with 10% sucrose (Ramesh and Kalaiselvam, 2011). The obtained CA concentrations were 80 g/L at pH 1.5, 30 g/L at pH 3.5 and 50 g/L at pH 3.0, respectively. After an economical comparative analysis, Ramesh and Kalaiselvam (2011) concluded that the medium with the algal biomass and 10% sucrose was the most viable alternative.

4.1.4. Succinic acid

Bioproduced succinic acid (SA) has an immense variety of market applications ranging from pharmaceuticals, resins, food industry to polyurethanes, cosmetics, de-icing solutions, solvents and fine

chemicals, showing a huge potential as platform chemical that can be derived from renewable resources.

Hydrolysates from the *Laminaria japonica* seaweed containing 10 g/L glucose and 10 g/L manitol were used as carbon source for SA production by the engineered *E. coli* BS002. The authors found that a higher yield was reached on mannitol, and, after a 72 h dual-step fermentation, a succinic acid concentration of 17.4 g/L was obtained (Bai et al., 2015). In a similar process, a higher succinic acid concentration (22.4 g/L) was attained with the recombinant *E. coli* KLPPP. The red algae *Palmaria palmate* was pre-treated and enzymatically hydrolysed originating a 12.6 g/L glucose and 18.0 g/L galactose sugar mixture. For this strain, the succinic acid yield on galactose was almost 3 fold higher than on glucose (Olajuyin et al., 2016).

Alvarado-Morales and co-workers (2015) proposed an integrated biorefinery approach, targeting the production of succinic acid and directing the by-products (post-hydrolysis solid residue and fermentation broth) for food (dietary food additive, fish feed), added value products and bioenergy production. In this study, the macroalgae *Laminaria digitata* was hydrolysed to soluble sugars with a total sugar recovery of 78.2%, that were converted to succinic acid by *Actinobacillus succinogenes 130Z* with a yield of 86.5% (g/g of total sugars) and a volumetric overall productivity of 0.50 g/(L h)(Alvarado-Morales et al., 2015). A recent paper reported a much higher succinic acid volumetric productivity (3.9 g/(L h)) corresponding to a 36.8 g/L concentration in the broth. This result was obtained feeding *Actinobacillus succinogenes* a hydrolysate produced with a blend of two different harvests of macroalgae biomass (*Saccharina latissima* hydrolysates) (Marinho et al., 2016). The authors suggest a co-production of antioxidants and fertilizers as a way towards process viability.

4.1.5. Lactic acid

Lactic acid (LA) can be produced by chemical synthesis or by fermentation, although presently over 95% of industrial production of lactic acid originates from this latter route (Taskila and Ojamo, 2013). LA finds its use in the chemical, food, cosmetics and pharmaceutical industries. The interest and high demand for biodegradable polymers as alternative to petroplastics has boosted LA production, as it is used as monomer for the production of poly(lactic acid) (PLA) (Taskila and Ojamo, 2013; Wee et al., 2006). For most applications, high purity L(+) lactic acid (L(+)-LA)) is essential, as this is the biologically active isoform in humans, however D(-)-LA is also bioproduced and commercialized. For PLA production, optical purity is desirable, since the polymer characteristics depend on the enantiomeric form of the monomers. Due to its properties and increased resistence, a stereo-complex PLA, including both P(L(+)-LA) and P(D(-)-LA) is preferably used for many applications. As for most fermentation processes, low cost raw materials play a major role in decreasing LA prices (Taskila and Ojamo, 2013).

Uchida and Murata (2004) firstly reported a method to produce lactic acid and ethanol by fermentation from seaweeds using cellulase for saccharification and lactic acid bacteria together with yeast as cultivation starter. The authors isolated, typed and characterized bacteria and yeasts colonies, and identified a consortium of *Lactobacillus brevis*, *Debaryomyces hanseni* var. *hansenii,* and a *Candida zeylanoides*-related specimen as the predominant micro-organisms in fermented *Ulva* biomass. *Ulva*-suspensions were treated with enzyme mixtures containing cellulase and abalone acetone powder (a visceral enzyme product effective for algal degradation) and concluded that *U. pinnatifida* was the most easily degraded seaweed during incubation (Uchida and Murata, 2004).

