Major commercial products from micro- and macroalgae

Melinda Griffiths[1], Susan T.L Harrison[1], Monique Smit[1,2] and Dheepak Maharajh[2]

[1] Centre for Bioprocess Engineering Research, Department of Chemical Engineering, University of Cape Town, Rondebosch, Cape Town, South Africa, 7701
[2] CSIR Biosciences, Bld 18, PO Box 395, Pretoria, South Africa, 0001

Melinda.Griffiths@uct.ac.za - 021 650 5523
Sue.Harrison@uct.ac.za - 021 650 4021
DMaharajh@csir.co.za – 012 841 2664

Abstract

Macro-and microalgae are used in a variety of commercial products with many more in development. This chapter outlines the major products, species used, methods of production, extraction and processing as well as market sizes and trends. Foods, nutraceuticals and feeds are the major commercial products from algae. Well-known culinary products include Nori, Wakame, Kombu and Dulse, from whole macroalgal biomass. The microalgae *Spirulina* and *Chlorella* have been widely marketed as nutritional supplements for both humans and animals. Several microalgae with a high nutritional value and energy content are grown commercially as aquaculture feed. The major processed products from macroalgae are the hydrocolloids, including carrageenan, agars and alginates, used as gelling agents in a variety of foods and health-care products. Pigments extracted from algae include β-carotene, astaxanthin and phycobiliproteins. These are generally used as food colourants, as additives in animal feed or as nutraceuticals for their antioxidant properties (Radmer, 1996; Pulz, 2004). Polyunsaturated fatty acids (PUFAs) are another high-value product derived from microalgae. Other potential products include fertilizers, fuels, cosmetics and chemicals. Algae also have application in bioremediation and CO_2 sequestration, as well as producing many interesting bioactive compounds. Algae have great potential to produce a wide range of valuable compounds, beyond their current exploitation. To date, commercialization of new products has been slow (Milledge, 2011; Wijffels, 2007; Radmer, 1996; Pulz, 2004; Spolaore, 2006), however, microalgal biotechnology is a relatively new industry, therefore, it is unsurprising that significant challenges remain to be solved. The advantages associated with algal production are likely to ensure that efforts continue.

Introduction

The term algae encompasses an extremely diverse taxonomic group, including both prokaryotic and eukaryotic organisms, with a wide genetic and metabolic range (Apt and Behrens, 1999; Radmer, 1996). Structurally, species range from the unicellular, colonial and filamentous through to complex multicellular structures resembling higher plants (e.g. kelp). The most general grouping divides these into the microalgae (those that are microscopic) and the macroalgae (those that can be seen with the naked eye). They are efficient cell factories, the majority operating photosynthetically to turn light energy and simple, inorganic nutrients into a range of complex biomolecules. Some species are heterotrophic, or able to grow in the dark on organic carbon sources such as sugar, similarly to yeast, fungi and bacteria. Occasionally species can switch between these modes (mixotrophy), although often grow better in one mode than the other. Common products of algal metabolism include proteins, carbohydrates, pigments, lipids, toxins and bioactive compounds (Radmer, 1996).

Foods, food additives, nutraceuticals and feeds are the major commercial products from algae. Well-known culinary products from macroalgae include Nori, Wakame, Kombu and Dulse. The microalgae *Spirulina* and *Chlorella* have been widely marketed as nutritional supplements for both humans and animals. Several microalgae with a high nutritional value and energy content are grown commercially as aquaculture feed. The major processed products from algae are the hydrocolloids, including carrageenan, agars and alginates. These polysaccharides are extracted from algal biomass and used as gelling agents in a variety of foods and health-care products. Pigments extracted from algae include β-carotene, astaxanthin and phycobiliproteins. These are generally used as food colourants, as additives in animal feed or as nutraceuticals for their antioxidant properties (Radmer, 1996; Pulz, 2004).

Production of algae for human consumption and nutraceuticals together make up a multi-billion dollar industry with the majority of production and consumption in the Far East (Radmer, 1996). Macroalgae, mostly certain species of red (*Rhodophyta*) and brown (*Phaeophyta*), have an established history of harvest and cultivation for the production of food and hydrocolloids. The annual world market for these products is approx. $600 billion, with over 7.5 million tonnes harvested per year (Pulz, 2004). Microalgal biotechnology is a much newer industry. *Spirulina*, possibly the most well-known microalgal product, has been collected and eaten by the Aztecs and people in Central Africa for centuries, however, commercial production in man-made ponds only began in 1978 (Belay, 1997). Microalgae are cultured mainly as health food, as the source of pigments such as beta-carotene and as a feed or feed additive in aquaculture (Borowitzka, 1997). In 2004, the market for microalgal biomass was estimated to be about 5000 tons per year with a value of about $1.25 billion (Pulz, 2004; Spolaore et al., 2006).

The production of commercial products from algae ranges from low-tech ocean farming (e.g. harvesting seaweed from the wild for production of food, feed or fertilizer) to high-tech bioprocess engineering (e.g. intensive culture of specific strains under sterile, controlled conditions in fermentation systems for the production of isotopically labeled compounds) (Radmer, 1996). With the expansion of the biotechnology industry has come an increase in the variety of tubes, columns, panels and hanging bags (collectively known as photobioreactors) in which microalgae are cultivated, however, the majority of commercially successful algal processes today continue to use open ponds. The high cost of closed photobioreactors can only be offset by a high value product, for example a pharmaceutical (Wijffels, 2007).

This chapter provides an overview of the major current commercial products from algae (summarized in Table 1) and those in development. It outlines the major species used, methods of production, extraction and processing as well as market sizes and trends.

Table 1: Major commercial algal products, species and market value ([a]Radmer, 1996; [b]Pulz, 2004; [c]Spolaore et al., 2006; [d]Borowitzka, 1992, assuming 1AU$ = 0.74US$ [1992 average]; [e]pers. comm. Leon Giese, 2013; [f]Metting, 1996; [g]Olaizola, 2003; [h]Singh et al., 2005;[i]Merril, 1993;[j]Watanabe and Nisizawa, 1984;[k]www.amazon.com;[l]Belarbi *et al.,* 1999; [m]Muller-Feuga, 2000;[n]www.made-in-china.com; [o]Bux, 2013)

Product	**Species**	**Application**	**Market value (million US$)**	**Approx. price (US$)**
Nori	*Porphyra*	Food (sushi)	2000[a]	66-166/kg sheets[i] 70-100/kg sheets[k]
Wakame	*Undaria pinnatifida*	Food	600[a] 150[j]	169/kg dried[k]
Kombu	*Laminaria japonica*	Food	600[a]	50-200/kg dried[k]
Health food	*Spirulina*	Nutraceutical	80[a] 20-25[f] 40[h]	10-20/kg bulk powder[d,e] 35/kg[k] powder 68-112/kg[k] tablets
Health food	*Chorella*	Nutraceutical	100[a]	100/kg[a] 100-120/kg[k] tablets
Alginates	*Laminaria*, *Macrocystis* and *Ascophyllum*	Thickening, gelling, water retention	230[a]	3-190/kg[k,n]
Carrageenans	*Eucheuma cottonii*, *E. spinosum* and *Chondrus crispus*	Gelling, thickening, stabilizing	100[a]	5-140/kg[k,n] powder
Agars	*Gracilaria*, *Gelidium* and *Pterocladia*	Gelling: food and biotechnology	160[a]	5-100/kg[k,n] powder
Agarose	From agar	Biotechnology	50[a]	Up to $25 000/kg[a]

