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Algal Polysaccharides, Novel Applications and Outlook

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1. Introduction

Marine algae contain large amounts of polysaccharides, notably cell wall structural, but also mycopolysaccharides and storage polysaccharides (Kumar et al. 2008b; Murata and Nakazoe 2001). Polysaccharides are polymers of simple sugars (monosaccharides) linked together by glycosidic bonds, and they have numerous commercial applications in products such as stabilisers, thickeners, emulsifiers, food, feed, beverages etc. (McHugh 1987; Tseng 2001; Bixler and Porse, 2010). The total polysaccharide concentrations in the seaweed species of interest range from 4-76 % of the dry weight (Table 1). The highest contents are found in species such as *Ascophyllum*, *Porphyra* and *Palmaria*, however, green seaweed species such as *Ulva* also have a high content, up to 65 % of dry weight.

Seaweeds are low in calories from a nutritional perspective. The lipid content is low and even though the carbohydrate content is high, most of this is dietary fibres and not taken up by the human body. However, dietary fibres are good for human health as they make an excellent intestinal environment (Holt and Kraan, 2011).

The cell-wall polysaccharides mainly consist of cellulose and hemicelluloses, neutral polysaccharides, and they are thought to physically support the thallus in water. The building blocks needed to support the thalli of seaweed in water are less rigid/strong compared to terrestrial plants and trees. The cellulose and hemicellulose content of the seaweed species of interest in this review is 2-10 % and 9 % dry weight respectively. Lignin is only found in *Ulva* sp. at concentrations of 3 % dry weight (Table 2).

The cell wall and storage polysaccharides are species-specific and examples of concentrations are given in Table 3. Green algae contain sulphuric acid polysaccharides, sulphated galactans and xylans, brown algae alginic acid, fucoidan (sulphated fucose), laminarin (β -1, 3 glucan) and sargassan and red algae agars, carrageenans, xylans, floridean starch (amylopectin like glucan), water-soluble sulphated galactan, as well as porphyran as

Brown Algae		Green Algae				RedAlgae	
Laminaria & Saccharina	Fucus	Asophyllum	Undaria	Sargassum	Ulva	Chondrus	Porphyrta
38 % ^b	62 % ^s	42-64 % ^s	35-45 % ^{d,e}	4 % ^f	15 % -65 % ^{g-i,q}	55-66 % ^s	40 % ^e
48 % ^s	66 % ^a	44 % ^c		68 % ^f	18 % ^p		41 % ^r
58 % ^a		70 % ^a			42-46 % ^s		50-76 % ^s
61 % ^s							54 % ^b
36 % ^l			35-46 % ^{l,m}	49-62 % ^{l,m}	38 % ^{l,m}		35-49 % ^{l,m}
		38 % ^c	30 % ^{l,m}	33 % ^{l,m}	21 % ^{l,m}		18 % ^{l,m}
					3 % ^g		
10 % in stipe ⁿ	2-4.5 % ^o	2 % ^o			9 % ^g		
4.5-9 % ^o		3.5-4.6 % ⁿ					
					9 % ^g		

n et al. 2006), c= (Tseng 2001), d= (Je et al. 2009), e= (Murata and Nakazoe 2001), f= (Marinho-Soriano et al. 2006), g= (Ortiz et al. 2006), i= (Sathivel et al. 2008), j= (Heo and Jeon 2008), k= (Mishra et al. 1993), l= (Dawczynski et al. 2000), o= (Black 1950), p= (Foster and Hodgson 1998), q= (Wong and Cheung 2000), r= (Arasaki and Arasaki 1983)

Brown Algae		Green Algae			Red Algae		
Laminaria & Saccharina	Fucus	Ascohyllum	Undaria	Sargassum	Ulva	Chondrus	Porphyrta
38 % ^b	62 % ^s	42-64 % ^s	35-45 % ^{d,e}	4 % ^f	15 % -65 % ^{g-i,q}	55-66 % ^s	40 % ^e
48 % ^s	66 % ^a	44 % ^c		68 % ^f	18 % ^p		41 % ^r
58 % ^a		70 % ^a			42-46 % ^s		50-76 % ^s
61 % ^s							54 % ^b
36 % ^l			35-46 % ^{l,m}	49-62 % ^{l,m}	38 % ^{l,m}		35-49 % ^{l,m}
		38 % ^c	30 % ^{l,m}	33 % ^{l,m}	21 % ^{l,m}		18 % ^{l,m}
					3 % ^g		
10 % in stipe ⁿ	2-4.5 % ^o	2 % ^o			9 % ^g		
4.5-9 % ^o		3.5-4.6 % ⁿ					
					9 % ^g		

Table 1. Content of total polysaccharides (% of dry weight) and structural and dietary fibres (% of dry weight) in seaweed species of interest in North-west Europe.

Class	Genus	Uses
<i>Chlorophyta</i>	<i>Monostroma</i>	edible, human food
	<i>Enteromorpha</i>	edible, human food
<i>Phaeophyta</i>	<i>Laminaria</i>	alginates, edible, human food
	<i>Undaria</i>	edible, human food
	<i>Cladosiphon</i>	edible, human food
<i>Rhodophyta</i>	<i>Asparagopsis</i>	medical applications
	<i>Gelidiella</i>	agar, food and medical
	<i>Gelidiopsis</i>	agar, food and medical
	<i>Gelidium</i>	agar, food and medical
	<i>Gracilaria</i>	agar, food and medical
	<i>Pterocladia</i>	agar, food and medical
	<i>Chondrus</i>	carrageenan, human food
	<i>Eucheuma</i>	carrageenan, human food
	<i>Kappaphycus</i>	carrageenan, human food
	<i>Gigartina</i>	carrageenan, human food
	<i>Hypnea</i>	carrageenan, human food
	<i>Iridaea</i>	carrageenan, human food
	<i>Palmaria</i>	human and animal feed
	<i>Porphyra</i>	human food

Table 2. Most common genera and uses of seaweeds produced in aquaculture.

Product	Global Production	Retail Price	Approximate Gross Market Value	Amount Used	
	(ton/year)	(US\$/kg)	(US\$million/year)	Food (%)	Pharmacy(%)
Agars	9,600	18	173	80	ca 15
Alginates	26,500	12	318	30	5
Carrageenans	50,000	10.5	525	80	10

Table 3. Phycocolloids and their global production, retail price, gross value and percentage used in food and pharmacy(Data; McHugh, 2003; Bixler and Porse, 2010)

mucopolysaccharides located in the intercellular spaces (Table 3; Kumar et al. 2008b; Murata and Nakazoe 2001). Contents of both total and species-specific polysaccharides show seasonal variations. The mannitol content varied markedly in the fronds of *Saccharina* and *Laminaria* species with maximum amounts found during summer and autumn, from June to November. The laminaran showed extreme variations during the year with very small amounts or none at all in February to June and maximum in September to November (Haug and Jensen 1954; Jensen and Haug 1956). The maximum content of alginic acid in the fronds of *Saccharina* and *Laminaria* species was generally found from March to June and the

minimum from September to October (Haug and Jensen 1954). However, highest contents of alginic acid were found during winter in other seasonal studies on *Laminaria* species from the same areas in Norway (Jensen and Haug 1956).

Further investigations on the hydrolysates of some brown algae showed complex mixtures of monosaccharides. The components of galactose, glucose, mannose, fructose, xylose, fucose and arabinose were found in the total sugars in the hydrolysates. The glucose content was 65 %, 30 % and 20 % of the total sugars in an autumn sample of 50 individual plants of *Saccharina*, *Fucus (serratus and spiralis)* and *Ascophyllum*, respectively (Jensen 1956).

Several other polysaccharides are present in and utilised from seaweed e.g. furcellaran, funoran, ascophyllan and sargassan, however these are not described in this chapter.

Seaweed polysaccharides are separated into dietary fibres, hydrocolloids etc. in the following paragraphs, even though the polysaccharides belong to more than just one of the functional groups.

2. Seaweed production and extraction

Harvesting or aquaculture of marine algae or seaweeds is an extensive global industry.

This seaweed industry provides a wide variety of products that have an estimated total annual production value of about US\$ 6 billion. Food products for human consumption contribute about US\$ 5 billion to this figure. Substances that are extracted from seaweeds – hydrocolloids – account for a large part of the remaining billion dollars, while smaller, miscellaneous uses, such as fertilizers and animal feed additives, make up the rest. The industry uses almost 20 million ton of wet seaweed annually (FAO, 2012 <http://www.fao.org/fishery/statistics/global-aquaculture-production/en>), harvested either from naturally growing (wild) seaweed or from cultivated (farmed) crops. The farming of seaweed has expanded rapidly as demand has outstripped the supply available from natural resources. Commercial harvesting occurs in about 35 countries, spread between the northern and southern hemispheres, in waters ranging from cold, through temperate, to tropical (Mc Hugh, 2003).

In Asia, seaweed cultivation is by far more important in terms of output and value than any other form of aquaculture. Looking at a global scale cultivated, seaweeds account for 87% of all seaweed harvested and processed of which the bulk is derived from aquaculture in Asia. The most valued of the cultivated seaweeds is the red alga *Porphyra*, or Nori. It is a major source of food for humans throughout the world, although it is almost exclusively cultivated in Japan, South Korea and China. Worldwide production has an annual value of over € 1.5 billion. In addition to *Porphyra*, other edible seaweeds include *Gracilaria*, *Undaria*, *Laminaria* and *Caulerpa* with their collective value exceeding € 3.0 billion. New applications of seaweeds and specific seaweed compounds in different sectors, such as food supplements, cosmetics, biomedicine and biotechnology are developed (Chritchley et al., 2006)

Seaweeds are also the industrial sources of polysaccharides such as carrageenans (*Chondrus*, *Eucheuma* and *Kappaphycus*), alginates (*Ascophyllum*, *Laminaria*, and

Macrocystis) and agars (Gelidium and Gracilaria; Table 2) and have a global value of approximately \$ US 1 billion (Table 3). These important polysaccharides are used in the food, textile, paint, biotechnological and biomedical industries and have recently come under the spotlight as functional food ingredients. (Critchley et al., 2006). The majority of these species are used in some form for food or, in a few cases, for chemical extracts. The costs of production of the biomass tend to exceed the value of the biomass as a raw material for phycocolloid extraction, although it is known that some Chinese kelps produced by aquaculture are used for the production of salts of alginic acid, and applying low-technology extensive forms of aquaculture are used to produce Gracilaria for agar extraction (table 2). The value of red seaweed produced by aquaculture showed a declining trend to US\$1.3 - 1.4 billion over the 1997 - 2000 period. This is probably due to the high volumes of carrageenophytes Kappaphycus and Eucheuma produced by cultivation in south-east Asia and, to some extent, Gracilaria cultivated in South America, and as such, showing trends towards commoditization.