Five different *Lactobacillus* strains were investigated by Hwang et al. (2012) for lactic acid (LA) production from *Enteromorpha prolifera* hydrolysates. Acid hydrolysis of algal biomass with H_2SO_4 for 2 h at 120 °C produced a total of 23.4 g/L monosaccharides (L-rhamnose (10.3 g/L), D-xylose (5.8 g/L), D-glucose (4.9 g/L), D-glucuronic acid (1.6 g/L), and D-glucuronic acid lactone (0.8 g/L)). The highest LA

production yield on total sugar consumption, 68.5%, was obtained by *Lactobacillus salivarius*. The authors also compared the LA yield from *E. prolifera* and from corn stover hydrolysates, suggesting that the tested strains used *E. prolifera* hydrolysate in a more efficient way, which is possibly related to differences in the monosaccharides composition and to the lower furan content in the macroalgae hydrolysate (Hwang et al., 2012). This study supports the idea that macroalgae carbohydrate hydrolysates are competitive as feedstock for biochemical production. In a previous study (Hwang et al., 2011), the same authors compared different macroalgae hydrolysates with promising results. As *Laminaria* sp. contains 38.3% non-fermentable sugars (alginate), and *Gelidium amansii* 42.9% of 3,6-anhydrogalactose, the authors anticipate that higher yields can be attained in the future, provided new technology, based on metabolic pathways optimization, is applied to convert the non-fermentable sugar into products.

An engineered *E. coli* strain, containing the *Streptococcus bovis/equinus* L-lactate dehydrogenase, was tested for L-lactate production from brown macroalgae *Laminaria japonica* hydrolysates as carbon source. The engineered strain allowed for a homofermentative route and utilized glucose and mannitol, producing 37.7 g/L of high optical purity L(+)-lactate at the end of 72 h in fed-batch operation, with a yield of 0.8 g/g, (80% of the maximum theoretical yield) (Mazumdar et al., 2014).

Jang et al. (2011) performed both acid (H_2SO_4) and alkaline (NH_4OH) hydrolysis of *L. japonica* to produce a substrate for LA production. The hydrolysates were mixed for neutralization and, for the tested experimental conditions, a 15.2 g/L mannitol concentration was obtained. This monosugar was then bioconverted by *L. rhamnosus* into 14.4 g/L of L-(+)-LA (97.9% optical purity) (Jang et al., 2011). The same authors tested a sulphuric acid hydrolysate of *Gelidium amansii* as carbon source for LA production by *L. rhamnosus* KY-3. Inhibitor components (5-HMF, furfural and phenol) were removed and the monosugars galactose and glucose were converted into lactic acid, together with a small amount of acetic acid, formic acid and etanol (Jang, 2013). A concentration of 12.5 g/L of LA with a 0.42 g/g yield was obtained, corresponding to a productivity of 0.09 g/(L h) (Jang, 2013).

4.1.6. Pyruvate

Besides its essential role as metabolite in living organisms, pyruvate is also an important comercial product, being used as raw material and building block in biochemical, chemical, pharmaceutical and food industries (Akita et al., 2016). Recently, Kawai et al. (2014) used *Sphingomonas* sp. strain A1 to produce pyruvate from alginate. Due to the deletion of the gene for d-lactate dehydrogenase, this bacterium was able to produce high concentrations of pyruvate and secrete it to the fermentation broth, at high aeration rates (Kawai et al., 2014). The higher pyruvate concentration and productivity were 4.56 g/L and 95.0 mg/(L h), respectively, at 5% (w/v) initial alginate concentrations. The authors suggest the use of alginate from brown algae as a sustainable C-source for pyruvate production.

4.1.7. 2,3-Butanediol

2,3 butanediol (BDO), commonly used as antifreeze agent, is an important platform chemical. Its dehydration products have many applications (e.g. as fuel additives, rubber production, food flavouring and bacteriostatic additive) (Celińska and Grajek, 2009).