				750-2600/kg[k]
Phycobiliproteins	*Arthrospira platensis*	Food colourant, nutraceutical	2[a] 12[b] 50[c]	370/kg[d] (food grade) 250-600/kg[e] (food grade) $5000/g[a] (reagent grade)
Aquaculture feed	*Isochrysis galbana*, *Phaeodactylum tricornutum*, etc. (Table 2)	Feed	700 [b]	50-300/kg[m]
β -carotene	*Dunaliella salina*	Pigment, feed, health supplement	280 [b] 44-133[d]	1400/kg[a] 300-3000/kg[c] 444/kg[d]
Astaxanthin	*Haematococcus pluvialis*	Pigment, feed additive, pharmaceuticals, health supplement	150 [b] 22-44[d] <5[g] (nutraceuticals)	2500/kg[c] 2220/kg[d] 2000/kg[g] (feed) >$100 000/kg[g] (nutraceutical)
Fatty acids, DHA, EPA, PUFA	*Odontella aurita*	Baby food, pharmaceuticals, cosmetics	1530 [b] 15[o] (DHA in the USA)	650/kg[l]
Isotopes		Biotechnology	5[b]; 13[c]	>100/g[a] 260-5900/g[c]

Food and nutraceuticals

Algae are rich in protein, lipids, carbohydrates, vitamins, minerals and essential nutrients, making them an excellent source of nutrition for humans and animals. Macroalgal food products, produced and sold primarily in the Far East, have the largest market value of any algal product (Radmer, 1996). Despite high hopes for the use of microalgae for food in the mid 20th century, this has not materialized to date, likely due to the high cost of production. While basic cost of production of microalgal biomass in raceway systems lies in the range US$ 0.23 – 0.6 per kg dry mass, in photobioreactors average predicted costs lie in the range US$ 3.2-9.5 per kg with data reported across the range US$ 0.42 to 30.4 per kg (Harrison et al. 2013). Nutraceuticals and health foods, where algal biomass can command premium prices, are the dominant markets for microalgae.

Macroalgae

The major algal foods are derived from macroalgae, generally harvested from wild, or from managed or cultivated populations. Indonesia, the Philippines, Malaysia and China are among the top macroalgae producing nations in the world (Hurtado, 2014). Whole algal biomass is often sun-, spray- or oven-dried, and sold as a sheet, powder, tablet or capsule, or incorporated into other foods. The only processing involved is the sorting, cleaning or preserving of the biomass (Radmer, 1996). Many types of macroalgae are eaten as foods. Those with the major commercial markets are listed here.

Nori

Produced from the blade or 'leaf' of the red algae *Porphyra*, Nori has been collected since the year 530, and actively cultivated since 1640 (Pulz, 2004). It is the dominant algal product on the market today, with an annual turnover of more than $1 billion (Pulz, 2004). In 1993, Nori production was 40 000 tons/annum (Jense, 1993). Its principle use is as a component of sushi. Toasted nori sheets (Fig. 1) are the most popular product. Nori cultivation, similarly to other macroalgal food products and hydrocolloid species, is essentially underwater farming. Conchospores (seed-like propagules) are seeded onto nets and hung in sheltered ocean areas, strung between poles or attached to surface buoys. More recently, devices designed to be raised out of the water have been developed, allowing more mechanized harvesting and processing (Radmer, 1996).

Wakame

Wakame is derived from the brown algae *Undaria pinnatifida*. It has been cultivated, primarily in Japan, Korea and China, since the 1950s. It is sold in many forms, the most popular product being boiled and salted. It is used as an ingredient in noodles, soups and salads. In 1990, approximately 20 000 tons were produced annually with a market value of $600 million (Radmer, 1996).

Kombu

Harvested from *Laminaria japonica* and related brown algal species, Kombu is another popular culinary alga, served with meat, fish, in soups or as a vegetable. Algae are collected, dried and boiled. The annual marked is estimated at $600 million (Radmer, 1996).

Other major macroalgae used for human consumption include Dulse, derived from the macroalgae *Palmaria palmata* in Europe and *Rhodymenia* sp. in North America, and *Nostoc*. Dulse has been consumed by people living on the coast of Europe for centuries (Radmer, 1996). *Nostoc* is a filamentous cyanobacteria that is cultivated and consumed for its high protein and vitamin content, mostly in Asia. *N. flagelliform* and *N. commune* are the most popular varieties in China and Japan, and *N. ellipsosporum* in Central Asia.

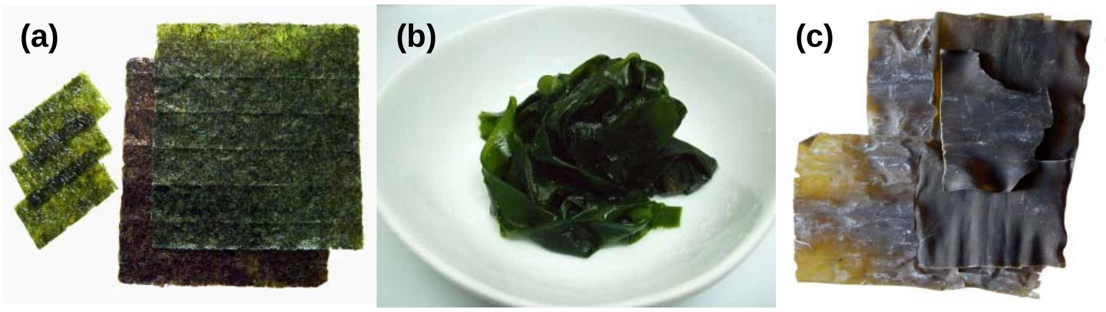

Figure 1: (a) dried Nori sheets, (b) boiled Wakame and (c) Kombu from *Laminaria japonica* ((a) and (c) Alice Wiegand, http://en.wikipedia.org/wiki/Nori#mediaviewer/File:Nori.jpg and http://en.wikipedia.org/wiki/Kombu#mediaviewer/File:Kombu.jpg; (b) えむかとー http://en.wikipedia.org/wiki/Wakame#mediaviewer/File:Boiled_wakame.jpg)

Microalgae

In the early 1950s, the rise in world population and concerns over a future shortage of food led to a search for alternative protein sources. Microalgae were identified as promising candidates due to their high protein content. Studies have shown the quality of algal derived protein to be superior to that from many conventional plants (Spolaore et al. 2006). Algae provide the full spectrum of amino acids. Algae are the original source of polyunsaturated fatty acids (PUFA) in the food chain, and thus provide essential fatty acids. Microalgae also represent a source of nearly all essential vitamins, and contain pigments such as chlorophyll, carotenoids and phycobiliproteins, which have been shown to have nutraceutical effects (Spolaore et al, 2006). Although microalgae and their extracts are used for food in certain niche applications, the large-scale production of algae to solve the world's food crisis and shortage of protein has not materialized (Milledge, 2011). This could be due in part to the relatively high cost of microalgal production in artificial containers, the undesirable taste, odor and strong colour of many dried algal powders, and the public perception of algae as 'slimy pond-scum' to be avoided.

Historically, nutritional supplements have been the largest commercial microalgal product. *Chlorella* and *Spirulina* (*Arthrospira platensis*) dominate the market and are predominantly used in health food products (Pulz, 2004). Either whole cell biomass or extracts are sold as nutraceuticals or functional foods, to be consumed on their own as a nutritional supplement, or combined into other products (Apt and Behrens, 1999).

Spirulina

The filamentous cyanobacteria *Arthospira platensis* and other species of *Arthrospira*, commonly known as *Spirulina*, has a long history of human consumption. It was originally harvested from natural alkaline lakes in Chad and Mexico (Radmer, 1996). The first commercial facility (Sosa Texcoco in Mexico) was established to harvest and enhance natural production in Lake Texcoco (Spolaore et al, 2006). Several large-scale commercial producers exist today e.g. Earthrise, Cyanotech and Dainappon Ink & Chemicals, each producing hundreds of tons of dry product per year.

Spirulina is a relatively easy microalga to cultivate due to its filamentous morphology (meaning it can be readily harvested by filtration) and the alkaline, saline conditions for cultivation prevent the growth of the majority of other algal species and contaminants (Radmer, 1996). *Spirulina* is commercially produced in large open raceway ponds (Fig. 2), harvested by filtration and dried by spray drying, solar drying or oven drying. Products include powder, tablets and capsules and are sold mainly in Europe, North America and Asia (Spolaore et al 2006. Metting, 1996). Prior to their consumption, algal biomass must be tested for safety in terms of heavy metal content, bacterial load and presence of toxins from contaminating microalgae (Spolaore et al, 2006).