3. Phycocolloid industry

The term phycocolloid is used to refer to three main products (alginate, carrageenan and agar) which are extracted from Brown and red seaweeds respectively. The estimated world market value for phycocolloids is \$ US 1 Billion (Bixler and Porse, 2010). European output of phycocolloids is estimated to have an annual wholesale value of around €130 million which is 97.5% of the total for all algal products in Europe (Earons, 1994).

3.1. Brown seaweeds and alginates

Global alginate production is ca. 26,500 tons and valued at US\$318 million annually, and is extracted from brown seaweeds, most of which are harvested from the wild. The more useful brown seaweeds grow in cold waters, thriving best in waters up to about 20 °C. The main commercial sources of phaeophytes for alginates are *Ascophyllum*, *Laminaria*, and *Myrocystis*. Other minor sources include *Sargassum*, *Durvillea*, *Eklonia*, *Lessonia*, and *Turbinaria* (Bixler and Porse, 2010)..

Brown seaweeds are also found in warmer waters, but these are less suitable for alginate production and are rarely used as food due to non-desirable chemical compounds such as terpenes. A wide variety of species are used, harvested in both the northern and southern hemispheres. Countries producing alginate include Argentina, Australia, Canada, Chile, Japan, China, Mexico, Norway, South Africa, the United Kingdom (mainly Scotland) and the United States. Most species are harvested from natural resources; cultivated raw material is normally too expensive for alginate production with the exception of China. While much of the *Laminaria* cultivated in China is used for food, when there is surplus production this can also be used in the alginate industry.

Seaweed hydrocolloids, such as alginate, agar and carrageenan, compete with plant gums (such as guar and locust bean) and cellulose derivatives (such as carboxy methyl cellulose (CMC) and methyl cellulose) that are often cheaper.

Alginates are used in the manufacture of pharmaceutical, cosmetic creams, paper and cardboard, and processed foods (Chapman, 1970). Alginate represents the most important seaweed colloid in term of volume. Grades of alginate are available for specific applications. Sodium alginate, pharmaceutical grade (US\$ 13-15.5 per kg), food grade (US\$ 6.5-11.0 per kg). In Japan and Korea, high demands for *Laminaria japonica* (trade name kombu) have resulted in high prices and necessitated the import of supplies for alginate extraction (Critchley, 1998). Annual growth rate for alginates is ca. 2-3 percent, with textile printing applications accounting for about half of the global market. Pharmaceutical and medical uses are about 20 percent by value of the market and have stayed buoyant, with 2-4 percent annual growth rates, driven by ongoing developments in controlled release technologies and the use of alginates in wound care applications. Food applications are worth about 20 percent of the market. That sector has been growing only slowly, and recently has grown at only 1-2 percent annually. The paper industry takes about 5 percent and the sector is very competitive, not increasing but just holding its own. The alginate industry faces strong competition from Chinese producers, whose prices do not reflect the real expense of cultivating *Laminaria japonica*, even in China, yet they do not appear to import sufficient wild seaweeds to offset those costs. The result is low profitability for most of the industry, with the best opportunities lying in the high end of the market, such as pharmaceutical and medical applications.

3.2. Red seaweeds and Carrageenans

Carrageenans are a group of biomolecules composed of linear polysaccharide chains with sulphate half-esters attached to the sugar unit. These properties allow carrageenans to dissolve in water, form highly viscous solutions, and remain stable over a wide pH range. There are three general forms (kappa, lambda and iota), each with their own gelling property. Kappa carrageenan today is almost exclusively obtained from farmed *Kappaphycus alvarezii* and iota from farmed *Eucheuma denticulatum* (Rasmussen and Morrissey 2007).

The Carrageenan market is worth US\$ 527 m with most Carrageenan used as human food-grade semi-refined carrageenan (90%) and the rest going into pet food as semi-refined carrageenan. *Chondrus crispus* and *Kappaphycus* sp. are species containing up to 71 % and 88 % of carrageenan, respectively (Chopin et al. 1999; Rodriguez and Montaña 2007). Food applications for carrageenans (E 407) are many, including canned foods, desert mousses, salad dressings, bakery fillings, ice cream, instant deserts and canned pet-foods. In the 1970s, an energy efficient process was developed in the Philippines to make a lower cost, strong-gelling kappa carrageenan and a weakly gelling iota. These semi-refined products gradually replaced the use of refined carrageenan as the gelling agent in canned meat pet foods. The process required lower capital investment than standard carrageenan refineries and the semi-refined extracts could be profitably sold for about two-thirds the price of conventionally refined carrageenan (Bixler and Porse, 2010). Industrial applications for purified extracts of carrageenans are equally diverse. They are used in the brewing industry for clarifying beer, wines and honeys, although less commonly than previously. There has been a fairly significant increase in production capacity for gel-press-refined carrageenan in recent years, particularly in Asia-Pacific.

3.3. Red seaweeds and agar

Agar is a mixture of polysaccharides, which can be composed of agarose and agaropectin, with similar structural and functional properties as carrageenans. It is extracted from red seaweed such as *Gelidium* spp. and *Gracilaria* spp. (FAO 2008; Rasmussen and Morrissey 2007; Jeon et al. 2005). A total of 9600 mt were sold in 2009 with a value of US\$ 173 m. Agar-agar is a typical and traditional food material in Japan and it is used as a material for cooking and Japanese-style confectionary. In addition, agar-agar is used in the manufacture of capsules for medical applications and as a medium for cell cultures, etc. Food applications continue to grow as shown by an increase of 2100 mt in over the last decade, and have been driven by the growth of processed foods in developing countries (Bixler and Porse, 2010).

Agar melts and gels at higher temperatures than carrageenan so it finds uses in pastry fillings and glazes that can be applied before the pastry is baked without melting in the pastry oven. In processed meats, carrageenan is the favored water binder or texturing agent, but agar hold on to the gelatine replacement market in canned meats and aspics. The texture of agar in fruit jellies also compete with kappa carrageenan jellies, but the agar texture is preferred in parts of Asia, particularly in Japan. Although significantly smaller, the markets for the specialty grades are quite attractive because of better profit margins. Agarose, a sulfate-free very pure form of agar finds widespread use today in gel electrophoretic analysis of the molecules of biotechnology.

4. Bioactive algal polysaccharides and functional properties

While food has long been used to improve health, our knowledge of the relationship between food components and health is now being used to improve food. Strictly speaking, all food is functional, in that it provides energy and nutrients necessary for survival. But the term “functional food” in use today conveys health benefits that extend far beyond mere survival. Food and nutrition science has moved from identifying and correcting nutritional deficiencies to designing foods that promote optimal health and reduce the risk of disease.

The combination of consumer desires, advances in food technology, and new evidence-based science linking diet to disease and disease prevention has created an unprecedented opportunity to address public health issues through diet and lifestyle. Widespread interest in select foods that might promote health has resulted in the use of the term “functional foods.” Although most foods can be considered “functional,” in the context of this Chapter the term is reserved for algal polysaccharides that have been demonstrated to provide specific health benefits beyond basic nutrition, such as, alginates, agars, carrageenans, fucoidan, mannitol, laminarin, and ulvan.

4.1. Dietary fibres

The dietary fibres are very diverse in composition and chemical structure as well as in their physicochemical properties, their ability to be fermented by the colonic flora, and their biological effects on animal and human cells (Lahaye and Kaeffer 1997). Edible seaweed

contain 33–50 % total fibres on a dry weight basis, which is higher than the levels found in higher plants, and these fibres are rich in soluble fractions (Lahaye 1991). The dietary fibres included in marine algae are classified into two types, i.e. insoluble such as cellulose, mannans and xylan, and water soluble dietary fibres such as agars, alginic acid, furonan, laminaran and porphyran. The total content of dietary fibres is 58 % dry weight for *Undaria*, 50 % for *Fucus*, 30 % for *Porphyra* and 29 % for *Saccharina* (Murata and Nakazoe 2001). *Fucus* and *Laminaria* have the highest content of insoluble dietary fibres (40 % and 27 % respectively) and *Undaria pinnatifida* (wakame), *Chondrus* and *Porphyra* have the highest content of soluble dietary fibres (15 % – 22 %; Fleury and Lahaye 1991).

The undigested polysaccharides of seaweed can form important sources of dietary fibres, although they might modify digestibility of dietary protein and minerals. Apparent digestibility and retention coefficients of Ca, Mg, Fe, Na and K were lower in seaweed-fed rats (Urbano and Goñi 2002). The seaweed dietary fibres contain some valuable nutrients and substances, and there has been a deal of interest in seaweed meal, functional foods, and nutraceuticals for human consumption (McHugh 2003), because among other things polysaccharides show anti-tumour and anti-herpetitic bioactivity, they are potent as an anti-coagulant and decrease low density lipid (LDL)-cholesterols in rats (hypercholesterolemia), they prevent obesity, large intestine cancer and diabetes, and they have anti-viral activities (Lee et al. 2004; Murata and Nakazoe 2001; Amano et al. 2005; Athukorala et al. 2007; Ghosh et al. 2009; Murata and Nakazoe 2001; Ye et al. 2008). Moreover, glucose availability and absorption are delayed in the proximal small intestine after the addition of soluble fibres, thus reducing postprandial glucose levels (Jenkins et al. 1978). Water insoluble polysaccharides (celluloses) are mainly associated with a decrease in digestive tract transit time (see also digestibility of polysaccharides; Mabeau and Fleurence 1993).

4.2. Alginates

Alginates were discovered in the 1880s by a British pharmacist, E.C.C. Stanford; industrial production began in California in 1929. Algins/alginate are extracted from brown seaweed and are available in both acid and salt form. The acid form is a linear polyuronic acid and referred to as alginic acid, whereas the salt form is an important cell wall component in all brown seaweed, constituting up to 40 % - 47 % of the dry weight of algal biomass (Arasaki and Arasaki 1983; Rasmussen and Morrissey 2007). Alginates are anionic polysaccharides. They contain linear blocks of covalently (1–4)-linked β -D-mannuronate with the C5 epimer α -L-guluronate. The blocks may contain one or both of the monomers and the ratio of monomers A and B, as well as a number of units (m and n) in a block is species dependent.

It has been reported that alginic acid leads to a decrease in the concentration of cholesterol, it exerts an anti-hypertension effect, it can prevent absorption of toxic chemical substances, and it plays a major role as dietary fibre for the maintenance of animal and human health (Kim and Lee 2008; Murata and Nakazoe 2001; Nishide and Uchida 2003). These dietary polysaccharides are not found in any land plants. They help protect against potential carcinogens and they clear the digestive system and protect surface membranes of the stomach

and intestine. They absorb substances like cholesterol, which are then eliminated from the digestive system (Burtin 2003; Ito and Tsuchida 1972) and result in hypocholesterolemic and hypolipidemic responses (Kiriyaama et al. 1969; Lamela et al. 1989; Panlasigui et al. 2003). This is often coupled with an increase in the faecal cholesterol content and a hypoglycaemic response (Dumelod et al. 1999; Ito and Tsuchida 1972; Nishide et al. 1993).