Mazumdar et al. (2013) engineered an *E. coli* strain to efficiently produce 2,3-butanediol (2,3-BDO) and acetoin (A) from a brown algae hydrolysate. The algal biomass (dried and pulverized *Laminaria japonica*) was acid pretreated and enzymatic hydrolysed with Viscozyme®L, Celluclast® 1.5 L and AMG®300 L to obtain a solution containing ca. 31.5 g/L of sugars, corresponding to glucose (ca. 20 g/L), mannitol (approx. 10 g/L), and 1.1 g/L of a mixture of galactose, mannose and xylose. The hydrolysates were 4 fold concentrated and clarified by centrifugation for fed-batch assays. The resulting metabolic pathway for microaerobic utilization of mannitol and glucose and synthesis of

fermentation products of the *E. coli* DSM02-B was designed to alter the mixed acid fermentation pathways. This allowed for the production of 2,3-BDO + A at high titers (14.1 g/L Meso and L-(+) isomers of 2,3-BDO and 4.8 g/L acetoin, corresponding to a product yield of 0.43 g (2,3-BDO + A)/g total sugar consumption)), as compared to other assays with modified *E. coli* and different residual carbon sources (Mazumdar et al., 2013).

4.1.8. 1,2-Propanediol

Another bioproduced valuable building block is 1,2-propanediol (1,2-PD), also known as 1,2-propylene glycol. This chemical is used in a wide range of applications including polyester resins production, antifreeze and de-icing agents, detergents, pharmaceuticals, cosmetics, and food nutrition products (Saxena et al., 2010).

A promising result was obtained by Merriman (2013), who worked on the optimization of 1,2-PD production by the bacteria *Thermoanaerobacterium thermosaccharolyticum*. A hollow fiber system was applied to an Algal Turf Scrubber® and tested for CO_2 efficient delivery, in order to increase algal biomass productivity. After production, sonic abrasion (a combination of ultrasonication with abrasive materials) (Woods et al., 2011) was performed to improve carbohydrates extraction. The composition of the extracts included C5 (xylose and arabinose) and C6 sugars (glucose, galactose, and mannose). Xylose accounted for approximately 60% of the recovered fermentable material (Merriman, 2013). Although real algal biomass was not included in the fermentation study, the author found, in preliminary 1.5 L bioreactor assays, a 65% increase in 1,2-propanediol titres when using 10 g/L of a synthetic algal sugar mixture, when compared to the same concentration of pure glucose as C-source. For further improvements, Merriman (2013) suggests the use of *U. lactuca* due to its high carbohydrates content.

As referred previously, Bikker and co-workers (2016) produced 1,2-PD from *U. lactuca* biomass in a cascading biorefinery approach. However, 1,2-PD was produced at low titers from the consumed rhamnose, when *Clostridium beijerinckii* NCIMB 8052 was grown in glucose, rhamnose and xylose mixtures. The conversion yield was 0.3 g $_{1,2\text{-PD}}$/g$_{\text{rhamnose}}$. ABE were the main products from the three sugars, and the rhamnose consumption was only 53%. The authors suggest that growth inhibition due to high ABE concentration and the lower metabolic efficiency of the rhamnose conversion route were responsible for this behavior.

4.1.9. PHAs

Polyhydroxyalkanoates (PHAs), a family of bioproduced and biodegradable polyesters, are considered good alternatives for petroplastics, provided their production cost is low enough to be competitive against traditional non-biodegradable polymers. Bacterial strains can synthesize PHAs from carbon-rich agricultural (Cesário et al., 2014; Cesário and de Almeida, 2015), industrial (Cavalheiro et al., 2009) or algal by-products (Alkotaini et al., 2016). The homopolymer poly-3-hydroxybutyrate (P3HB) is the most commonly occurring PHA, although many other polyesters, with different alkanoate monomers, can be biosynthesized.