Figure 2: *Spirulina* being cultivated in a raceway pond

It is sold primarily as a health food, or as an additive in animal feed. There is a large body of literature examining the nutritional value of *Spirulina* in human and animal diets (Radmer, 1996). As a source of usable protein, *Spirulina* contains in excess of 50% protein. This is superior to plants, comparable to meat and dairy products, but inferior to poultry and fish. It contains the full range of amino acids, but has low levels of sulfur-containing essential amino acids (Metting, 1996). It also contains some essential fatty acids, unusually high levels of the vitamins A and B_{12}, and many useful minerals. *Spirulina* biomass has been suggested to have various health-promoting effects e.g. immune boosting, antiviral, promotion of growth of healthy intestinal flora (Spolaore et al 2006), however, most of these have not yet been validated in clinical studies.

Chlorella

Chlorella is a green microalga marketed primarily as a health food product. It was first commercially cultivated in the early 1960s by Nihon Chlorella (Taiwan). Today more than 70 companies produce it commercially (Spolaore et al, 2006). Success in its cultivation in open reactors relies on its fast growth rate to outcompete contaminants, as it grows under moderate conditions. It can also be grown heterotrophically on glucose or acetate, usually for the production of high value compounds e.g. pharmaceuticals. Biomass is harvested by centrifugation and dried to a powder (Radmer, 1996). It is purported to have a variety of health promoting effects e.g. immune-stimulation, reduction in blood lipids, antioxidant properties, efficacy on gastric ulcers, wounds and constipation (Spolaore et al 2006). However, these investigations are often initiated by the *Chlorella* producing company itself and have not been conclusively demonstrated.

Feed

Aquaculture

The growth in the production of farmed fish and shellfish has been strong, increasing in one decade from 10Mt to 29 Mt in 1997. Nutrition of these farmed fish has been reliant on by-products of wild fish and terrestrial agriculture. Enhancing availability of algae for fish feed enables independence from wild fish by-products to be approached in a sustainable manner (Naylor et al. 2000). Algae are an important food and feed additive in the aquaculture of a variety of animals, including fish, molluscs (clams and oysters), crustaceans (shrimp) and zooplankton (e.g. rotifers and *Artemia*), which serve as live food for fish and shellfish (Borowitzka, 1997; Metting, 1996). Microalgae are required during a brief but vital period in larval nutrition, either for direct consumption by molluscs or shrimp, or as food for the live prey fed to fish larvae (Spolaore et al 2006). Algae are the preferred food source as they are the natural food of these animals, although alternatives are available (e.g. yeast and artificial feeds) (Borowitzka, 1997).

There are three slightly differentiated markets for algae in aquaculture (Pulz, 2004):

- the unialgal cultivation of specific microalgal species as a starting food for larvae and young vertebrates
- the addition of small amounts of algae e.g. *Spirulina*, *Chlorella*, and *Dunaliella* into fish feed, similarly to animal feed, to enhance health and colour
- the use of macroalgae as whole grazing fodder, or incorporated into feed mixtures/pellets for feeding to larger aquaculture stocks.

Microalgae represent the largest market for aquaculture feed. More than 40 microalgal species are used in aquaculture worldwide, depending on the local requirements (Pulz, 2004). Cultures of specific algal strains are necessary in the production of a defined food with characterized properties to ensure the survival and high productivity of larvae. Those commonly used are listed in Table 2. The nutritional content of microalgae can be altered through adjusting the culture conditions. This can be exploited to enhance the nutritional content of the algae in order to improve larval survival rates. For example, the content and ratio of the long-chain fatty acids DHA, EPA and arachidonic acid in feed have been recognized to affect the larval development. This can have a large impact on the economics of the aquaculture facility (Apt and Behrens, 1999).

Table 2: Algae commonly used as aquaculture feed (Apt and Behrens, 1999; Borowitzka, 1997; Khatoon et al. 2009; Pulz, 2004; Spolaore et al. 2006)

Species	**Aquaculture crop**
Amphora	Shrimp
Chaetoceros	Molluscs, Crustaceans
Chlorella	Fish
Cyclotella	Molluscs
Dunaliella	Crustaceans
Haematococcus	Fish, mainly salmon
Isochrysis	Molluscs
Nannochloropsis	Rotifers
Navicula	Abalone, Shrimp
Nitzschia	Abalone
Pavlova	Molluscs
Phaeodactylum	Fish
Skeletonema	Molluscs, Crustaceans
Spirulina	Fish e.g. koi, tilapia
Tetraselmis	Crustaceans

Macroalgae used as grazing fodder for animals such as urchins and abalone include *Ecklonia maxima*, *Ulva lactuca* and *Gracilaria gracilis* (Naidoo et al. 2006).

Of the 1000 tons of microalgae produced for aquaculture in 1999, 62% was used for molluscs, 21% for shrimps and 16% for fish (Spolaore et al. 2006). Microalgae are often grown on-site in aerated, hanging plastic bags or tanks, and are fed fresh or dried, alone or combined with other nutrients. To be a good aquaculture feed, algae must contain the right nutrients for the target organism at the required growth stage. Protein and fatty acid content are of particular importance in larval nutrition, as well as vitamins. In addition, they must be the right size and shape for the consumer to feed on, have a digestible cell wall, be non-toxic and be cost-effective to cultivate at a medium scale (Borowitzka, 1997; Spolaore et al, 2006).

Microalgal production is costly and can contribute 30-40% of the aquaculture production cost (Borowitzka, 1997). Bivalve and shrimp hatcheries require substantial amounts of algae on a continuous basis. Photosynthetic production costs are reported to be as high as >$160/kg dry weight, representing a substantial part of the production cost (Radmer, 1996), suggesting the need for focus on improved algal productivity and efficiency of production of the required scale to improve competitiveness, as their value in aquaculture is well recognised. The reliability of feed supply is critical, as an interruption could lead to the death of the aquaculture crop (Borowitzka, 1997).

The addition of microalgae to the tanks of aquaculture organisms has been found to have unexpected additional positive benefits, e.g. algae helps to improve the quality of the water, leading to higher survival and growth rates. This may be due to uptake of nutrients, oxygen formation, pH stabilization, regulation of bacterial population and/or probiotic and immune boosting effects (Spolaore et al., 2006).

The colour of aquaculture organisms can be an important factor in their desirability by consumers and hence impact their market price (Apt and Behrens, 1999). In the natural food chain, algae are the primary source of pigments that impart this coloration, e.g. the characteristic orange-pink colour of salmon, trout and shrimps. Artificial aquaculture feeds generally lack these pigments, and hence artificial supplements such as canthaxanthin are often added. The pigment astaxanthin is most commonly used. Astaxanthin is sourced from the microalga *Haematococcu*s (see also the astaxanthin section under pigments). Several companies have successfully produced and marketed natural astaxanthin from *Haematococcu*s for aquaculture feeds (Apt & Behrens, 1999).

Pets and farm animals

Microalgae can be incorporated into the feed of a variety of animals, including fish, pets and farm animals, with beneficial outcomes. In 2006, approximately 30% of world microalgal production (including 50% of *Spirulina* production) was sold as feed supplement (Spolaore et al. 2006). Feed quality has a large influence on the survival, development, growth and fertility of animals. There is evidence that small amounts of *Spirulina*, *Chlorella* and *Scenedesmus* incorporated into the feed can have a positive effect, particularly in boosting the immune response (Pulz, 2004). Poultry can be fed up to 5-10% *Spirulina* and this has been shown to enhance the yellow colour of the skin and egg yolks due to the carotenoid content (Milledge, 2011). *Spirulina* is easily digested due to its soft cell wall, however, some other algal species show poor digestibility due to the high content of cellulosic cell wall material. Ruminates such as sheep and cattle are capable of digesting cellulosic material, but algae as a major part of the feed has not gained much commercial favor yet (Milledge, 2011).