Alginates, fucoidans and laminarin extracts were tested against nine bacteria, including *Escherichia coli*, *Staphylococcus*, *Salmonella* and *Listeria*. They appeared to be effective against *E. coli* and *Staphylococcus*. Sodium alginate seemed to demonstrate a strong anti-bacterial element. It not only binds but also kills the bacteria. Studies conducted on seaweed extracts found that fucoidan appeared to function as a good prebiotic (a substance that encourages the growth of beneficial bacteria in the intestines). An anti-inflammatory effect from some of the extracts has also been found, and so far no toxic effects have emerged in use for human health (Hennequart 2007). Furthermore, alginates with molecular weights greater than or equal to 50 kDa could prevent obesity, hypocholesterolemia and diabetes (Kimura et al. 1996). Clinical observations of volunteers who were 25 % - 30 % overweight showed that alginate, a drug containing alginic acid, significantly decreased body weight (Zee et al. 1991). In type II diabetes treatment, taking 5 g of sodium alginate every morning was found to prevent a postprandial increase of glucose, insulin, and C-peptide levels and slowed down gastric transit (Torsdottir et al. 1991). Meal supplemented with 5 % kelp alginates decreased glucose absorption balance over 8 hours in pigs. Similar studies have been done on rats and humans (Vaugelade et al. 2000). Another health effect is that the binding property of alginic acid to divalent metallic ions is correlated to the degree of the gelation or precipitation in the range of $Ba < Pb < Cu < Sr < Cd < Ca < Zn < Ni < Co < Mn < Fe < Mg$. No intestinal enzymes can digest alginic acid. This means that heavy metals taken into the human body are gelled or rendered insoluble by alginic acid in the intestines and cannot be absorbed into the body tissue (Arasaki and Arasaki 1983). In several countries such as the USA, Germany, Japan, Belgium and Canada, the use of alginic acid and its derivatives for the treatment of gastritis and gastroduodenal ulcers, as well as the use of alginates as anti-ulcer remedies, is protected by patents (Bogentoff 1981; Borgo 1984; Reckitt and Colman Products Ltd 1987; Sheth 1967). Several products of alginate containing drugs have been shown to effectively suppress postprandial (after eating) and acidic refluxes, binding of bile acids and duodenal ulcers in humans. Examples are "Gaviscon" (sodium alginate, sodium bicarbonate, and calcium carbonate), "Algitec" (sodium alginate and cimetidine, an H₂ antagonist) and "Gastralgin" (alginic acid, sodium alginate, aluminium hydroxide, magnesium hydroxide and calcium carbonate) (Khotimchenko et al. 2001; Washington and Denton 1995). Clinical trials showed that sodium alginate promotes regeneration of the mucous membrane in the stomach, suppresses inflammation, eradicates colonies of *Helicobacter pylori* in the mucous membrane and normalizes non-specific resistance of the latter in 4 to 15-year-old children. It also promotes restoration of the intestinal biocenosis (Miroshnichenko et al. 1998). Other studies show positive dietary effects of alginates on faecal microbial fauna, changes in concentrations of compounds and acids, and prebiotic properties that can promote health (Terada et al. 1995; Wang et al. 2006).

Sodium alginate is often used as a powder, either pure or mixed with other drugs, on septic wounds. The polysaccharide base stimulates reparative processes, it prepares the wound for scarring, and it displays protective and coating effects, shielding mucous membranes and damaged skin against irritation from unfavourable environments. Calcium alginate promotes the proliferation of fibroblasts and inhibits the proliferation of microvascular endothelial cells and keratinocytes (Doyle et al. 1996; Glyantsev et al. 1993; Swinyard and Pathak 1985). Profound wound healing effects have also been reported for a gelatine-alginate sponge impregnated with anti-septics and anti-biotics (Choi et al. 1999).

Another use of alginates is the absorbing hemostatic effect exploited in surgery. Gauze dressings, cotton, swabs, and special materials impregnated with a solution of sodium alginate are produced and used for external use and for application onto bleeding points during abdominal operations on parenchymatous organs (Khotimchenko et al. 2001; Savitskaya 1986). Studies on the effect of alginate on prothrombotic blood coagulation and platelet activation have shown that the degree of these effects depends on the ratio between the mannuronic and guluronic chains in the molecule, as well as on the concentration of calcium. However, a zinc ion containing alginate was shown to have the most profound hemostatic effects (Segal et al. 1998). A “poraprezinc-sodium alginate suspension” has been suggested as a high performance mixture for the treatment of severe gingivostomatitis (cold sores) complicated by hemorrhagic erosions and ulcers (Katayama et al. 1998). When applied to the tooth surface, alginate fibres swell to form a gel like substance, a matrix for coagulation. Alginate dressings are used to pack sinuses, fistulas, and tooth cavities (Reynolds et al. 1982).

Furthermore, the algin have anti-cancer properties (Murata and Nakazoe 2001) and a bioactive food additive “Detoxal”, containing calcium alginate, has anti-toxic effects on hepatitis. This drug decreases the content of lipid peroxidation products and normalizes the concentrations of lipids and glycogen in the liver (Khotimchenko et al. 2001).

4.3. Carrageenans

From a human health perspective it has been reported that carrageenans have anti-tumour and anti-viral properties (Skoler-Karpoft et al. 2008; Vlieghe et al. 2002; Yan et al. 2004; Zhou et al. 2006b). Furthermore, Irish Moss or Carrageen (*C. crispus* and *Mastocarpus stellatus*) has a large number of medical applications, some of which date from the 1830s. It is still used in Ireland to make traditional medicinal teas and cough medicines to combat colds, bronchitis, and chronic coughs. It is said to be particularly useful for dislodging mucus and has anti-viral properties. Carrageenans are also used as suspension agents and stabilisers in other drugs, lotions and medicinal creams. Other medical applications are as an anti-coagulant in blood products and for the treatment of bowel problems such as diarrhoea, constipation and dysentery. They are also used to make internal poultices to control stomach ulcers (Morrissey et al. 2001).

New research on the biocide properties shows that applications of carrageenan gels from *C. crispus* may block the transmission of the HIV virus as well as other STD viruses such as

gonorrhoea, genital warts and the herpes simplex virus (HSV) (Caceres et al. 2000; Carlucci et al. 1997; Luescher-Mattli 2003; Shanmugam and Mody 2000; Witvrouw and DeClercq 1997). In addition, carrageenans are good candidates for use as vaginal microbicides because they do not exhibit significant levels of cytotoxicity or anti-coagulant activity (Buck et al. 2006; Zeitlin et al. 1997). Results of sexual lubricant gels raised the possibility that use of such lubricant products, or condoms lubricated with carrageenan-based gels, could block the sexual transmission of HPV (human papillomavirus) types that can cause cervical cancer and genital warts. However, carrageenan inhibition of herpes simplex virus and HIV-1 infectivity were demonstrated as about a thousand-fold higher than the IC₅₀s observed for genital HPVs *in vitro* (Witvrouw and de Clerck 1997; Luescher-Mattli et al. 2003). A carrageenan-based vaginal microbicide called Carraguard has been shown to block HIV and other sexually transmitted diseases *in vitro*. Massive clinical trials by the Population Council Centre began in two severely affected African countries; Botswana and South Africa in 2002. Carraguard entered phase III clinical trials involving 6000 non-pregnant, HIV-negative women in South Africa and Botswana in 2003 (Spieler 2002).

Many reports exist of anti-coagulant activity and inhibited platelet aggregation of carrageenan (Hawkins et al. 1962; Hawkins and Leonard 1963; Kindness et al. 1979). Among the carrageenan types, λ carrageenan (primarily from *C. crispus*) has approximately twice the activity of unfractionated carrageenan and four times the activity of κ -carrageenan (*Eucheuma cottoni* and *E. spinosum*). The most active carrageenan has approximately one-fifteenth the activity of heparin (Hawkins et al. 1962), but the sulphated galactan from *Grateloupa indica* collected from Indian waters, exhibited anti-coagulant activity as potent as heparin (Sen et al. 1994). The principal basis of the anti-coagulant activity of carrageenan appeared to be an anti-thrombotic property. λ -carrageenan showed greater anti-thrombotic activity than κ -carrageenan, probably due to its higher sulphate content, whereas the activity of the unfractionated material remained between the two. It was found that toxicity of carrageenans depended on the molecular weight and not the sulphate content. Similar results were obtained with λ -carrageenan of *Phyllophora brodiaei* which gave the highest blood anti-coagulant activity (Sen et al. 1994). In addition, the hypoglycaemic effect of carrageenan may prove useful in the prevention and management of metabolic conditions such as diabetes (Dumelod et al. 1999). The use of carrageenan for food applications started almost 600 years ago. Due to its long and safe history of use, carrageenan is generally recognised as safe (GRAS) by experts from the US Food and Drug Administration (21 CFR 182.7255) and is approved as a food additive (21 CFR 172.620). The World Health Organisation (WHO) Joint Expert Committee of Food Additives has concluded that it is not necessary to specify an acceptable daily intake limit for carrageenans (van de Velde et al. 2002). Although carrageenans are used widely as a food ingredient, they are also used in experimental research in animals where they induce pleurisy and ulceration of the colon (Noa et al. 2000). Furthermore, carrageenans can cause reproducible inflammatory reaction and they remain a standard irritant for examining acute inflammation and anti-inflammatory drugs. Two test systems are used widely for the evaluation of non-steroidal anti-inflammatory drugs and cyclooxygenase activity: (1) The carrageenan air pouch model

and (2) The carrageenan-induced rat paw edema assay (Dannhardt and Kiefer 2001). The role of carrageenans in promotion of colorectal ulceration formation is controversial and much seems to depend on the molecular weight of the carrageenan used. The international agency for research on cancer classified degraded carrageenan as a possible human carcinogen but native carrageenan remains unclassified in relation to a causative agent of human colon cancer and as mentioned it has GRAS status (Carthew 2002; Tobacman 2001).

4.4. Agar

The agar content in *Gracilaria* species can reach 31 % . It has been reported that agar-agar leads to decreases in the concentration of blood glucose and exerts an anti-aggregation effect on red blood cells. It has also been reported to affect absorption of ultraviolet rays (Murata and Nakazoe 2001). Anti-tumour activity was also found in an agar-type polysaccharide from cold water extraction of another *Gracilaria* species and hydrolysates of agar resulted in agaro-oligosaccharides with activity against α -glucosidase and antioxidant ability (Chen et al. 2005; Fernandez et al. 1989). Agarose can be separated from the agar with a yield of 42 %, and the agar content varied seasonally from 26 % - 42 % in *Gelidium* spp. in another experiment (Mouradi-Givernaud et al. 1992; Jeon et al. 2005). Agaro-oligosaccharides have also been shown to suppress the production of a pro-inflammatory cytokine and an enzyme associated with the production of nitric oxide (Enoki et al. 2003).