P3HB production from acid treated (10% w/v) *Gelidium amansii*, a red marine macroalgae, was studied by Alkotaini and co-workers (2016). Six *Bacillus megaterium* strains have been screened for PHA production from the obtained hydrolysates composed of 25.5 g/L galactose, 3.6 g/L glucose, 6 g/L 5-HMF, and 1.05 g/L levulinic acid. Different cultivation conditions were tested and the best results were obtained with *B. megaterium* KCTC 2194 in fed-batch operation mode (54.5% P3HB accumulation, corresponding to 5.4 g$_{\text{P3HB}}$/L). For the tested cell biomass concentration, the authors found a total growth inhibition for concentrations higher than 4 g/L for 5-HMF and 0.7 g/L for levulinic acid in the broth, suggesting the use of 1.2 g/L glucose, 8.5 g/L galactose, 2 g/L 5-HMF, and 0.35 g/L levulinic acid as the optimal hydrolysate composition in the initial media (Alkotaini et al.,

2016). (Alkotaini et al., 2016). A recent study showed that P3HB can be bioproduced from red seaweed *Gelidium amansii* by *Saccharophagus degradans* 40-2 without the need for biomass hydrolysation steps ((Sawant et al., 2017). This bacterial strain synthesizes enzymes such as glycoside hydrolase, glycosidetransferases, polysaccharide lyases, and carbohydrate esterases, being able to process complex polysaccharides, namely agar (Ekborg et al., 2006). The final P3HB concentrations and productivities were very low (Table 4). Nevertheless, the fact that no pretreatment nor enzymatic hydrolysis is needed makes it a promising choice for process optimization.

Azizi et al. (2017) focused on P3HB production based on *Sargassum* sp. hydrolysates. The brown seaweed biomass was subjected to dilute acid hydrolysis, followed by an enzymatic step for monomeric sugars production yielding 201 mg reducing sugars/g biomass dw. *Cupriavidus necator* PTCC 1615 was cultivated in this hydrolysate supplemented with a nitrogen source (ammonium sulphate) and sodium chloride, used as a stress factor to improve polymer production. Different NaCl concentrations were tested with a 20 g/L reducing sugar hydrolysate. The best results were a total biomass of 5.4 g/L containing 3.9 g/L P3HB (Azizi et al., 2017). Although the sugar conversion yields into P3HB are over 0.7 g/g, further work is needed to improve polymer productivity to attain commercial viability.

Poly-3-hydroxybutyrate-*co*-hydroxyvalerate (P(3HB-3HV)) was synthesized by *Halomonas hydrothermalis* MTCC 5445 from *Jatropha* biodiesel by-products (crude glycerol and oil cake hydrolysate) and a seaweed-derived crude levulinic acid (SDCLA) from *Kappaphycus alvarezii* (Bera et al., 2015). After a screening using different media compositions, the optimized conditions at shake flask scale were found to be a mixture of Zobell marine broth (ZMB) supplemented with 0.35% of SDCLA, 2.0% crude glycerol residue and 10% of hydrolysate. 10.7 g/L of P(3HB-81%3 HV) were obtained, corresponding to 73% of total cell dry weight. In previous assays with biodiesel residues, only 4 g/L of PHA had been produced. The authors state that *H. hydrothermalis* appears to perform better with crude glycerol and crude levulinic acid (LA), as compared to the pure glycerol and LA, suggesting that the impurities play an important role on PHA production.

In an exploratory study, Cesário and co-workers (Cesário et al., 2017) (unpublished results) tested sugar mixtures simulating the seaweed hydrolysates of the green macroalga *Ulva lactuca* and residues of the red macroalga *Gelidium sesquipedale* obtained after agar-agar extraction, as sugar sources for the production of poly-3-hydroxybutyrate (P3HB) by two different marine bacterial strains. The hydrolysates were obtained after enzymatic hydrolysis of the seaweed biomass. The best enzyme cocktail, enzyme/biomass ratio and duration of the enzymatic reaction were determined for each type of seaweed. In both cases, a pretreatment using dilute acid hydrolysis was carried out to assess its contribution on the total sugars released. The enzyme cocktail used for *Gelidium* residues hydrolysis was a mixture of cellulase and β-glucosidase while for *Ulva* a mixture of cellulase, β-glucosidase glucoamylase and xylanase was used. Enzymatic hydrolysis alone of *Gelidium* residues achieved a yield of 85–90% of total released sugars (galactose and glucose) and thus the pretreatment was considered redundant. However, *Ulva lactuca* requires a mild acid pretreatment for L-rhamnose release. The combined hydrolysis achieved a yield of 90% of total released sugars (glucose, xylose and rhamnose). *Ulva latuca* and *Gelidium* hydrolysates with ca. 20 g/L total sugars were produced that were easily uptaken by *Halomonas* sp. for PHA production. *Halomonas* species known to be able to consume the main sugars present in each algal hydrolysate were selected from literature. For the purpose, the bacterial ability to accumulate PHA (% w/w) based on those sugars, as well as high specific growth and production rates were key factors. The obtained *U.lactuca* and *G.sesquipedale* hydrolysates, with ca. 20 g/L total sugars, were successfully used for PHA production by *Halomonas elongata* and *H. boliviensis*, respectively.