Another promising application for microalgal biomass or extracts is in the pet food market. Studies on minks and rabbits have shown evidence of health-promoting effects (Pulz, 2004). *Spirulina* has been included in the diets of cats, dogs, aquarium fish, ornamental birds, poultry, horses, cows and pigs, with positive effects on physiology e.g. improved immune response and fertility, as well as improved appearance of coats and skin (Spolaore et al., 2006).

Hydrocolloids

Several useful polysaccharides, collectively known as hydrocolloids, are extracted from macroalgae. Together they make up the largest market ($500 million) for algal extracts (Pulz, 2004). Hydrocolloids are produced by species of red and brown macroalgae, either harvested from the wild or cultivated in stands, similarly to those for food. The hydrocolloids are extracted through a series of steps involving hot solvent extraction and purification. Agarose is derived from agar by further separation and purification (Radmer, 1996). The hydrocolloids are used for their thickening and gelling properties in a variety of food and industrial applications (Pulz, 2004).

Alginates

Alginates, polymers of D-mannuronic acid and L-gluronic acid, are salts of alginic acid. The exact composition varies in different sources. Alginates are used as thickeners, to form gels and for their water-retaining properties in the food, paper, biomedical and biotechnology fields. In 1990, the market for alginates was approximately $230 million (27 000 tons). The major sources of alginates are brown macroalgae, particularly *Laminaria*, *Macrocystis* and *Ascophyllum*. A typical process involves extraction of the alginic acids in hot sodium bicarbonate, followed by filtration and purification (Radmer, 1996).

Carrageenans

Carrageenans are extracted primarily from the red macroalgae *Eucheuma cottonii*, *E. spinosum* and *Chondrus crispus*. Carrageenans are complex polysaccharides, made of sulfonated galactose polymers, typically extracted with hot water. They are used to gel, thicken, suspend and stabilize foods, cosmetics, pharmaceuticals and other products. Approximately 15 500 tons of carrageenans, with a market value of $100 million, were sold annually (data from 1990, Radmer, 1996).

Agars and Agarose

The first reports of agar production date back to 1658 in Japan (Pulz, 2004). Agars are a mixture of polysaccharides extracted from red algae. Similarly to carrageenans, they are also composed of galactose-related monomers, with varying amounts of sulfate, pyruvate and methoxy groups. The content varies with the source species and processing procedure. Primary sources include *Gracilaria*, *Gelidium*, *Pterocladia*, *Acanthopeltis* and *Ahnfeltia*. Agars are usually extracted with hot water, concentrated and dried before being milled for packaging. Agar forms a gel that is stable under a range of temperatures, humidities and chemical conditions. Agars are used in food and to produce solid growth media for microorganisms in laboratory studies. Agars command a significantly higher price than alginates and carrageenan. Annual sales are in the range of $160 million, with a volume of 11 000 tons (Radmer, 1996). Agaroses are refined from agar by isolating the less ionic fractions of agar. The main applications of agarose are in the field of biotechnology (Radmer, 1996).

Pigments

Chlorophyll, carotenoids and phycobiliproteins are the major pigments produced by algae (Metting, 1996). The types and ratios present are dependent on species, light, nutrition and other environmental factors (Metting, 1996). In addition to chlorophyll, algae contain various other pigments in order to increase the efficiency of light usage (e.g. phycobiliproteins) and to protect against solar radiation and free radicals (e.g. carotenoids) (Pulz, 2004).

There is increasing demand for natural colourants from sustainable sources for use in the food, cosmetics, pharmaceuticals, textile and printing industries (Dufosse et al. 2005). Few natural colourants have the tinctorial values and persistence required for industrial use (Sekar and Chandramohan, 2008). Carotenoids and phycobiliproteins are among these. The major pigments of commercial interest are β-carotene from *Dunaliella*, astaxanthin from *Haematococcus* and the phycobiliproteins from Cyanobacteria and some red algae.

Carotenoids

Carotenoids are tetraterpene, lipid soluble pigment molecules, most often in the brown-red-orange-yellow colour range. The colouration of many brown and golden algae is produced by carotenoids. The variety found in algae is much greater than in land plants (Metting, 1996). Only very few of the 40+ known carotenoids are produced commercially (Metting, 1996). These are mainly β-carotene and astaxanthin, and, to a lesser extent, lutein, zeaxanthin and lycopene (Spolaore et al, 2006).

β-carotene

β-carotene is produced naturally by the halotolerant green alga *Dunaliella*. Species of the *Dunaliella* are generally grown in hypersaline, open ponds. Once the biomass has accumulated, it is subjected to high salt, high light environments. These stressful conditions cause accumulation of β-carotene up to 14% dry weight (Radmer, 1996; Spolaore et al. 2006). The extreme growth conditions assist in limiting the growth of other algae and microorganisms. Harvesting of the dilute cells from a large volume of media represents a major expense in the process (Radmer, 1996). The biomass is processed by a variety of techniques to produce products ranging from high-carotene content biomass to β-carotene in oil (Radmer, 1996).

β-carotene is used as a natural food colourant and feed additive. It is also marketed as a health food and finds application in cosmetics for its putative health benefits. Carotenoids are precursors to Vitamin A and have antioxidant and anti-inflammatory properties (Radmer, 1996; Pulz, 2004). β-carotene occurs in many higher plants as well as algae and can be produced synthetically. As the synthetic form can be produced at a lower cost, the market for the natural form of β-carotene relies on the consumer perception that the natural form is superior. The natural form supplies cis-isomers in the natural ratio, which is generally accepted to be superior to the all-trans synthetic form (Spolaore et al. 2006).

Figure 3: Aerial view of open raceway ponds used for the production of β-carotene near Uppington, South Africa

Astaxanthin

Astaxanthin is produced commercially from *Haematococcus pluvialis*. This alga can accumulate astaxanthin up to 3% dry weight (Pulz, 2004). It is primarily sold as an additive in aquaculture feed for colouration purposes (Pulz, 2004; Spolaore et al., 2006). The largest astaxanthin consumer is the salmon feed industry (Olaizola, 2003). Human neutraceuticals has also expanded as a new market for astaxanthin since the 1990s. The annual aquaculture market of this pigment is estimated at $200 million, with an average price of $2500/kg (Spolaore et al. 2006).

Much research has been done on the production of *Haematococcus* for astaxanthin. In Hawaii and China, it is produced in open ponds, while other manufacturers, e.g. in Israel, have opted for closed systems (Pulz, 2004). A combination of closed and open systems is also possible, the first to generate uni-algal biomass and the second to provide stress conditions in the final pigment accumulation stages of cultivation. *Haematococcus* is produced in a two-stage culture process: an initial algal growth stage under condition favourable for biomass productivity, where the algae are green and flagellated, followed by a pigment accumulation stage where a stress condition or combination of stressors, usually high light and low nutrient conditions, are applied to trigger accumulation of pigment to high levels (1.5-3% dry weight), yielding red, non-motile cells (Spolaore et al., 2006).

Astaxanthin can also be manufactured synthetically. Natural production cannot compete with the synthetic form on price, but still finds a market due to consumer preference for natural products in the nutraceutical and cosmetics markets. There is little consumer influence in the aquaculture market as consumers remain uninformed about rearing practices, however, natural astaxanthin does offer enhanced performance in some applications, e.g. the natural form leads to enhanced deposition in the tissues of carp, chicken and red sea bream (Olaizola, 2003; Spolaore et al 2006).

Phycobiliproteins

Phycobiliproteins are unique to algae. They are a group of water-soluble, light harvesting proteins found in cyanobacteria, rhodophytes, cryptomonads and glaucophytes (Sekar and Chandramohan, 2008; Erikson, 2008). They 'close the gap' in light absorption by absorbing light in the range that chlorophyll and carotenoids do not (495 – 650nm, Apt & Behrens, 1999). The three major groups of phycobiliproteins, classified according to their spectral properties, are phycocyanin (PC), phycoerythrin (PE) and allophycocyanin (APC) (Table 3). The different phycobiliproteins are organized into complexes called phycobilisomes on the outer surface of thylakoid membranes (Fig. 4) (Sekar and Chandramohan, 2008).