4.5. Fucoidan/fucans/fucanoids

Fucoidans are a group of polysaccharides (fucans) primarily composed of sulphated L-fucose with less than 10 % of other monosaccharides. They are widely found in the cell walls of brown seaweed, but not in other algae or higher plants (Berteau and Mulloy 2003).

Fucoidan is considered as a cell wall reinforcing molecule and seems to be associated with protection against the effects of desiccation when the seaweed is exposed at low-tide. Fucoidans were first isolated by Kylin almost one century ago and have interesting bioactivities (Kylin 1913). According to Table 4b the species *Fucus vesiculosus* contains the highest concentration of fucoidans (up to 20 % on a dry weight basis). Fucanoids can make up more than 40 % of dry weight of isolated algal cell walls and can easily be extracted using either hot water or an acid solution (Berteau and Mulloy 2003). Fucoidan is viscous in very low concentrations and susceptible to breakdown by diluted acids and bases. Fucoidans are produced as complex, heterogeneous polysaccharides, which contribute to intercellular mucilage. Their structural complexity varies in the degree of branching, substituents, sulfatation and type of linkages, the fine structure depending on the source of the polysaccharide. Although their composition varies with species and geographical origin, fucoidans always contain fucose and sulfate with small proportions of uronic acids, galactose, xylose, arabinose and mannose. Algal fucoidans have one of two types of homofucose backbone chains, with either repeated (1→3)-linked α -L-fucopyranosyl residues or alternating (1→3)- and (1→4)-linked α -L-fucopyranosyl residues (Cumashi et al 2007). Sulfonato- and acetyl-groups as well as α -L-fucopyranosyl, α -D-glucuronopyranosyl and

some other sugar residues may occur at O-2 and/or at O-4 of the α -L-fucose units of the backbone. Fucoidans with backbones of first type have been isolated from seaweeds *Saccharina latissima*, *Laminaria digitata*, *Chorda filum*, and *Cladosiphon okamuranus*. The second type of backbone was found in fucoidans from *Fucus evanescense*, *Fucus distichus*, and *Ascophyllum nodosum*.

A Tasmanian company, Marinova Pty Ltd, is able to supply commercial volumes of fucoidan extract and their derivatives, formulated to purity levels of up to 95 %. All fucoidans of the species *Undaria* sp., *Lessonia* sp., *Macrocystis* sp., *Cladosiphon* sp., *Durvillea* sp., *Laminaria* sp., *Ecklonia* sp., *Fucus* sp., *Sargassum* sp., *Ascophyllum* sp., and *Alaria* sp. are Halal and Kosher certified. Marinova has isolated fucoidans from a range of species (species-specific), and can provide characterised fractions for either investigational research or as ingredients for nutraceutical and cosmetic applications. Different therapeutic profiles are primarily due to the molecular structure. The company has developed the Maritech™ coldwater extraction process, which maintains the integrity of fucoidans, and produces nature-equivalent high molecular weight molecules with optimal bioactivity. Solvent-based extraction, which is commonly used, causes degradation of fucoidans, and limits the activity of these molecules in biological assays.

Sector	% Demand	Sector Growth	Ton	Value US\$
Textile printing and technical grades	41.5	Flat	11,000	140.0m
Food and Pharma	49	60%	13,000	150.0m
Animal feed	3.8	-75%	1,000	18.0m
PGA	5.7	-25%	1,500	10.0m
Total	100		26,500	\$318m

Table 4. Alginate markets (2009) – Tonnage, value and sector growth over last decade (Data; Bixler and Porse, 2010)

Although the major physiological purposes of fucans in the algae are not thoroughly understood, they are known to possess numerous biological properties with potential human health applications (Berteau and Mulloy 2003). The list of bioactivity of fucoidan for human health is long. Fucoidan found in seaweed such as *Undaria* and *Laminaria* shows anti-coagulant, anti-viral and anti-cancer properties; Chevolot et al. 1999; Zhuang et al. 1995).

Fucoidan preparations have been proposed as an alternative to the injectable anti-coagulant heparin, because fucoidan originates from plant matter and is less likely to contain infectious agents such as viruses (Berteau and Mulloy 2003). No toxicological changes were observed when 300 mg/kg body weight per day fucoidan was administered to rats, however, significantly prolonged blood-clotting times were observed when concentrations were increased three-fold (Li et al. 2005). The biological activity (e.g. antioxidant and anti-coagulant) of sulphated polysaccharides is not only related to molecular weight and sulphated ester content (role in the charge of the molecule), as previously determined, but also to glucuronic acid and fucose content, together with the position of the sulphate groups on the sugar residues (Berteau and Mulloy 2003; Li et al. 2005; Zhao et al. 2008). A large

molecular weight is required to achieve anti-coagulant activity, as fucoidan needs a long sugar chain in order to be able to bind the thrombin (coagulation protein in the blood stream). Some researchers have measured fucoidan's molecular weight at approximately 100 kDa, while others have observed a molecular weight of 1600 kDa (Rioux et al. 2007). The native fucoidan from *Lessonia vadosa* with a molecular weight of 320 kDa showed good anti-coagulant activity compared to a smaller depolymerised fraction with a molecular weight of 32 kDa, which presented weaker anti-coagulant activity (Li et al. 2008a). Some structural features of fucoidan are most likely required for certain specific activities.

Fucoidan stimulates the immuno system in several ways, and the numerous important biological effects of fucoidans are related to their ability to modify cell surface properties (Usov et al. 2001). Oral intake of the fucoidans present in dietary brown seaweed might take the protective effects through direct inhibition of viral replication and stimulation of the immune system (innate and adaptive) functions (Hayashi et al. 2008). Fucoidan has been found to restore the immune functions of immune-suppressed mice, act as an immunomodulator directly on macrophage, T lymphocyte, B cell, natural killer cells (NK cell; Wang et al. 1994), promote the recovery of immunologic function in irradiated rats (Wu et al. 2003), induce the production of interleukin (IL-1) and interferon- γ (IFN- γ) *in vitro*, and promote the primary antibody response in sheep red blood cells *in vivo* (Yang et al. 1995).

The mechanism of anti-viral activities of fucoidan is to inhibit viral sorption so as to inhibit viral-induced syncytium formation (Mandal et al. 2007). Sulphate is necessary for the anti-viral activity and sulphate located at C-4 of (1-3)-linked fucopyranosyl units appears to be very important for the anti-herpetic activity of fucoidan (Mandal et al. 2007). Some anti-viral properties of sulphated fucans have also been characterised, for example inhibition of infection of human immunodeficiency virus (HIV), Herpes Simplex Virus (HSV) (Iqbal et al. 2000; Mandal et al. 2007; Witvrouw and DeClercq 1997), poliovirus III, adenovirus III, ECH06 virus, coxsackie B3 virus, coxsackie A16 (Li et al. 1995), cytomegalovirus and bovine viral diarrhea virus (Iqbal et al. 2000).

Fucoidan is known to have anti-tumour effects but its mode of action is not fully understood. A study done by Alekseyenko et al. 2007 demonstrated that when 10 mg/kg of fucoidan was administered in mice with transplanted Lewis lung adenocarcinoma, it produced moderate anti-tumour and anti-metastatic effects (Li et al. 2008b). These polyanionic polysaccharides have anti-angiogenesis, antiproliferation for tumour cells, they inhibit tumour growth and reduce tumour size (Ellouali et al. 1993; Li et al. 2008a), inhibit tumour cell adhesion to various substrata (Liu et al. 2005), and have direct anti-cancer effects on human HS-Sultan cells through caspase and ERK pathways (Aisa et al. 2005).

Besides directly inhibiting the growth of tumour cells, fucoidans can also restrain the development and diffusion of tumour cells through enhancing the body's immunomodulatory activities, because fucoidan mediates tumour destruction through type 1 T-helper (Th1) cell and NK cell responses (Maruyama et al. 2007). In addition, at a dose of 25 mg/kg, fucoidan potentiated the toxic effect of cyclophosphamide used to treat various types of cancer and some auto-immune disorders (Alekseyenko et al. 2007).

Many studies suggest that fucoidan has potential for use as an anti-inflammatory agent. A study showed that fucoidan treatment led to less severe symptoms in the early stages of *Staphylococcus aureus*-triggered arthritis in mice, but delayed phagocyte recruitment and decreased clearance of the bacterium (Verdrengh et al. 2000). Additionally, injection of fucoidan into sensitized mice before hapten challenge can reduce contact hypersensitivity reactions (Nasu et al. 1997). Furthermore, recruitment of leukocytes into cerebrospinal fluid in a meningitis model is reduced by fucoidan (Granert et al. 1999) as is IL-1 (interleukin-1) production in a similar model (Ostergaard et al. 2000).

Fucoidan can act as a ligand for either L- or P- selectins, both of which interact with the sulphated oligosaccharides and this interaction has physiological consequences that could be therapeutically beneficial (Omata et al. 1997). Selectins are a group of lectins (sugar-binding proteins) that interact with oligosaccharides clustered on cell surfaces during the margination and rolling of leukocytes prior to firm adhesion, extravasation and migration to sites of infection (Lasky 1995).

In addition, fucoidan is an excellent natural antioxidant and presents significant antioxidant activity in experiments *in vitro*. In recent years, sulphated polysaccharides from the marine algae *Porphyra haitanesis* (Zhang et al. 2003), *Ulva pertusa* (Qi et al. 2005b; Qi et al. 2005a), *Fucus vesiculosus* (Ruperez et al. 2002), *Laminaria japonica* (Xue et al. 2004) and *Ecklonia kurome* (Hu et al. 2001) have been demonstrated to possess antioxidant activity. There are few reports however detailing the relationship between structure and antioxidant activity of sulphated polysaccharides from marine algae. Fucan showed low antioxidant activity relative to fucoidan (Rocha de Souza et al. 2007) and as mentioned previously, the ratio of sulphate content/fucose and the molecular weight were effective indicators to antioxidant activity of the samples (Wang et al. 2008). Fucoidan may have potential for preventing free radical mediated diseases such as Alzheimer's and the aging process. Previously, fucoidan was extracted from *L. japonica*, a commercially important algae species in China. Three sulphated polysaccharide fractions were successfully isolated through anion-exchange column chromatography and had their antioxidant activities investigated employing various established *in vitro* systems, including superoxide and hydroxyl radical scavenging activity, chelating ability, and reducing power (Wang et al. 2008). All fractions were more effective than the unprocessed fucoidan. Two galactose-rich fractions had the most potent scavenging activity against superoxide (generated in the PMS-NADH system) and hydroxyl radicals with EC_{50} values of $1.7 \mu\text{g mL}^{-1}$ and 1.42 mg mL^{-1} , respectively. One of these fractions also showed the strongest ferrous ion chelating ability at 0.76 mg mL^{-1} (Wang et al. 2008). Additionally, fucoidan (homofucan) from *F. vesiculosus* and fucans (heterofucans) from *Padina gymnospora* had an inhibitory effect on the formation of hydroxyl radical and superoxide radical (Rocha de Souza, et al. 2007). Healing of dermal wounds with macromolecular agents such as natural polymers is a growing area of research interest in pharmaceutical biotechnology. Fucoidan has been shown to modulate the effects of a variety of growth factors through mechanisms thought to be similar to the action of heparin. Fucoidan-chitosan films can promote re-epithelization and contraction of the wound area. Moreover, fucoidan-chitosan films may be suitable for use in hydrogel formulations for the treatment of dermal burns (Sezer, et al. 2007).