4.1.10. Pigments

Saccharina (Laminaria) japonica was tested as substrate for red and yellow pigments production by *Talaromyces amestolkiae* GT11. This fungus was cultivated in submerged fermentation at different values of pH and temperature, and both intra and extracelular pigments were quantified (General et al., 2014b). The authors concluded that the pigments are thermostable in a range of 40–60 °C at pH 6 (General et al., 2014a, 2014b).

4.2. Challenges on the effective use of complex seaweed carbohydrates

So far, most of the productivities obtained in algae-to-bioproduct studies are quite low and further optimization is needed (Table 4). The highest reported productivity (3.9 g/(L h)) was obtained by Marinho et al. (2016) for succinic acid production from *S. latissima* hydrolysate in a 3 L (total volume) bioreactor. In fact, as most of the studies are still being run at laboratory scale, mainly in shake flasks, an improvement in yields and productivities is expected when cultivation parameters are on-line monitored and better controlled in bioreactor experiments. The reported yields of product on sugars are based on the sugars released during the saccharification process, which typically features an acid pre-treatment followed (in some cases) by enzymatic hydrolysis with cellulase cocktails. The released monosugars are glucose from the enzymatic hydrolysis of the cellulosic fraction (although glucose can also be originated from the acid hydrolysis of laminarin in brown seaweed) and the sugars that are produced during the acid hydrolysis from algal polysaccharides, such as mannitol from brown seaweed, galactose from red, and rhamnose, xylose and glucuronic acid from green. Some of these sugars are not easily metabolized by standard industrial microbes plus carbon catabolie repression (CCR) phenomena take place when sugar mixtures are to be uptaken. These aspects are discussed below.

Although macroalgae possess high carbohydrate contents (25–50% dw in green, 30–60% in red and 30–50% in brown (Jung et al., 2013)), the complex nature of typical algal polysaccharides prevents the effective utilization of the total carbohydrates in bioprocesses, due to the inability of industrial microbes to metabolize these molecules. A typical example is alginate. In brown seaweed, alginate comprises 30–60% of the total sugars and the non-exploitation of this polysaccharide as substrate in fermentations results in a substantial loss of productivity. Aiming at this genetic modification of industrially used microbes is required to utilize marine algae more effectively. Some groups have engineered *E.coli* and *S. cerevisiae* to metabolize alginate from brown macroalgae.

Representative carbohydrates in brown algae include glucan (laminarin and cellulose), mannitol and alginates. *E.coli* is a natural consumer of glucose and mannitol released after chemical or enzymatic hydrolysis. However, *E. coli* wild species lack the ability to degrade alginate. Researchers of Bio Architecture Lab (BAL) have genetically engineered *E. coli* to break down and ferment alginate from the brown seaweed *Saccharina japonica* and to generate the intermediate pyruvate, the precursor of various fuels and commodity chemical compounds produced in microbial processes. The gene encoding alginate lyase was cloned from a marine bacterium *Pseudoalteromonas* sp. and expressed in *E.coli*, enabling the modified strain to break down alginate into oligomers without chemical pre-treatment or enzymatic saccharification (Wargacki et al., 2012). The next step was to further modify *E. coli* so that it could take up the alginate oligomers and further break them down into pyruvate. This was accomplished by introducing a gene from the marine bacteria *Vibrio splendidus* 12B01 into the genome of the modified *E. coli*. Finally, the researchers added ethanol production genes from *Zymomonas mobilis*, enabling the pyruvate conversion into ethanol. When the fully engineered *E. coli* was fed with a *Saccharina japonica* slurry, the cells produced ethanol through co-fermentation of glucose, mannitol and alginate, up to a concentration of 5% ethanol—comparable to the benchmark. Fermentation of alginate to ethanol consumes the excess reducing equivalents (NADH or NADPH)