Table 3: characteristics and origin of the major phycobiliproteins (Eriksen, 2008; Sekar and Chandramohan, 2008; Abalde et al., 1998; Bermejo et al., 2006)

Phycobiliprotein	**Colour**	**Max absorption (nm)**	**Max emission (nm)**	**Common sources**	**Commercial applications**
Phycocyanin (PC)	Blue	610-620	650	*Spirulina*, *Anabaena*, Synechococcus, *Nostoc*, *Galdieria*	Food colourant, Nutraceutical
Phycoerythrin (PE)	Red	540-570	577	*Porphyridium*, *Porphyra*	Fluorescence
Allophycocyanin (APC)	Blue-green	650-655	660	*Spirulina*, *Microcystis*	Fluorescence

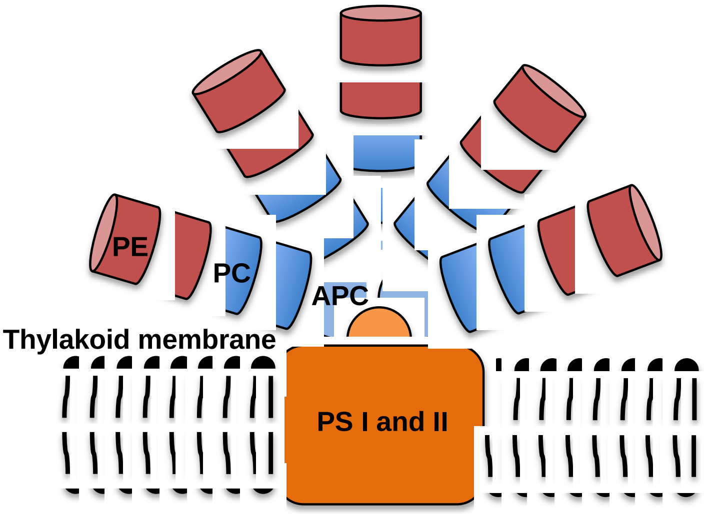

Figure 4: typical structure of a phycobilisome**.** PS = photosystem, PC = phycocyanin, PE = phycoerythrin, APC = allophycocyanin

Phycobiliproteins are some of the most abundant proteins in many cyanobacteria (Eriksen, 2008). The nature of the culture conditions, particularly light and nutrients, can influence the cellular content of phycobiliproteins. Glucose and acetate have been found to enhance cell growth and PC production in *Spirulina platensis* (Chen et al. 1997). PE production in *Porphyridium* was shown to depend on the concentration of chloride and nitrate, and to a lesser extent, sulphate and phosphate in the culture medium (Kathiresan et al 2006).

The extraction of phycocyanin as a food colourant is fairly straightforward. Water-soluble components are extracted, generally from dry biomass, into buffer and the solids removed.

Preparation of pure phycobiliproteins such as PE for use in fluorescent applications requires further separation and purification, generally with chromatographic techniques. The high cost of this process is offset by the high price of the purified product (up to $5000/g). The market size for the reagent is estimated to be $2 million (Radmer, 1996).

Phycobiliproteins are used as food colourants and natural dyes: phycoycanin mostly in the food industry and phycoerythrin mainly in cosmetics. Phycocyanin has a bright blue colour (Fig. 5) and is considered more versatile than alternative natural blue colourants such as gardenia and indigo, although it has a lower stability to heat and light (Sekar and Chandramohan, 2008). It is used in ice cream, soft drinks, candies, chewing gum, desserts, cake decorations, icings and frostings, milk shakes as well as lipsticks and eyeliners (Sekar & Chandramohan, 2008; Eriksen 2008; Sarada et al 1999).

Figure 5: Phycocyanin extracted from *Spirulina platensis* dissolving in water

Phycobiliproteins also have fluorescent properties that make them useful as fluorescent dyes and markers. PE and APC are most commonly used, although other phycobiliproteins also fluoresce at different wavelengths, yielding a toolbox of colours for multicolour detection systems. They can be conjugated with molecules with specificity to certain substrates, e.g. antibodies, receptions, strepavidin and biotin, yielding fluorescent tags or probes that will bind to a specific protein, tissue or cell type. Fluorescent phycobiliproteins are used in fluorescent microscopy, flow cytometry, fluorescence-activated cell sorting, diagnostics, immunolabelling and immunohistochemistry (Eriksen, 2008; Sekar and Chandramohan, 2008). They have also been proposed for use as a light sensitive agent in cancer tumour photodynamic therapy (Niu et al 2007).

As with many pigments, phycobiliproteins also exhibit pharmaceutical activity. Studies have indicated them to have the following properties: antioxidant, anti-inflammatory, neuroprotective, hepatoprotective, immunomodulatory, antiviral and antitumour (Eriksen, 2008; Sekar and Chandramohan, 2008). Applications are expanding into the cosmetics, nutrition and pharmacy markets (Pulz, 2004).

Polyunsaturated fatty acids (PUFAs)

Polyunsaturated fatty acids include the omega-3 fatty acids Eicosapentanoic (EPA) acid and Docosahexanoic acid (DHA) as well as arachidonic acid (AA) and are well known for their nutritional importance. They confer flexibility and selective permeability properties to cellular membranes, which have been shown to be vital for brain development, are beneficial to the cardiovascular system and form important nutraceutical and pharmaceutical targets in both human and animal subjects (Yongmanitchai and Ward, 1989; Agostoni et al., 1995; Ward and Singh, 2005). Health benefits associated with these fatty acids include brain health and development, prevention of cardiovascular disease, stroke, asthma, as well as rheumatoid arthritis prevention (Agostoni *et al.*, 1995; Kris-Etherton *et al.*, 2003; Ward and Singh, 2005; Jude *et al.*, 2006). Ultimately the beneficial effects of omega-3 fatty acids result from both the competitive inhibition of compounds produced from omega-6 fatty acids, and the direct production of beneficial compounds (eicosanoids) from the omega-3 fatty acids (Simopoulos, 2002).

PUFA's are almost exclusively synthesized *de novo* by plants, microalgae and some bacteria. Animals can convert one form of PUFA to another through elongation and desaturation, but very few can synthesize these fatty acids (Brett and Müller-Navarra, 1997). Longer chained PUFAs, with 20 or more carbon atoms, occur commonly in the lipids of microalgae such as phytoflagellates and dinoflagellates, green and red microalgae and in diatoms (Radwan, 1991). PUFA's such as EPA and DHA are commonly found in deep-sea cold water microalgae. This is largely due to the fact that EPA and DHA increase the cell membrane fluidity, as they have very low melting points compared to other biolipids, in effect acting as membrane lipid anti-freeze for fish and algae living in cold environments (Brett and Müller-Navarra, 1997; Thompson Jr, 1996).

The advantages of using algal biotechnology to produce PUFA's include easier extraction and purification (lower operational costs) as well as an increase in product content, quality and safety. Fungi, especially of the order *Mucorales*, and bacteria of the genera *Shewanella*, *Alteromonas*, *Flexibacter* and *Vibrio* can accumulate relatively large amounts of EPA (Yongmanitchai and Ward, 1989), however, the ability of bacterial and fungal fermentations to compete economically with traditional sources of omega-3 fatty acids is limited by low productivities and excessively long fermentation times (Barclay et al., 1994). Additionally, long chain omega-3 fatty acid productivities reported for the microalgal fermentation systems are 1-2 orders of magnitude greater than productivities reported for fungal or bacterial systems (Table 4).

The current market price of EPA and DHA ethyl ester (95% pure) in bulk quantities is about $650/kg and any new source would need to compete with that price (Belarbi et al., 1999). This article goes on to state that for microalgal EPA to be competitive with fish oil derived material, the price of microalgal biomass (dry basis) must not exceed about $5/kg. The estimated market size for DHA is estimated at US$15 million in the USA (Bux, 2013).