4.6. Mannitol

Mannitol is an important sugar alcohol which is present in many species of brown algae, especially in *Laminaria* and *Ecklonia*. The mannitol content is subject to wide seasonal fluctuations and varies with environment. Mannitol is the sugar alcohol corresponding to mannose. It usually constitutes less than 10 % of the dry weight in both *Ascophyllum nodosum* and *L. hyperborea* stipe. In autumn fronds of *L. hyperborea*, however, the content may be as high as 25 % of the dry weight .

Applications of mannitol are extremely diverse. It is used in pharmaceuticals, in making chewing gum, in the paint and varnish industry, in leather and paper manufacture, in the plastics industry and in the production of explosives. The US, the UK, France and Japan are the main centres of production. Mannitol can be used in a variety of foods, candies and chocolate-flavoured compound coatings because it can replace sucrose to make sugar-free compound coatings. Sugar-free chocolates are especially popular for people with diabetes, a growing problem in modern society. It is used as a flavour enhancer because of its sweet and pleasantly cool taste. Mannitol can be used to maintain the proper moisture level in foods so as to increase shelf-life and stability, because it is non-hygroscopic and chemically inert. Mannitol is the preferred excipient for chewable tablets due to its favourable feel in the mouth. It is non-carcinogenic and can be used in pediatric and geriatric food products, as it will not contribute to tooth decay (Nabors 2004).

4.7. Laminarin

Laminaran is a glucan, built up from (1→3)- and (1→6)- β -glucose residues. It is a linear polysaccharide, with a $\beta(1\rightarrow3):\beta(1\rightarrow6)$ ratio around 3:1. Laminarin is found in the fronds of *Laminaria*/*Saccharina* and, to a lesser extent, in *Ascophyllum* and *Fucus* species and *Undaria*. The content varies seasonally and with habitat and can reach up to 32 % of the dry weight. Laminaran does not gel nor form any viscous solution and its main potential appears to lie in medical and pharmaceutical uses.

Commercial applications of the extract have so far been limited, although some progress has been made in France as an anti-viral in agricultural applications (Goemar 2010) or as dietary fiber (Deville et al. 2004). Especially the use of laminarin as substratum for prebiotic bacteria seems to have a good commercial application (Deville et al. 2004). Laminarin does not gel or form any viscous solution, and its main potential appears to lie in medical and pharmaceutical uses. It has been shown to be a safe surgical dusting powder, and may have value as a tumour-inhibiting agent and, in the form of a sulphate ester, as an anti-coagulant (Miao et al. 1999). The presence of anti-coagulant activity in brown algae was first reported in 1941, when *Laminaria* showed anti-coagulant properties with its active compound being located in the holdfasts (Shanmugam and Mody 2000). There are about 60 brown algal species identified to have blood anti-coagulant properties. Laminarin only shows anti-coagulant activity after structural modifications such as sulphation, reduction or oxidation. The anti-coagulant activity is improved chemically by increasing the degree of sulphation (Shanmugam and Mody 2000).

Preparations containing 1→3:1→6-β-D-glucans, laminarin, and fucoidan are manufactured by the health industry and marketed for their beneficial properties on the immune system. The producers of these tablets cite numerous papers discussing the biological activity of these glucans.

Laminarin provides protection against infection by bacterial pathogens, and protection against severe irradiation, it boosts the immune system by increasing the B cells and helper T cells, reduces cholesterol levels in serum and lowers systolic blood pressure, among other effects (Table 4c; Hoffman et al. 1995) lower the levels of total cholesterol, free cholesterol, triglyceride and phospholipid in the liver (Miao et al. 1999; Renn et al. 1994a; 1994b). The hypocholesterolemic and hypolipidemic responses are noted to be due to reduced cholesterol absorption in the gut (Kiriyaama et al. 1969; Lamela et al. 1989; Panlasigui et al. 2003). This is often coupled with an increase in the faecal cholesterol content and a hypoglycaemic response (Dumelod et al. 1999; Ito and Tsuchida 1972; Nishide et al. 1993). A high level of low density lipid (LDL) cholesterol can lead to plaque forming and clog arteries and lead to cardiovascular diseases and heart attacks or strokes, a major cause of disease in the US. Laminarin as a potential cancer therapeutic is under intensive investigation (Miao et al. 1999).

4.8. Ulvan

The name ulvan is derived from the original terms ulvin and ulvacin introduced by Kylin in reference to different fractions of *Ulva lactuca* water-soluble sulphated polysaccharides. It is now being used to refer to polysaccharides from members of the Ulvales, mainly, *Ulva* sp. Ulvans are highly charged sulphated polyelectrolytes, composed mainly of rhamnose, uronic acid and xylose as the main monomer sugars, and containing a common constituting disaccharide; the aldobiuronic acid, [→4)-D-glucuronic acid-(1→4)- L-rhamnose3-sulfate-(1→]. Iduronic acid is also a constituent sugar. The average molecular weight of ulvans ranges from 189 to 8,200 kDa (Lahaye 1998). The cell-wall polysaccharides of ulvales represent 38 % to 54 % of the dry algal matter (Lahaye, 1998). Two major kinds have been identified: water soluble ulvan and insoluble cellulose-like material.

The mechanism of gel formation is unique among polysaccharide hydrogels. It is very complex and not yet fully understood. The viscosity of ulvan solutions as isolated polysaccharides has been compared to that of arabic gum. Whether ulvans present other functional properties of this gum remains to be established. The gelling properties of ulvans are affected by boric acid, divalent cations and pH. They are thermoreversible without thermal hysteresis. The gelling properties can be of interest for applications where gel formation needs to be precisely controlled (by pH or temperature), like the release of trapped molecules or particles under specific conditions (Percival and McDowell 1990; Lahaye et al. 1998). As already mentioned, highly absorbent, biodegradable hydrocolloid wound dressings limit wound secretions and minimise bacterial contamination. Polysaccharide fibres trapped in a wound are readily biodegraded. In the context of BSE (mad cow disease) and other prion contamination diseases, macromolecular materials from

algal biomasses such as ulvans can constitute an effective and low-cost alternative to meat-derived products, because their rheological and gelling properties make them suitable as a substitute for gelatin and related compounds (Choi et al. 1999).

Ulvans are a source of sugars for the synthesis of fine chemicals. In particular, they are a potential source of iduronic acid, the only occurrence to date of this rare uronic acid in plants (Lahaye and Ray 1996). Iduronic acid is used in the synthesis of heparin fragment analogues with anti-thrombotic activities, and obtaining it requires a lengthy synthetic procedure that could be more cost-effectively replaced by a natural source (Lahaye 1998). Oligosaccharides from *Ulva* could be used as reference compounds for analyzing biologically active domains of glycosaminoglycans (GAG) like heparin. The use of oligo-ulvans as anti-coagulant agents could be expected since other, rarer, sulphated polysaccharides, like dermatan sulphate or fucan in brown algae, have shown this anti-thrombinic activity. Regular oligomers from ulvans may provide better-tolerated anti-thrombinic drugs (Paradossi et al. 2002).

Rhamnose, a major component of ulvans, is a rare sugar, used as a precursor for the synthesis of aroma compounds. Combinatorial libraries in glycopeptide mimetics are another example of the use of L-rhamnose in the pharmaceutical industry. The production of rhamnose from *Monostroma*, a Japanese species of Codiales, has been patented. Rare sulphated sugars such as rhamnose 3-sulphate and xylose 2-sulphate are also of interest (Lahaye and Robic 2007).

Other potential applications of ulvan oligomers and polymers are related to their biological properties. Recent studies have demonstrated that ulvans and their oligosaccharides were able to modify the adhesion and proliferation of normal and tumoural human colonic cells as well as the expression of transforming growth factors (TGF) and surface glycosyl markers related to cellular differentiation (Lahaye and Robic 2007). Earlier work demonstrated strain-specific anti-influenza activities of ulvan from *U. lactuca* and the use of rhamnan, rhamnose and oligomers from desulphated *Monostroma* ulvans has been patented for the treatment of gastric ulcers (Fujiwara-Arasaki et al. 1984; Nagaoka et al. 2003).

Oligomers from seaweed species such as *Laminaria* sp. or *Fucus serratus* have also been studied as plant elicitors. These are natural compounds which stimulate the natural defences of plants. Several products derived from brown algae are already marketed worldwide. The success is because of their size and availability rather than their chemical composition. *Ulva* cell walls bind heavy metals and ulvans are the main contributors with 2.8 to 3.77 meq g⁻¹ polysaccharide. The ion-exchange property of ulvans explains why they have been chosen as bioindicators for monitoring heavy metal pollution in coastal waters (Nagaoka et al. 1999).

4.9. Porphyrans and Xylans

In red algae, the fibrillar network is made of low crystalline cellulose, mannan or xylan and represents only about 10% of the cell wall weight. It can also contain minor amounts of

sulphated glucans, mannoglycans and complex galactans. Most of our current knowledge of red algal cell wall polysaccharides is on the gelling and thickening water soluble galactans, agars and carrageenans, used in various applications. Unlike most red seaweed generally studied, *Palmaria palmata* does not produce matrix galactans, but instead (10/4)- and (10/3)-linked b-D-xylan together with a minor amount of fibrillar cellulose and b-(10/4)-Dxylan. Xylans can be 35 % of the dry weight of *Palmaria* (Lahaye et al. 2003). Xylans have not yet been of economic interest and only few applications are known. Species of *Porphyra* contain a sulphated polysaccharide called porphyran; a complex galactan. Porphyran is dietary fiber of good quality, and chemically resembles agar. A powder consisting of 20 % nori mixed with a basic diet given orally to rats prevented 1,2-dimethylhydrazine-induced intestinal carcinogenesis. Porphyran showed appreciable anti-tumour activity against Meth-A fibrosarcoma. In addition, it can significantly lower the artificially enhanced level of hypertension and blood-cholesterol in rats (Noda 1993).