generated during the metabolism of mannitol providing thus a counterbalance in the intracellular redox environment. This counterbalance improved also the rate of mannitol fermentation and the resulting ethanol titre (Wargacki et al., 2012).

In another work, Enquist-Newman et al., 2014 have engineered *Saccharomyces cerevisiae*, the standard microbe in the bioethanol industry, to metabolize alginate and mannitol (Enquist-Newman et al., 2014). Genes encoding the alginate metabolism pathway in bacteria were expressed in *S. cerevisiae*. An alginate monomer (4-deoxy-ʟ-erythro-5-hexoseulose uronate, or DEHU) membrane transporter isolated from the alginolytic eukaryote *Asteromyces cruciatus* was introduced. Finally, the efficiencies of the mannitol and alginate metabolism pathways were synchronised for redox control.

These studies show that industrial microbes such as *E. coli* and *S. cerevisiae* can thus work as synthetic biology platforms for the production of other biofuels and renewable chemicals with further genetic modifications. For instance, *E.coli* has already been modified to produce higher value renewable chemicals such as 1, 3-propanediol (Nakamura and Whited, 2003) and 1,4-butanediol (Yim et al., 2011).

The ability of these engineered strains to depolymerize alginate into oligomers and metabolize these oligomers to a variety of biofuels and commodity biochemicals without thermal/chemical pre-treatment or enzymatic saccharification is economically very attractive and an example of a consolidated bioprocess (CBP), i.e. a process that includes hydrolysis and fermentation to the desired products in a single step.

CBP constitutes an alternative to another fermentation scheme namely the Simultaneous Saccharification and Fermentation (SSF) system. This is a process that combines the saccharification and fermentation processes in the same vessel: specialized microorganisms/enzymes degrade complex macroalgae polysaccharides to simple sugars, while other microbes metabolize those sugars to the product of interest. It was shown that higher ethanol yields can be obtained using SSF compared to Separate Hydrolysis and Fermentation (SHF), i.e. the configuration featuring a sequential process where the hydrolysis and fermentation are carried out in different vessels (Jang et al., 2012a). The principal advantages of performing the enzymatic hydrolysis together with the fermentation are: i) the sugar released from the polysaccharides can be utilized directly instead of being accumulated which could inhibit the activity of ethanol producers and ii) the reduced investment costs. The drawbacks, on the other hand, are the need to assure favourable conditions (e.g. temperature and pH) for both the enzymatic hydrolysis and the fermentation and the difficulty to recycle the fermenting organism and the enzymes (Olofsson et al., 2008).

CBP was found to be a promising strategy for effective ethanol production from lignocellulosic materials because it allowed for the reduction in utilities, substrate and other raw materials and simplification of the operation. However, effective CBP microorganisms must be able to fullfill several conditions: i) be able to produce the polysaccharide hydrolysing enzymes to fermentable sugars, ii) be efficient end-product producers (high titer, yield and productivity), iii) be able to consume a mixture of sugars, iv) have resistance to fermentation inhibitors and v) resistance to stressful environments, e.g.: high osmotic pressure, low pH, high temperature, fluctuating processes. A good review comparing SSF and CBP for ethanol production from lignocellulosic biomass is given by Hasunuma and Kondo, 2012 (Hasunuma and Kondo, 2012).