Table 4. Polyunsaturated C20-fatty acid content of representative fungi, microalgae, macroalgae and mosses (Radwan, 1991)

	Percentage of Total Fatty acids[a]		
Species	20:3	20:4	20:5
Fungi (lower Phyomycetes)			
Allamyces javonicus		10.3 (0.3	
Blastocladiella emersonii	3.2 (0.2)	16.4 (1.1	
Synchytrium endobioticum		4.8	5.9
Achlya americana		9.9	7.1
Sparolegnia ferax	8.9 (0.6)	16.6 (1.2)	
Pythium debaryanum	11.9 (1.3)		
Phytophthora erythroseptica	12.1 (0.5)		
Microalgae			
Euglena gracilis		8.0	9.0
Peridinium trochoideum		1.0	13.0
Chlorella minutissima			45.0 (22.5)
Dunaliella tertiolecta		4.0	10.0
Porphyridium cruentum	2.0	36.0	17.0
Skeletonema costatum	2.0	2.0	30.0
Lauderia borealis		1.0	30.0
Navicula pelliculosa			26.0
Macroalgae (brown and red)			
Fucus platicarpus (brown)		11.0	8.0
F. serratus (brown)		10.0	8.0

F. versiculosus (brown)		10.0	8.0
Sargassum salicifolium (brown)		24.9 (4.3)	
S. boveanum (brown)		12.6 (0.6)	13.3 (0.7)
Layengaria stellata (brown)		6.9 (0.4)	10.0 (0.5)
Colpomenia sinousa (brown)		8.5 (0.7)	10.2 (0.8)
Plocamium coccinium (red)		12.0	22.0
Rhodomella subfusa (red)		14.0	24.0
Gelidium latifolium (red)		10.1 (0.9)	24.6 (2.1)
eolysiphonia coacta (red)		6.4 (0.5)	4.4 (0.4)
P. lanosa (red)		10.2 (0.1)	38.6 (0.4)
Chondrus crispus (red)		25.0	26.9
Mosses			
Pogonatum urnigerum	75.7 (3.0)	2.8 (0.1)	0.4
Ctenidium molluscum	10.0 (0.4)	22.9 (1.0)	5.3 (0.2)

[a] Values in parentheses are expressed in g/100 g biomass, and were calculated considering total lipid contents

PUFA production

Microalgal species such as *Skeletonema costatum* and *Chlorella minutissima* can accumulate up to 45% of their total fatty acid as EPA or DHA (Radwan, 1991). Triacylglycerides (TAG's) fall under the saturated storage or neutral lipid group. This is of interest to the biofuel industry, whereas PUFA's tend to consist of membrane structural 'polar' lipids (Berge et al., 1995). Average cell age also has a profound effect on lipid classes, producing changes in the amounts of TAGs and polar lipids at different cultivation stages. In general, the content of polar lipids (which generally make up PUFA's) tends to decrease with culture age (Wen & Chen, 2003). This does not however avoid the complication of various EPA and DHA levels in different environments and in different species of microalgae.

The lipid and fatty acid contents of microalgae also vary depending on culture conditions and strain. It has been found that, in some cases, lipid accumulation and composition can be enhanced by various growth conditions such as nitrogen starvation, silicon deficiency, phosphate limitations, high salinity and heavy metal stress (Guschina & Harwood, 1996). In one study, supplementation of media with 100ng of vitamin B12 per litre produced a 65% increase in yield of EPA from *Phaeodactylum tricornutum* compared with the control (Yongmanichai and Ward, 1990). Changes in the lipid composition, lipid production rate and growth of algae often occur as a result of variations in environmental or culture conditions. This is, in turn, complicated by different algal species responding uniquely to these environmental fluctuations.

Fertilizers and soil conditioners

Macroalgae have historically been used as soil fertilizer in coastal regions throughout the world. The carbohydrate polymers in macroalgae assist in particle adherence and water retention, as well as enhancing the mineral composition of the soil (Pulz, 2004). They can be applied in the form of seaweed meal, liquid fertilizer or concentrated plant supplements. The nitrogen and phosphates in the biomass act as fertiliser, and, perhaps most beneficially, the high concentration of plant growth hormones (e.g. auxins and cytokinins) in algal biomass can have a stimulating effect on plant growth (pers. comm. Kelpak, 2014). Extracts from macro and microalgae have been shown to promote germination, growth or flowering (Pulz, 2004). Liquid extracts from algae are used to aid plant establishment and growth, particularly in the remediation of mining areas. (Pulz, 2004). Polysaccharide producing species of *Chlamydomonas* have been used as soil-conditioning agents to prevent erosion (Metting, 1996).

Nitrogen (N) fixing microalgae are able to absorb and transform nitrogen from the atmosphere into a form accessible to higher plants (Pulz, 2004). Several species of N-fixing algae (e.g. *Anaebena*, *Nostoc*, *Aulosira*, *Tolypothrix* and *Scytonema*) are regularly used in rice cultivation in China, India and Asia. They can provide in excess of 20 kg N/Ha/yr, or up to a third of the requirements of traditional rice cultivars (Metting, 1996). Despite the demonstration of algae as useful plant growth and soil enhancers, there has been limited research effort in this area to date.

Fuels

Aside from foods and feeds, the other commodity industry where algae have received much focus is in the production of fuel. World energy demand continues to rise and the necessity to reduce fossil fuel use is becoming more urgent, hence there is currently much focus on the production of sustainable energy. Algae are a potential source of a variety of renewable fuels (Harun et al, 2010). Algal biomass could be combusted directly or converted via liquefaction, gasification or pyrolysis into 'green crude', syngas, heat, electricity and liquid fuels. Algae can also produce significant quantities of lipids and carbohydrates that could be converted into biodiesel or bioethanol respectively. There has also been some research into the use of algae for biological hydrogen production (Benemann, 2000). In addition, algal biomass could form the substrate for anaerobic digestion to produce methane (Chisti, 2013).

Despite considerable academic and commercial interest algal fuels are not widely-available currently on the market, largely due to the practical and economic challenges of producing a low-value, commodity product from algae on a very large scale. Over fifty algal biofuel companies have existed and as yet none are producing commercial-scale product at competitive prices (Milledge, 2011). Much of the initial work on production of fuel from algae was conducted by the Solar Energy Research Institute under the Aquatic Species Program (Sheehan et al., 1998). Although the technical feasibility of algal biofuels has been demonstrated (Miao and Wu, 2006; Mata et al., 2010), at present, the process appears to be uneconomic. This is largely due to the artificially low cost of energy derived from fossil fuels, which relies on a large historical storage reserve of energy, accumulated from biomass such as microalgae, over millennia, and hence does not take into account the cost of producing the fuel, nor that of CO_2 emissions. It is essential that the net energy balance of algal fuel production be positive (Walker, 2009). The reduction in process energy input will be vital here.

To date, commercially successful algal companies have focused on products with a high sales price. For commodity algal products such as fuels to be successful, the cost of algal production needs to be dramatically reduced from current costs. To produce a reasonable quantity of fuel, the scale of production would also need to be orders of magnitude greater than current production (Wijffels, 2007).

Bioactive compounds

The intricacy of microalgal chemical composition and range of biochemical products has led to the interest and potential use of these compounds in the food, feed, pharmaceutical and research industries (Pulz 2004). Bioactive compounds are defined by Bhatnagar (2010) as *secondary metabolites produced by certain microalgae displaying a degree of bioactivity either against another microorganism, or acting against certain physiological states of a diseased body* (Bhatnagar 2010). Algae produce a variety of secondary metabolites with anticancer, antifungal, antibiotic as well as anti-viral properties, as well as a variety of toxins.

Anticancer compounds

Anti-cancer bioactive compounds include polyunsaturated fatty acids (specifically EPA), carotenoids and lipopeptides. Polyunsaturated fatty acids have been used in cancer treatments as an adjuvant for chemotherapy, as compounds with direct anti-cancer effects, or as supplements to alleviate the effects of radiation and chemotherapy (Vaughan 2013). The anti-inflammatory nature of EPA is likely the source of the anti-cancer effects seen, as it reduces damage caused by oxidative stress (Vaughan 2013). Furthermore, the direct anti-cancer effects of these compounds have been suggested to work specifically against tumours through the inhibition of angiogenesis and metastasis (Baracos 2004). Carotenoids also act as antioxidants, reducing stress from oxidative damage. *Dunaliella salina* is a good natural source of β-carotene, which has been shown to reduce the risk of cancer and degenerative diseases in humans (Ben-Amotz, 1999).