4.10. Digestibility of polysaccharides

The majority of edible seaweed fibres are soluble anionic polysaccharides which are little-degraded or not fermented by the human colonic micro flora (Lahaye and Kaeffer 1997). The amount of dietary fibres in marine algae not digested by the human digestive tract is higher than that of other food materials (Murata and Nakazoe 2001). Most of the total algal fibres disappeared after 24 h (range 60 % - 76 %) in *in vitro* fermentation of e.g. *L. digitata* and *U. pinnatifida* using human faecal flora. However, unlike the reference substrate (sugar beet fibres), the algal fibres were not completely metabolized to short chain fatty acids (SCFA; range 47 % - 62 %). Among the purified algal fibres, disappearance of laminarins was approximately 90 % and metabolism to SCFA was approximately 85 %, in close agreement with the fermentation pattern of reference fibres. Sulphated fucans were not degraded. Sodium alginates (Na-alginates) exhibited a fermentation pattern quite similar to that of the whole algal fibres, with a more pronounced discrepancy between disappearance and production of SCFA: disappearance was approximately 83 % but metabolism was only approximately 57 %. Laminarin seemed to be a modulator of the intestinal metabolism by its effects on mucus composition, intestinal pH and short chain fatty acid production, especially butyrate. The characteristic fermentation pattern of the total fibres from the brown algae investigated was attributed to the peculiar fermentation of alginates (Michel et al. 1996; Deville et al. 2007).

Phycocolloids are more or less degraded following adaptation of the human micro flora, but none of the seaweed polysaccharides have been shown to be metabolized, although some may be partly absorbed. Nothing is known about the fate of other algal polysaccharides in the human digestive tract, except that they cannot be digested by human endogenous enzymes. Results of fermentation *in vitro* with human faecal bacteria, indicate that brown seaweed fibres exhibit an original fermentation pathway (Mabeau and Fleurence 1993). Carrageenan is a good source of soluble fibre (Burtin 2003). Rats excrete carrageenan quantitatively in the faeces, if it is administered in the diet at levels of 2 % - 20 % and it therefore has no direct nutritive value (Hawkins and Yaphe 1965). Weight gain was

significantly reduced, especially at higher levels. Furthermore, food efficiency showed interference with utilization of other nutrients in the diet. Only 10 % - 15 % appeared digestible from faecal examination (Hawkins and Yaphe 1965). An experimental feeding with *L. digitata* seaweed extract in pig resulted in a higher production of butyric acid in the caecum and colon compared to the control group (Reilly et al. 2008). Butyrate is a beneficial metabolite for intestinal bacteria, because it is quickly metabolised by colonocytes and accounts for about 70 % of total energy consumption of the colon (Reilly et al. 2008). Therefore, it is desirable to promote butyrate production in the colon by laminarin fermentation.

The particular chemical structure of ulvan (and of *Ulva*) is responsible for the resistance of this polysaccharide to colonic bacterial fermentation. Consumption of dietary fibres from *Ulva* sp. could be expected to act mainly as bulking agents with little effect on nutrient metabolism due to colonic bacterial fermentation products (short-chain fatty acids; Bobin-Dubigeon et al. 1997). All soluble fibre fractions of *P. palmata* consisted of linear beta-1,4/beta1,3 mixed linked xylans containing similar amounts of 1,4 linkages (70.5 % - 80.2 %). The insoluble fibres contained essentially 1,4 linked xylans with some 1,3 linked xylose and a small amount of 1,4-linked glucose (cellulose). Soluble fibres were fermented within 6 hours by human faecal bacteria into short chain fatty acids (Lahaye et al. 1993).

4.11. Commercial products, patents and applications

Due to their plethora of bioactive molecules, marine macroalgae have great potential for further development as products in the nutraceutical, functional food, and pharmaceutical markets. Patent activity in this area has increased and several novel products based on macroalgae have entered the market in recent years. In respect of carbohydrates for example, the Kabushiki Kaisha Yakult Honsha company, Japan has patented a polysaccharide derivative (which contains fucoidan and rhamnan or rhamnan sulphate polysaccharides), extracted from the marine brown macroalgae, such as, *Cladosiphon okamuranus*, *Chordaricles nemacystus*, *Hydrilla* sp., *Fucus* sp., and a green alga *Monostroma nitidum*. The purpose of this compound is as a therapeutic agent for the prevention and treatment of gastric ulcers (specifically inhibiting the adhesion of *Helicobacter pylori* and administered as tablets, granules, powders or capsules (Nagaoka et al., 2003). Furthermore, Takara Shuzo Company, Kyoto, Japan has developed a medicinal composition exemplified by viscous polysaccharides isolated from red algae (specifically: *Gelidium amansil*, *G. japonicum*, *G. pacificum*, *G. subcostatum*, *Pteocladia tennis* and *Hypneaceae* species consisting of at least one 3,6- anhydrogalactopyranose. This compound is proposed for the treatment or prevention of diabetes, rheumatism, cancer and contains various inhibitory factors (Enoki et al., 2003). Sulphated fucans from *Fucus vesiculosus* and *Ascophyllum nodosum* have been patented as anticoagulant substances (Smit, et al., 2004). In practical gastroenterology, mixtures of alginic acid and alginates with antacids are used to prevent gastro-esophageal reflux and to cure epigastric burning (Klinkenberg- Knol et al., 1995; Zeitoun et al., 1998). Indeed, in several countries such as the US, Germany, Japan, Belgium and Canada the use of alginic acid and its derivatives for the treatment of gastritis and gastroduodenal ulcers, as

well as the use of alginates as atulcer remedies, is protected by patents (Bogentoff et al, 1981; Borgo et al., 1984; Reckitt et al., 1987; Sheth et al., 1967). The medical application of pure fucoidan fraction has also been patented by the French research institute IFREMER-CNRS (PATENT WO/32099, however the extract of brown seaweeds (containing fucoidan fractions) can still be applied in cosmetology as fibroblast proliferation activators in the context of treatments aimed at aesthetics, for example of antiwrinkle treatments or of prevention of skin ageing without patent infringement.

Company	Compound	Activity / disorder	Development
Various	Heparin and derivatives	Anti-coagulants	Since 1940's
Astellas	Auranofin (Ridaura)	Anti-rheumatic	1983
GSK	Zanamivir (Relenza)	Anti-influenza	1992
Johnson & Johnson	Topiramate (Topamax)	Anti-epileptic	1987
Bayer	Acarbose (Glucobay) (Pseudo-oligosaccharide)	Type II diabetes (α -glucosidase, α -amylase inhibitor)	1990
Ortho-McNeil Janssen Pharmaceutical	Elmiron (Pentosan polysulfate)	Cystitis (for CJD)	1996
Alfa Wassermann	Sulodexide (Vessel™)	cardiovascular indications	Marketed since 1980's
Hunter Fleming (now Newron)	HF0420 – low MWt oligosaccharide	neuroprotective	Phase I
Progen (Australia)	PI-88 (Phosphomannopentaose sulphate, Heparan sulfate mimetics)	Anti-angiogenic/anti- metastatic. (hepatocellular carcinoma)	Phase III
	PG500 series (Heparan sulfate mimetics)	Anti-angiogenic/anti- metastatic.	Preclinical
Endotis Pharma	EP42675 (org) EP224283 (org) EP37	Anticoagulant Neutralizable antithrombotic venous and arterial thromboses	Phase I Preclinical Preclinical
	EP8000 programme	Anti-angiogenesis, anti- tumour growth /metastasis	Preclinical
Biotech Pharmacpn (Norway)	SBG (Soluble beta glucan – beta-1,3/1,6 glucan)	Immune stimulation Anti-cancer	GRAS nutraceutical

Table 5. Examples of carbohydrate-based drugs in use or development

The macroalgae polysaccharides described in this chapter show that these can be an interesting natural source of potential functional ingredients. As the content of proteins,

carbohydrates, lipids and fiber can be influenced by the growing parameters (water, temperature, salinity, light and nutrients), macroalgae can be considered bioreactors that may be able to provide different polysaccharides at different quantities (Rui *et al.*, 1990). From the descriptions above it is clear that macroalgae polysaccharides possess a multitude of bioactivities that might have antioxidant, antibacterial, antiviral, anticarcinogenic, anticoagulant and other bioactive properties for use as functional foods, pharmaceuticals and cosmeceuticals. The use of carbohydrate-based drugs is in its infancy, although there are several well-known examples (Table 5). Heparin is the key example of a major medically used carbohydrate based molecule. Low molecular weight heparins (eg. Certoparin, Dalteparin) and various derivatives (Fondaparinux – fully synthetic) have been developed to improve efficacy and half-life, and some are now being trialed for non-thrombotic/vascular applications (eg. Certoparin for inflammatory aspects of Alzheimers disease). It is only comparatively recently that the anti-inflammatory properties of heparin have been discovered. The presence of abundant 3-O-sulfated glucosamines in Heparan sulfates from human follicular fluid has suggested that some biological activities could be mimicked by other sulfated polysaccharides or derivatives (de Agostini *et al.*, 2008). Low molecular weight fucoidan (LMWF) has been used to demonstrate this (Collic *et al.*, 1991, Millet *et al.*, 1999 and Durand *et al.*, 2008).

The drugs listed in Table 5 have overcome some of the perceived limitations of sugar-based molecules in terms of delivery, synthesis and immunogenicity. Although many still require intravenous delivery, several are available orally (e.g. Pentosan polysulfate), and further research is targeting to the improvement of oral availability by reducing compounds to their smallest active components, or by combining with other molecules (e.g. sulodexide). Improved synthesis has meant that some compounds can be synthesised (e.g. Fondaparinux), and small active components can be selected or modified to improve efficacy. No major problems have been reported with immunogenicity either in animal trials or as approved drugs. Sulphated polysaccharides may also be good anticoagulants through non-antithrombin mediated mechanisms, such as inhibition of thrombin mediated by heparin cofactor II (HC2). Like heparin, sulphated polysaccharides modulate cell growth-related activities, with inhibitory or promoting effects, depending on their carbohydrate backbones, degrees of sulphation, and distribution of sulphate groups. These activities can be usefully modulated through modification of sulphation patterns (Casu *et al.*, 2002; Naggi *et al.*, 2005). Several biological activities of sulphated polysaccharides are also molecular weight-dependent and the average length of their chains should be carefully controlled in order to maximize the desired activity. In some systems, opposite activities can be achieved with chains of different lengths of the same sulphated polysaccharide. Typically, relatively small oligosaccharides of heparin and heparinoids bind to growth factors and act as inhibitors of angiogenesis. On the other hand, the corresponding higher molecular weight species induce growth factors activation through oligomerization and binding with their receptors, thus promoting cell growth signalling (Casu and Lindahl, 2002; Goodner *et al.*, 2008). Also inhibition of heparanase and heparanase-related biological activities such as angiogenesis and metastasis by heparin species (Naggi *et al.*, 2005) and sulfated

hyaluronates (Naggi *et al.*, 2005) are dependent on molecular weights and sulfation patterns. Similarly, sulphated polysaccharides are expected to be exploitable as drugs either in their natural form or with depolymerization and/or chemical derivatisation. Macroalgal sulphated polysaccharides need to be fully characterised and the biological activities associated with specific structures determined so that their development can progress.