Another important issue concerning seaweed carbohydrate metabolism is related to the effective and efficient conversion of mixed sugars. This matter is also relevant in lignocellulosic biomass where the consumption of the hexoses and pentoses of lignocellulosic hydrolysates is limited by carbon catabolite repression (CCR) (Cesário et al., 2014). Metabolic engineering has been applied to *S. cerevisiae* to improve the consumption of mixed sugars from the hydrolysis of the red seaweed Ceylon moss i.e. *Gracilaria lichenoides*, namely glucose and galactose for ethanol production. That work focused not only to improve galactose metabolism but also on co-fermentation of glucose and galactose.

Different approaches were followed. However, they failed to achieve simultaneous fermentation of glucose and galactose because of the tight regulation of galactose metabolic enzymes by galactose and the strong transcriptional repression of galactose permease (*GAL2*) by glucose (Ha et al., 2011). Repression was circumvented by avoiding the formation of glucose during the carbohydrate hydrolysis of the red seaweed *Gelidium amansii* and allowing cellobiose (a dimer of glucose) to accumulate during hydrolysis. Genes coding to cellobiose transport and intracellular β-glucosidase synthesis from *Neurospora crassa* were expressed in *S. cerevisiae* (Ha et al., 2011). Cellobiose did not repress galactose metabolism and ethanol productivity increased as compared to the two-stage sequential consumption of glucose and galactose. This also reduced the enzyme cost because there was no need for addition of β-glucosidase in the enzyme cocktail during the carbohydrate hydrolysis.

5. Future perspectives

Althought great effort has been dedicated to genetically modify microorganisms aiming at the metabolization of complex algal polysaccharides, the utilization of those polysaccharides for the production of ethanol or other commodities may, in the long term, compromise the multibillion seaweed hydrocolloid industry. That technology is valuable in case of oversupply of phycolloids or to upgrade the polysaccharides of seaweed that cause eutrophication of marine ecosystems as well as of seaweed that are not adequate for applications in the food and feed area. Examples are macroalgae species that have been used to treat waters polluted with heavy metals due to their high biosorption capacity (Ahmady-Asbchin et al., 2009).

The 'biorefinery' concept may improve the economics of sustainable production of biofuels, biochemicals or biomaterials. The fractionation and selective utilization of cellulose for the production of these commodities would be the best option, preventing any negative impacts on the present hydrocolloid industry. Algae competitiveness could be further increased by maximising the extraction of all available high-value components through cascading biorefinery (proteins, lipids, pigments and finally ashes as fertilizer). Additional research to develop new methods that will enable the step extraction of the different algal components and lower processing costs is required.

Another strategy to enhance the productivity of biochemicals or bioproducts from algae feedstocks is to increase the carbohydrate content of algae. Several authors have looked into the genetic transformation of algae to improve biomass productivity and bioproducts accumulation benefiting thus industry and medicine. Many studies have been undertaken to develop molecular biotechnology on macroalgae and various transformation methods were used such as trans-conjugation, electroporation, microinjection, and DNA viruses as transformation vectors. The first report on stable genetic transformation was done in the red marine seaweed *Porphyra yezoensis* (Cheney et al., 2001). The current progress in establishing both transient and genetic transformation systems in macroalgae is reviewed by Mikami, (2013).

There have also been recent efforts to genetically improve microalgae to obtain high value products, but most of them are directed towards fresh water species, namely *Chlamydomonas reinhardtii* (Lauersen et al., 2016; Morales-Sanchez et al., 2017). The Biocore team at the French Institute for Research and Automatic Control (Inria), led by Dr. O. Bernard, has been using evolution engineering for enhancing the productivity of microalgae. At Inria, wild strains are being improved to increase their productivity by applying, for instance, thermal stress. A strain of the marine microalga *Tisochrysis lutea* was obtained that presents a high resistence to large thermal amplitudes (Bonnefond et al., 2017).

6. Economic feasibility

Algae are, undoubtedly, an underexplored resource. The economic

feasibility of the use of both micro and macroalgae as renewable and environment friendly feedstock relies, according to several authors, on upgrading the various fractions in a cascade biorefinery (Wijffels et al., 2010; (Bleakley and Hayes, 2017; Wijffels et al., 2010). In fact, several studies have pointed out the value of the so-called algal "by-products" (Bleakley and Hayes, 2017). Conventional methods of, for instance, agar extraction, still leave behind *G. sesquipedale* residues that contain 48% carbohydrates (Cesário et al., 2017).