In the cyanobacteria, nitrogen-containing compounds (lipopeptides) occur that target tubulin or actin filaments in eukaryotic cells, making them an attractive source of anticancer agents (Jordan and Wilson, 2004). An example of these anti-microtubule agents are curacin A and dolastatin 10, currently in pre-clinical and/or clinical trials as potential anticancer drugs (Gerwick et al., 2001). The majority of these biomolecules are found in *Nostocales*, and members belonging to the genera *Lyngbya, Oscillatoria,* and *Symploca* (Tan 2007).

Antifungal and antibiotic compounds

Bioactive compounds with antifungal and antibiotic activity are currently investigated for use as components in anti-fouling paints for maritime industries around the world (Bhadury 2004). Microalgal species tested for antibiotic activity included *Rhodophyta*, *Chlorophyta*, and *Phaeophyta* and exhibit inhibition against the gram-positive bacteria *Bacillus subtilus* and *Staphylococcus aureus* (Falch 1992).

Antiviral compounds

The antiviral bioactive compound cyanovirin-N (CV-N) was discovered as a constituent of the cultured cyanobacterium, *Nostoc ellipsosporum*, which irreversibly inactivates diverse primary strains of HIV-1 during sexual transmission of HIV. CV-N also blocks the cell-to-cell transmission of HIV infection (Burja 2001; Yang 1997). Another bioactive compound, shown to protect human lymphoblastoid T-cells from the cytopathic effect of HIV infection, was extracted from blue-green algae *Lyngbya lagerheimeii* and *Pormidium tenue* (Gustafson 1989).

Table 6: Consolidated group of bioactive compounds applicable to the pharmaceutical sector: bioactive compounds and microalgal producers

Bioactive Type	Bioactive compound	Microalgal producer	Reference
Anti-cancer			
Anti-microtubule	Dolastatin-10* Curacin-A* Dolastatin-15*	*Cyanobacteria*	Tan 2007 Gerwick 2001
Anti-tumor			Tan 2007 Mita 2006 Burja 2001
Anti-fungal	Toyocamycin Ciguatoxin Okadaic acid	*Cyanobacteria* *Gambierdiscus toxicus* *Prorocenrum lima*	Bhatnagar 2010
Anti-parasitic			
Anti-malaria	Calothrixin A	*Cyanobacteria*	Rickards 1999
Anti-protozoal	Viridamides A Crude extracts	*Cyanobacteria* Green-algae (*Cladophora, Codium* and *Ulva*)	Simmons 2008 Allmendinger 2010
Anti-viral			
	Cyanovirin-N	*Nostoc ellipsosporum*	Burja 2001 Yang 1997
	Extract	*Lyngbya lagerheimeii* *Phormidium tenue*	Gustafson 1989

*Bioactive compounds in various stages of clinical trials

Toxins

Marine cyanobacteria are also a source of potent neurotoxins that target the eukaryotic voltage gated sodium channel (VGSC) and may act as either blockers (lalkitoxin and jamaicamide A) or as activators (antillatoxin) of the eukaryotic VGSC. Hepatotoxin includes the cyclic peptides microcystin and nodularin. *Microcystis aeruginosa* and *Nodularia spumigena* synthesize these toxins that are able to destroy liver cells by the inhibition of protein phosphatases (Burja 2001). To date, over 50 different variants of microcystins have been isolated from the species *Anabaena*, *Hapalasiphon*, *Microcystis*, *Nostoc* and *Oscillatoria* (Singh 2005).

The potential contribution of microalgae to the discovery of new bioactive compounds is large. Natural products have been isolated from a wide variety of organisms and tested for various biological activities. Cyanobacteria are regarded as good candidates for drug discovery, with applications in the pharmaceuticals and cosmetic sector. Despite the vast diversity of microalgae and the plethora of bioactivity displayed, no drug of microalgal origin is on the market as of yet, although several compounds are currently in clinical trials (Table 6,*). With novel research investigating the effects of these (and new) compounds, more can be expected to enter clinical trials, which may eventually lead to the market entry of these bioactive compounds.

Cosmetics

In addition to the use of algal-derived hydrocolloids as thickening and water-retaining agents in cosmetics, macro- and micro-algal extracts and bioactive compounds such as antioxidants, pigments and essential fatty acids are incorporated into a variety of cosmetic products ranging from anti-aging creams and anti-UV products to hair care products and anti-irritant skin peels (Spolaore et al. 2006). Other cosmetics incorporating algae include microalgal whole cell shampoo and conditioner, oil and salt scrubs, clay masks and soaps (Oilgae.com). Current market products (Table 7) usually do not specify the exact formulation of each product, however, research investigating compounds and possible cosmeceutical effects suggest they are likely microsporines and microsporine-like amino acids, tocopherols, phenolic compounds and terpenoids that aid in skin protection against UV radiation that behave as antioxidants, emollients and diuretics (Kim 2008). *Chlorella* is most commonly used in cosmetics production but other microalgae currently investigated for their biochemical properties include *Parachlorella*, *Neochloris*, *Bracteacoccus*, *Scenedesmus, Anabaena, Ankistrodesmus, Chlorococcum, Schizochytrium, Spirulina, Crypthecodinium, Cryptomonas, Isochrysis, Rhodococcus* and *Nannochloropsis* (oilgae.com). The global cosmetics industry is worth more than $40 billion – and ever increasing. Prominent companies currently involved are Heliae, Solazyme, Indeed Laboratories, Biotherm and Algalscientific.

Table 7: Cosmetics incorporating algal extracts: products and microalgal producers (adapted from Spolaore et al. 2006 and Kim et al. 2008)

Cosmetic effect	Market product	Microalgal producer
Anti-aging	Protulines®	*Arthospira (Spirulina)*
Stimulates collagen synthesis	Dermochlorella®	*Chlorella vulgaris*
Skin tightening	Pepha®-Tight	*Nannochloropsis oculata*
Stimulates cell proliferation	Pepha®-Ctive Blue Retinol™	*Dunaliella salina*
Repairs UV damaged skin	Remergent™	*Anacystis nidulans*

Chemicals

The adaptation of classical fermenters to photosynthetic organisms and the use of heterotrophic algae have allowed the production of biomass of consistent quality under controlled conditions. This allows for the potential production of high quality, higher value pharmaceuticals and specialty biomolecules from algae (Apt and Behrens, 1999). Algae grow more slowly than other microorganisms such as bacteria and yeast, and reactor design is complicated by the requirement for efficient light distribution throughout the culture. Therefore, there must be a compelling reason to use algae for the production of biologics that could be produced in other microorganisms. Such reasons could include:

- reduced cost and improved sustainability through the use of light energy to power production
- a reduced risk of infection and contamination by production of animal proteins in plant cells.

Stable isotopically labeled compounds

Microalgae are ideally suited to the production of stable, isotopically labeled compounds. Cultivated under strictly controlled conditions, algae can be used to convert ^{13}C, ^{15}N and ^{2}H from simple inorganic compounds ($^{13}CO_2$, $^{15}NO_3$ and $^{2}H_2O$) into more complex labeled organic substrates (e.g. amino acids, sugars, lipids and nucleic acids). These labelled compounds have application as substrates for investigating the metabolism of other microorganisms, in elucidating the structure of proteins, and for noninvasive diagnostics. They can be used to investigate the metabolism of microorganisms, e.g. bacteria, yeast and mammalian cells, by tracing the labeled carbon through metabolic pathways and evaluating its incorporation into macromolecules e.g. lipids, carbohydrates and proteins. NMR technology, together with stable-isotope-editing techniques can also be used to elucidate the structure of proteins into which isotopes have been incorporated. Another application of microalgal-produced isotopes is breath tests for medical diagnosis. A substrate labeled with ^{13}C is ingested, and the rate at which it is absorbed, metabolized and expelled as $^{13}CO_2$ is measured. The rate and amount of appearance of $^{13}CO_2$ in the breath is indicative of the patient's physiological state (Apt and Behrens, 1999; Radmer, 1996). The market for labeled compounds is approx. $5 million, with prices in excess of $100/g (Radmer, 1996).