5. Brown algae – Growth, cultivation and productivity

Due to the relatively high carbohydrate content in percentage of the dryweight (Table 1) brown macroalgae in particular species of the Laminariaceae have come under the spotlight for mass-cultivation. Specifically in respect of carbohydrate production for fermentation purposes for ethanol and or biodegradable plastics production or other medical and food applications. Brown macroalgae exploitation in Europe is currently restricted to manual and mechanised harvesting of natural stocks, although several EU projects explore mass cultivation in European waters. The majority of Asian seaweed resources are cultivated. The most common system in Europe to obtain seaweed biomass is by harvesting natural stocks in coastal areas with rocky shores and a tidal system. The natural population of seaweed is a significant resource. Depending on water temperature, some groups will dominate, like brown seaweeds in cold waters and reds in warmer waters. In Europe the main harvesting countries are Norway and France. Between 120,000 and 130,000 tonnes of *Laminaria* are harvested annually in Norway. The standing stock is estimated to be 10 million tonnes (Vae and Ask, 2011). France harvests about 30,000 – 50,000 tonnes annually, mainly *Laminaria* species for hydrocolloid production. The green alga *Ulva*, is commonly encountered in estuaries and inshore coastal areas. When mass proliferation occurs they are known as 'green tides.' They tend to develop at more and more locations along European coasts, due to eutrophication, and are used to a small extent as fertilisers but not yet used for industrial applications. Nevertheless they may form an interesting source and feedstock for carbohydrates (Kraan & Guiry, 2006).

In Europe, knowledge of seaweed cultivation is scattered across several R&D groups and a few industrial groups. The amount of cultivated seaweed is very low, mainly very small companies with local facilities for cultivating high value species. Existing industries having large scale cultivation are located in Asian countries (China, Philippines, Korea, Indonesia, and Japan) and in Chile. The main obstacle in European countries will be labour cost. Development of mechanized seaweed cultivation will be required in Europe to achieve cost objectives. In Ireland also the aquaculture sector is gradually building know-how and basic infrastructure for *Laminaria* cultivation. Technologies to cultivate *Laminaria* are well known. For instance, the FAO publish a guide to *Laminaria* culture which is very detailed (Chen, 2005). The main producers of *Laminaria* are located in China, Korea and Japan, where preservation of natural stocks is not always sustainably managed. The main reason for an increased harvest is increased productivity due to selection of the best performing strains, improved crop-care, less variability, fertilizing techniques and faster harvesting. This strategy will also be required to achieve the low material cost with a high carbohydrate content needed for biofuel applications.

Brown seaweeds from the *Laminariaceae* or *Fucaceae* families have a generative cycle including *microstages*, i.e. zoospores and gametophytes, before fertilization results into new sporophytes. Gametophytes are independently living organisms which can be grown and propagated vegetatively. The interesting thing is that the gametophytes can be grown in biofermentors in a controlled way like microalgae or bacteria. This procedure is used for example for production of fucoxanthin from *Undaria pinnatifida* cultivated under microscopic gametophyte form. Because cloning of these gametophyte structures is possible, genetically uniform strains can be obtained quickly. These clones can be used also for breeding purposes towards varieties of the seaweed in their mature stage. Selection and breeding is an essential step to reach a uniform crop and to optimize yield of the targeted compounds. Little is known about the composition of these microstages, but based on their survival rate in non favorable (a)biotic conditions interesting levels of bioactive polysaccharides may be obtained. Cultivation is the most efficient solution to guarantee consistent contents in bioactive compounds, whereas seaweeds harvested from wild resources undergo uncontrolled composition variations due to changes in growing environment. If highly valuable molecules such as fucoidans are aimed as products, seaweed aquaculture is therefore a very interesting option. The current cultivation methods are still based on Asian techniques although currently programs are initiated in the EU to develop open sea based seaweed cultivation technology. Although seaweeds are known for their richness in bioactive substances like polysaccharides, proteins, lipids, vitamins and polyphenols and have been shown to have a wide range of potential cosmetic, pharmaceutical and medical applications, their economic potential is still insufficiently developed.

5.1. Eutrophication and suitable carbohydrate feed stocks

Inputs of biodegradable organic matter and inputs deriving from fertilizer run-off together with run off or dilution from finfish and shellfish rearing in near-shore waters and land based activities have many effects on the quality of coastal inshore waters and are a primary cause of eutrophication due to increased availability of nutrients (EPA, 2003). In estuaries and shallow coastal bays, this can lead to the proliferation of vast green algal mats, known as 'green tides' (Fletcher 1996). Kelp farms (inshore and near-shore) are able to act as bio-filters and are able to remove nitrates and phosphates from the surrounding eutrophic inshore waters. This allows for increased production of farmed seaweed as demonstrated by Chopin et al., 2001, 2008b. Eutrophic waters are high in ammonia and phosphorous which can be stripped from the water by seaweed at rates varying from 60% up to 90% of the nutrient input. Macroalgae are able to take up nitrogen from seawater with rates to allow for a biomass increase of ca. 10% day⁻¹. It is well documented that in tank systems the green alga *Ulva* is able to remove 90% of the nitrogenous compounds and the red alga *Gracilaria* up to 95% of dissolved ammonium from fish effluent (Neori et al., 2000). By cultivating and harvesting macroalgae as biofilters integrated with other shellfish or fish production systems, nutrient pollution from these aquaculture systems could be alleviated through a process called IMTA (Integrated Multitrophic Aquaculture) while increasing production

and carrying capacity (Chopin et al., 2001, 2008; Troell et al., 2009). Production of macroalgae in near-shore sea cultivation can be harvested for the bioethanol market to produce a value added marketable product acting as both an economic incentive and environmental incentive.

5.2. Effect of enrichment on carbohydrates

High ammonia and nitrate concentrations will alter the proximate composition in the macroalgae and cause a shift with higher protein and generally lower carbohydrate levels such as starch or dietary fiber as demonstrated by Rosenberg and Ramus, (1982); Pinchetti et al., (1998). How exactly it effects the carbohydrate composition is not known but might be an interesting way to manipulate carbohydrate content and composition in algae. In the red carrageenophyte *Kappaphycus alvarezii* the effect of ammonium addition in an otherwise nitrogen starved environment had a profound impact on the carrageenan content and showed increased gel strength of the carrageenan with an increase of ammonia (Rui et. al., 1990).

6. From a hydrocarbon society to carbohydrate society

Global demand for bio-fuels continues unabated. Rising concerns over environmental pollution and global warming, has encouraged the movement to alternate fuels, the world ethanol market is projected to reach 100 billion litres this year. Bioethanol is currently produced from carbohydrates from land-based crops such as corn and sugar cane. A continued use of these crops drives the food versus fuel debate. An alternate feed-stock which is abundant and carbohydrate-rich is necessary. The production of such crop needs to be sustainable and reduce competition with production of food, feed and industrial crops on agricultural inputs (pesticides, fertiliser, land, water). Macroalgae, in specific brown seaweeds could meet these challenges, being an abundant and carbon neutral renewable resource with potential to reduce green-house gas (GHG) emissions and the man-made impact on climate change.

Macroalgae are fast growing marine plants that can grow to considerable size (up to tens of meters in length in the case of Pacific kelp species), although Atlantic species would be smaller at ~ 3 m length (Lüning 1990). Growth rates of marine macroalgae far exceed those of terrestrial biomass. The large brown algae of kelp forests found on rocky shores inhabit an environment of vigorous water movement and turbulent diffusion. This allows very high levels of nutrient uptake, photosynthesis and growth. Highest productivity of kelp forests is found along the North American Pacific coast, which out-performs that of the most productive terrestrial systems (Velimirov et al. 1977). *Laminaria*-dominated communities of the European coasts have an annual productivity of approximately 2 kg carbon per m², which is still higher than, for example, temperate tree plantations or grasslands with a productivity of generally less than 1 kg carbon per m² (Thomas 2002). Production figures have been reported in the range of 3.3 – 11.3 kg dry weight m⁻² yr⁻¹ for non-cultured and up to 13.1 kg dry weight m⁻² over 7 month for cultured brown algae compared with 6.1 – 9.5 kg

fresh weight $\text{m}^{-2} \text{yr}^{-1}$ for sugar cane, a most productive land plant. In addition marine biomass does not require fertilisation as currents and water exchange provide a continuous flow of a base level of nitrates and phosphates and large scale cultures may be useful in alleviating increased nitrogen levels in inshore waters. Due to the absence of lignin and a low content of cellulose, brown macroalgae carbohydrates may be easily convertible in biological processes compared to land plants.

Seaweeds are already farmed on a massive scale in Asia and substantial quantities are also harvested from natural populations. Recent research has shown the potential for large scale culture of macroalgae in Atlantic waters (Germany; Buck and Buchholz 2004; Canada; Chopin et al. 2008; France; Kaas 2006; Ireland; Kraan et al. 2000; Isle of Man, UK; Kain et al. 1990, Spain; Peteiro et. al., 2006). The challenges now lie in further developing cost-effective methodologies to grow, harvest transport and process large quantities of macroalgae.

A large body of research on fermentation of seaweeds into methane exists starting in the early 1980s and is extensively reviewed in Kelly and Dworjanyn (2008).

The only commercially available biofuels today are first generation biofuels, mainly bioethanol and biodiesel, produced from e.g. sugar cane and corn, and rapeseed, respectively. However, continued use of these crops will drive the food versus fuels debate even more as demand for ethanol increases. Not only does the large-scale production of corn and sugar cane damage the environment by the use of pesticides, it uses two other valuable resources: arable land and enormous quantities of water. For instance, the production of corn in the USA uses over 3 trillion litres of water a year in 2007 (Chiu et al. 2009).

Increased demand and the competition with food production has called for the development of second generation biofuels, based on utilization of lignocellulosic biomass, such as wood and agricultural waste. Second generation biofuels do not compete with food as a feedstock, but they compete for land and fresh water resources. Therefore the challenge is to find a feedstock which is abundant and carbohydrate-rich. This crop must be sustainable, use no agricultural inputs (pesticides, fertiliser, land, water), and must not be part of the human or animal food chain. Such a feedstock and an alternative to terrestrial biomass are marine macroalgae or seaweeds. Macroalgae and aquatic biomass are emerging as one of the most promising potential sources for biofuels production.