With regard to microalgae, Norsker et al. (2011) report an economic analysis on the production cost of microalgae according to the type of bioreactor used (Norsker et al., 2011). Three configurations were considered, namely open ponds, and horizontal tubular and flat panel photobioreactors. The conclusion from this simulation analysis was that, under Dutch weather conditions, the tubular photobioreactor is the most economical with regard to the sum of capital and operating costs, with a unit production cost of 4.15 €/kg (dw) of algal biomass. Moreover, these authors conducted a sensitivity analysis of the decrease of mixing and nutrient costs, as well as of improvements in irradiation and photosynthetic efficiencies, which were taken as realistic in the short term. The result was the decrease in cost to 0.68 €/kg of dry microalgae. This cost level makes microalgae a competitive feedstock for the production of biodiesel and bulk chemicals. From the conclusions of this study, it may be extrapolated that high added value microalgal final products or intermediates, obtained either by biochemical or chemical conversion, will be economically feasible if the algae are cultivated under optimized conditions.

In a recent paper, Baghel and co-workers claim that both the encouraging and the support of large-scale macroalgal farming will stimulate ocean-based industries and mitigate coastal eutrophication in many areas (Baghel et al., 2015). Additionally, these authors pointed out that macroalgal farming might be converted into a source of sustainable income, and, concomitantly, decrease the global warming and climate change arising from the use of fossil fuels.

Murthy Konda et al. (2015) conducted an important evaluation on the economic feasibility of macroalgal as feedstocks for biorefineries under different scenarios, namely ethanol production, alginate extraction and macroalgae-to-sugar processes, for the production of chemicals under market conditions of high sugar prices. Interestingly, the later scenario appears to lead to an economically viable platform, provided advances are made in the hydrolytic step by focusing on solids and enzyme loadings, as well as on the sugar yield (Konda et al., 2015).

In this context, there seems to be a promising economic viable future on the use of algal carbohydrates as carbon source to produce valuable biomaterials, biochemicals and chemical intermediates.

7. Conclusions

Marine algae have a huge potential as carbon source for biobased products because they are a carbohydrate rich-feedstock. Moreover, they are able to grow in saline and blackish water, requiring light, CO_2 and some common elements (N, P, K), that they convert into lipids, carbohydrates, and proteins with no need for arable land or fresh water. They have higher growth rates compared to terrestrial biomass because of their high photosynthetic rates (Jung et al., 2013). Another advantage of macroalgae is the low content of hemicelluloses and the absence of lignin, simplifying carbohydrate extraction and hydrolysis steps. Despite these advantages, the main challenge related to seaweed biomass is their unique carbohydrates (e.g. alginate) making the existing lignocellulosics-based technologies, i.e. hydrolysis and fermentation processes, inadequate. Besides, the cost of production and harvest of seaweed and marine microalgae is currently still too high (de Jong and Jungmeier, 2015), making marine biorefinery economically feasible only when the different algal fractions (carbohydrates, proteins and lipids) are utilized/upgraded using a cascade-biorefinery approach. Until recently, efforts have mainly been directed to obtain sustainable biofuels (e.g. biodiesel) from microalgae lipids and bioethanol from

macroalgae carbohydrates, and the encouraging results have paved the way for further studies. Many research groups are currently focusing on algal biomass up-grade into biochemicals and biomaterials. Nevertheless, this review shows that the potential of marine microalgae carbohydrate rich fraction (e.g. by-product from algal biodiesel production) as carbon source biological processes is still to be explored. From this review, it is anticipated that the advances in saccharification methods and biological utilization of complex sugars and sugar mixtures will greatly enhance product yields. These new methods are currently being optimized at lab and pilot scale mainly for marine macroalgae carbohydrate rich fractions. As a result, it is highly plausible that an environmentally conscious applied biorefinery strategy will soon both greatly boost marine resource exploitation and foster new markets in bioeconomy.

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