Biomanufacturing and specialty chemicals

Green microalgae have attracted interest recently as a potential host for the production of recombinant proteins. Algae can be used as cell factories to produce enzymes, vaccines, biologics and bioactive molecules such as antioxidants, toxins and molecules with pharmaceutical properties such as anti-cancer or anti-microbial activity. The advantages of using photosynthetic plant cells include safety, low cost of substrates and ease of genetic transformation (Rasala and Mayfield, 2014). Chlamydomonas reinhardtii is the most widely used species due to well established transformation methods and genetic tools, as well as a fully sequenced genome, but other species such as *Chlorella* and *Dunaliella* are also potential candidates (Rasala and Mayfield, 2014).

Microalgae have been investigated for the production of vitamins and vitamin precursors e.g. ascorbic acid, riboflavin and tocopherol, for food, cosmetics and aquaculture (Metting, 1996). Heterotrophic production of ascorbic acid (vitamin C) by *Chlorella* was demonstrated by Running et al. (1994). Microalgae have also been investigated as a source of polysaccharides, lipids and hydrocarbons for use in food, cosmetics, and as lubricants. For example, the green alga *Botryococcus braunii*, although a slow grower, produces and excretes a range of interesting long-chain hydrocarbons. Investigations of non-disruptive harvesting by 'milking' are currently underway (Moheimani et al. 2013).

Feedstock for industrial bioprocesses

Microalgae could potentially be used as a nutrient source for other microorganisms in the production of industrial chemicals. The photosynthetic production of algal biomass, particularly if produced for environmental reasons e.g. CO_2 sequestration, could provide a cheap and sustainable source of nutrients, e.g. carbohydrates, sugars, proteins, lipids, for fermentation by other microorganisms to produce valuable products (Harun et al 2010). For example, Nakas et al (1983) found the yield of solvents (propanediol, butanol and ethanol) from *Clostridium pasteurianum* was improved when fed on *Dunaliella* sp. A yield of 14-16 g.L^{-1} of mixed solvents was achieved on the microalgal substrate.

Environmental applications

CO_2 sequestration

Microalgal biomass contains about 50% carbon, usually obtained photosythetically from CO_2. This, coupled with their ability to grow on non-arable land, utilizing waste nutrients, have made them an attractive potential vehicle for carbon sequestration (Milledge, 2011). The global rise in CO_2 levels is an international problem demanding feasible solutions. The ultimate goal should be to drastically curb CO_2 emissions through decreasing dependency on fossil fuels, as well as investigating options for CO_2 sequestration. Microalgae, due to their high growth rates and the fact that they do not require arable land, appear to be a promising option for fixation of CO_2 photosynthetically from point source emissions (Harun et al, 2010). Several companies and research groups have investigated this process. It has been shown to be technically viable but not economically feasible and has yet to be implemented on a large scale (Pulz, 2004). Significant challenges include the large land areas required (and in close proximity to the power station), due to the low biomass concentrations achieved in the currently most economically feasible cultivation option: open ponds, and the large requirement for water and fertilizers such as nitrogen and phosphates. The area remains promising, as several valuable products could potentially be produced from the algal biomass, and one of the limitations to algal cultivation generally is CO_2 provision. Nutrients may need to be recycled, e.g. through anaerobic digestion of the residual biomass after product extraction, and hypersaline species sought which do not require a supply of fresh water (Sing et al., 2014).

Wastewater treatment and bioremediation

Algae are important components of biological treatment methods for municipal and industrial effluents. They contribute oxygen from photosynthesis e.g. in facultative ponds and high rate oxidation ponds, to aerobic microorganisms. These algal communities are commonly dominated by green microalgae (e.g. *Chlorella*, *Scenedesmus*, *Ankistrodesmus*) (Metting, 1996). Algae have been investigated for the final polishing of municipal wastewater, or the direct treatment of a variety of industrial wastewaters. Algae are very efficient at removing nutrients such as nitrogen and phosphates, as well as environmental contaminants e.g. heavy metals and toxins, from water, (Harun et al, 2010). However, microalgae have a much slower growth rate than bacteria, which limits the speed of their bioremediation. The biosorption and removal of metals such as chromium, cadmium, nickel and zinc is an important function in the bioremediation of industrial effluents such as mining or ash dam waters (Murphy et al 2008).

The use of immobilized algae, particularly as biofilms e.g. in an algal turf scrubber, appears to be a promising strategy for water treatment and bioremediation. The quality of large amounts of relatively dilute wastewater can be improved, while the biomass is retained. Depending on the nature of the wastewater, the resulting algal biomass could be used for animal feed, or anaerobically digested to produce methane (Spolaore et al, 2006). Cyanobacteria were reported to be effective in the treatment of wastewater from pulp and paper processing. Treatment of wastewater containing phenols with bacteria requires addition of an organic carbon source, whereas algal cells can efficiently remove phenols from wastewater using CO_2 as the carbon source. *Scenedesmus* has also been investigated for degrading cyanide from mining process waters (Harun et al 2010).

Conclusions

Algae are responsible for over 50% of the photosynthesis on earth. They are the primary producers in the oceans and are efficient and adaptable sunlight factories, producing a range of useful products. It has been estimated that at least 200 000 algal species exist worldwide, with roughly 36 000 algal species known. The enormous diversity of algal species remains commercially underutilized. Current commercial production relies on only 10 to 20 species, most of them macroalgae (Radmer, 1996; Pulz, 2004; Milledge, 2011). While humans have taken advantage, to a limited extent, of naturally harvested algae, such as seaweeds and *Spirulina,* as a food source for centuries, it is only relatively recently that microalgal biotechnology has offered the potential to produce a wide array of products from industrial chemicals to pharmaceuticals (Olaizola, 2003). Dozens of useful products from microalgae have been identified, but there have been few successes in scale-up and marketing (Olaizola, 2003). Major commercial products are limited to the food and feed industries, despite the wide range of potentially useful metabolites produced, beyond the current exploitation (Milledge, 2011).

General limitations to the commercialization of algal products include their low growth rates compared to other microorganisms such as bacteria or yeast, and low maximum biomass densities in outdoor algal culture (Apt and Behrens, 1999). As a result, large culture volumes are required per unit product, which implies large capital and running costs, particularly in terms of pumping, mixing and harvesting, as well as large volumes of fresh water. Much research is being directed towards enhancing the biology of the algae as well as engineering cheaper and more efficient photobioreactors. At present, however, a financially viable algal production process requires either an easily harvestable species (e.g. filamentous *Spirulina*), a high value product that can cover the relatively high cost of production (e.g. pigments, PUFAs and other fine chemicals), a suite of products derived from a unit of biomass which collectively cover the costs (the biorefinery concept), or very low costs of production, such as in natural lagoons (e.g. β-carotene production in Hutt lagoon, Australia). Another challenge to commercialization of algae as foods, food additives or in the production of pharmaceuticals is the regulatory environment, and customer acceptance. Successful authorization of a new species, product or application can take several years (Pulz, 2004).

None of the challenges associated with large-scale production of algal products appear insurmountable, however, a concerted, coordinated effort across disciplines is required for commercial success. Algae have great promise as the next generation of biological factories, with the potential to produce a wide range of valuable compounds for food, feed, fuel, pharmaceutical and research use (Milledge, 2011; Wijffels, 2007; Radmer, 1996; Pulz, 2004; Spolaore, 2006). With the ever-increasing world population, the requirement for agricultural land is becoming a global challenge. In addition to photosynthetic production, algae have the advantage of not requiring arable land, which could help to displace some non-food related production processes to arid and semi-arid regions. The industry focus should be on those products with a large potential market, where production using microalgae leads to clear competitive advantages (Milledge, 2011). It is also imperative that the research focus around algae perseveres to address the technical hurdles that currently prevent a broader commercial exploitation of this valuable natural resource.

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