6.1. Suitable species and production

Several species of macroalgae accumulate high levels of carbohydrates, which are suitable as substrate for microbial conversion processes, e.g. for production of bioethanol, biobutanol as biofuels or other desirable chemicals with an attractive high product value. Green algae species such as the *Ulva* sp Linnaeus with high levels of the polysaccharide Ulvan (Lahaye and Ray 1996; Lahaye 1998) have been used in ethanol and methane production production (Morand et al. 1991, Adams et al. 2009). Brown macroalgae in particular kelp contain 50-60% carbohydrates of the dryweight and cultivation techniques have been firmly established for the last 50 years. Moreover, kelp is cultivated in large quantities up to 15.5 million wet tonnes in the Far East (FAO 2010).

Five Atlantic kelp species are suitable for cultivation and have a high carbohydrate level, i.e., *Saccorhiza polyschides* (Lightfoot) Batters; *Alaria esculenta* (L.) Greville; *Laminaria hyperborea* (Gunnerus) Foslie; *Laminaria digitata* (Hudson) J.V. Lamouroux; and *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders (Werner and Kraan 2004). They differ in various aspects, such as morphology, ecophysiology and longevity. *Laminaria digitata* and *Laminaria hyperborea* are the only species that form extended monospecific kelp beds.

The biomass productivity of macroalgae ranges converted to carbon is about 1 to 3.4 kg carbon m⁻² year⁻¹ (Gao and McKinley 1994; Mann 1982; Mohammed and Fredriksen 2004). Seaweed communities of the North Atlantic coasts have an annual productivity of approximately 2 kg C per m², which is far higher than, for example, temperate tree plantations or grasslands with a productivity of generally less than 1 kg C m⁻², year⁻¹ (Mann 1982; Chapman 1987; Thomas 2002; Lüning and Pang 2003; Mohammed and Fredrikson 2004), and 2.8 times higher than for sugar cane (Gao and McKinley 1994). Macroalgae can be cultivated in the open sea (Zhang et al. 2008; Bartsch et al. 2008; Kelly and Dworjanyn 2008). Ocean farming of seaweed does not depend on fresh water and does not occupy land areas (Yarish and Pereira 2008). Sustainable utilization of algal biomass - a largely unexplored feed stock resource can be a complement to terrestrial biomass for the future global energy and carbon security and thereby also strengthen the maritime economies.

Ocean farming of seaweed has the potential to produce in the order of 40 ton dry weight biomass per hectare per annum. An area of 2500 km², the size of Luxembourg, would be able to provide 10 million ton dry biomass, representing 5.6-5.8 million ton carbohydrates. With current 90% enzymatic conversion into ethanol (Wargacki et al., 2012) this would yield close to 2 billion litres of bioethanol. This is about 2-3 % of the world's global bioethanol production (F.O. Licht 2009); however, it would cover about 50% of the EU's ethanol demand (Annon 2008b). The use of algal biomass has the potential to not only replace fossil resources, and thereby mitigate climate change, but also aid in the recycling potential of nitrates and phosphates in near and inshore waters.

6.2. Processing and fermentation of macroalgal biomass

The water content in macroalgae is higher than in terrestrial biomass (80-85 %), making seaweeds more suited for microbial conversion than for direct combustion or thermo-chemical conversion processes, which is an alternative for land-based biomass (Horn et al. 2000a; Ross et al. 2007). Seaweed carbohydrates may be used as substrates for microbial production of a wide range of fuels and chemicals. Ethanol production from hexose sugars such as glucose, sucrose, laminarin etc. derived from e.g. corn stover or sugar cane, is a well-known process. However, hexose-based polysaccharides constitute only about 30-40% of the carbohydrates in kelp. The remaining fraction is composed of C-5 sugars that until now have not been applied as substrates for industrial microbial production processes. However, recent breakthroughs have been made in C5 sugar fermentation technology allowing up to 90% of the available carbohydrates to be fermented (Wargacki, et al., 2012).

6.3. Pre-treatment of the seaweed biomass and hydrolysis of the polysaccharides of brown seaweed biomass

Fresh harvested brown seaweed contains about 15-20 % carbohydrates of the total wet weight, which equals about 200 g carbohydrates per kg wet weight, which is an appropriate substrate concentration for microbial conversion processes (Horn et al. 2000a). Lack of lignin in seaweeds implies that the harsh pre-treatment applied for release of fermentable sugars from lignocellulosic biomass is not required. Laminaran and mannitol can easily be extracted by water (Horn et al. 2000b). Alginates (consisting of polymer blocks of Uronic and guluronic acid) is present in the macroalgae biomass at 30-40% (Honya et al. 1993; McHugh 2003). Sodium alginate can be removed from the initial extraction solution by adding a calcium salt. This causes calcium alginate to form with a fibrous texture; it does not dissolve in water and can be separated from it. The separated calcium alginate is suspended in water and acid is added to convert it into alginic acid. This fibrous alginic acid is easily separated, placed in a planetary type mixer with alcohol, and sodium carbonate is gradually added to the paste until all the alginic acid is converted to sodium alginate. The paste of sodium alginate is sometimes extruded into pellets that are then dried and milled (McHugh 2003). Due to the high viscosity, dilution with large amounts of water is required. The process is operating at alginate concentrations in the order of ~2 %. Such a dilution cannot be applied to processes aimed at use of alginate as fermentation substrate. Preferably, no water should be added, as it will increase the downstream processing costs (McHugh 2003).

Bioethanol production from cellulosic materials can be achieved by running simultaneously enzymatic hydrolysis and fermentation. For the wet seaweed biomass it is not as easy due to the alginates which are harder to release from the biomass causing enzymatic degradation of un-treated biomass and will be rate-limiting step if combined with fermentation. Hydrolysis should therefore be a part of the biomass pre-treatment. This hydrolysis can be carried out mechanically through grinding and emulsifying equipment, chemically using acid or alkali, or enzymatic. Several methods for partial or complete degradation of alginate as an integrated part of the mechanical pre-treatment of the biomass are known. Chemical hydrolysis should follow existing technologies, e.g. by modification and adaptation of methods used for acid and alkali pre-treatment of wood biomass (e.g. Ballesteros 2001; Klinke et al. 2001) and by combination of acid- or alkali with steam treatment. Other studies with macroalgae demonstrated the need for pre-treatment at 65°C, pH 2 for 1 h prior to fermentation (Horn, 2000a; 200b, Percival and McDowell, 1967). This in contrast with Adams et al. (2009) who found that these pre-treatments are not required for the fermentations with *Saccharina latissima* conducted, with higher ethanol yields being achieved in untreated fermentations than in those with altered pH or temperature pre-treatments. This result was seen in fresh and defrosted macroalgae samples using *Saccharomyces cerevisiae* and 1 unit of the enzyme laminarinase per kg of defrosted macroalgae. Nevertheless, the easiest and environmental friendliest way of pre-treatment of algal biomass is through a combination of mechanical and enzymatic hydrolysis (Doubet and Quatrano 1982).

6.4. Ethanol and butanol from brown seaweeds

Ethanol production from fermentation is the most obvious one as it has a direct application in the replacement of fossil fuels. However other products such as butanol and itaconic acid can be produced as well which can substitute and/or replace similar products produced from fossil resources. There are many microorganisms in the marine environment that can degrade and utilize algal carbohydrates as a carbon source for energy. Often these microorganisms are associated with the seaweeds being present on the blade surface or in tissue as many kelp species produce exo-polysaccharides as mucus layer or shed entire skin. This would imply that these organisms possess the necessary enzymes for cleavage of the algal polysaccharides. However, compounds such as ethanol and butanol are produced by anaerobic fermentation that require the presence of specific metabolic pathways generating these compounds as end products, e.g. yeast for ethanol production and *Clostridia* for butanol production. Limited information is available on the efficiency of these processes with seaweed carbohydrates (Horn et al. 2000a; 2000b), although several brake troughs have recently been made in respect of ethanol production from brown seaweeds (Wargacki, et al., 2012).

6.4.1. Ethanol

The potential of ethanol production from seaweeds can be calculated and is based on the following assumptions: A carbohydrate content of 60 % of dry weight and a 90 % conversion ratio to ethanol. Through fermentation one gram of sugar can yield 0.4 g ethanol. This will yield 0.22 kg or 0.27 l ethanol from 1 kg dry weight seaweed biomass, corresponding to approximately 0.05 l ethanol per kg wet weight.

Bacteria can be metabolize uronic acids to pyruvate and glyceraldehyde-3-P, which may then be fermented to ethanol by the glycolytic pathway (van Maris et al. 2006). In anaerobic fermentation processes, as ethanol and butanol production, oxygen is not available for removal of excess hydrogen generated. This implies that the conversion reaction from substrate to product must be red-ox balanced. Ethanol-production from hexose sugars is red-ox balanced, while production from pentoses or mannitol generates excess hydrogen. In many bacteria but not in yeast the enzyme transhydrogenases, solves this problem. Yeasts can avoid the problem by receiving a small, controlled supply of oxygen. However, oxygen leads to complete oxidation of the substrate to CO₂ and water, and reduced ethanol yields. Another strategy is introduction of transhydrogenase into strains that lack this, through genetic engineering (Fortman et al. 2008; Lee et al. 2008). Prospecting macroalgae (seaweeds) as feedstocks for bioconversion into biofuels and commodity chemical compounds is limited primarily by the availability of tractable microorganisms that can metabolize alginate polysaccharides. Wargacki, et al., (2012) present the discovery of a 36-kilo-base pair DNA fragment from *Vibrio splendidus* encoding enzymes for alginate transport and metabolism. The genomic integration of this ensemble, together with an engineered system for extracellular alginate depolymerization, generated a microbial platform that can simultaneously degrade, uptake, and metabolize alginate. They further engineered for

ethanol synthesis, this platform enables bioethanol production directly from macroalgae via a consolidated process.

6.4.2. Butanol

Butanol is an alternative to ethanol with a higher energy content (butanol 29.2 MJ/l, ethanol 19.6 MJ/l), compared to gasoline (32 MJ/l). It can be used to supplement both gasoline and diesel fuels and can be handled by existing infrastructures (Fortman et al. 2008). Butanol is an important industrial chemical and is currently produced via petrochemical processes. In the last century butanol was produced through bacterial fermentation of starch rich compounds using *Clostridia* strains (Zverlov et al. 2006), which can use hexose as well as pentose sugars.

7. Outlook

Macroalgae are an interesting source for a myriad of different bioactive polysaccharides ranging from industrial applications to novel food applications. They possess many different interesting and often exotic polysaccharides that are currently explored for their functional properties in food and biomedicine. However, a far larger application would be the use of carbohydrates from cultivated seaweeds for alternative fuel sources. It is the exploitation of nature's energy cycle, photosynthesis and the resulting plant biomass that can accelerate this application. Society has to make a transition from a hydrocarbon to a carbohydrate economy, with the accrued benefits of carbon neutral biofuel, plastics and medicine. Macroalgae are efficient solar energy converters, and can create large amounts of biomass in a short-term, however, marine biomass is often an overlooked source, and potentially represents a significant source of carbohydrates as a renewable energy source.

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