1

Microalgae: A Renewable Resource for Food and Fuels and More

Susan I. Blackburn[1] *and Kim Jye Lee-Chang*[2]

[1] *Commonwealth Scientific and Industrial Research Organisation (CSIRO) National Collections and Marine Infrastructure, Australian National Algae Culture Collection, Castray Esplanade, GPO Box 1538, Hobart, TAS 7001, Australia*
[2] *CSIRO Oceans and Atmosphere, Algal Ecology and Resources, Castray Esplanade, GPO Box 1538, Hobart, TAS 7001, Australia*

Abstract

Microalgae are a large and diverse group of microscopic aquatic plants that are responsible for over half of the global primary productivity. They constitute a major food source for organisms at the base of the marine and other aquatic food webs and are important components of many ecosystems. Cultivation of microalgae offers a renewable resource for foods, fuels, aquaculture feeds, and other bioproducts and bioapplications. Bioproducts include a feedstock for biofuels and high-value lipids such as long-chain omega-3 oils, carotenoid pigments, and squalene, as well as other non-lipid materials, including exopolysaccharides. Successful commercial cultivation is dependent on the knowledge of the microalgal strain biology, matching the growth conditions according to the particular geographic sites and culturing and processing methods for the bioproducts of interest. Marine microalgae, as well as those from brackish and hypersaline environments, are grown in seawater at various salinities. Both autotrophic and heterotrophic cultivations are important, offering options in strain biology, chemistry, and production methods. Detailed fatty acid profiles demonstrate characterization of the microalgae into different chemotaxonomic groups, giving useful tools for identifying candidates with favorable bioproduct qualities for further optimization, for example, fast growth, high oil content, and suitable lipid composition. This chapter provides a perspective on the cultivation and qualities of microalgae for their renewable bioproduct and bioapplication potential, which offers great future potential in renewable marine resources.

Blue Biotechnology: Production and Use of Marine Molecules,
First Edition. Edited by Stéphane La Barre and Stephen S. Bates.

1.1 Introduction

Microalgae are single-celled microscopic plants found in marine and other aquatic environments globally. They have a very high biodiversity, including ancient prokaryotes such as the blue-green microalgae (cyanobacteria) and the more complex eukaryotes with a diverse phylogeny, including green, brown, and red microalgae [1]. The majority of, but not all, microalgae are phytoplankton – floating plants.

Microalgae have been estimated to include ~300 000 different species, with some published estimates of diatom numbers of over 200 000 species [2]. Microalgae constitute a major food source for organisms at the base of the aquatic food web and are responsible for over half of the global primary productivity, converting the sun's energy to organic compounds and oxygen and using carbon dioxide as part of the process, thus underpinning the health of the planet [3]. Microalgae biomass contains lipids (oils), carbohydrates, proteins, and other biochemical constituents such as antioxidants, polysaccharides, and vitamins, and thus they are a recognized source of current and potential bioproducts.

Microalgae, along with other ancient sedimentary organic matter, formed petroleum through processes involving underground heat and pressure over geological time scales [4]. Over human history, microalgae have been sourced as wild foods and, in the past decades, cultivated as a source of food supplements in human nutrition, and developed for live aquaculture feeds for a rapidly developing aquaculture industry. However, microalgae remain a relatively untapped source for a range of bioproducts and bioapplications. With recent improved cultivation and harvesting technologies and better understanding of biosynthesis pathways, combined with metabolic engineering, microalgae have the potential to be a sustainable renewable resource of considerable economic potential and value to humanity.

1.2 Sourcing Microalgae: Algal Culture Collections

Of the vast diversity of species of naturally occurring microalgae, only several thousands have been isolated from nature and cultured. Many of these cultured microalgal strains are maintained in algal culture collections in various parts of the world. Information on the major algal culture collections is available from the World Federation for Culture Collections (http://www.wfcc.info/collections/). Some of the major culture collections are listed in Table 1.1. The physiological, biochemical, and genetic characterization of cultured microalgae, including type species held long term in algal culture collections, highlights the value of collections as repositories of global microalgal biodiversity. Many of the culture collections make their strains available to researchers, educational institutions, and industry, often for a modest charge, thus providing a reliable base for microalgal applications, including bioproducts. Recognizing the "treasure chest" of potential oils and coproducts in microalgae, the Culture Collection of Algae and Protozoa (CCAP) screened 175 strains from their collection, contributing

Table 1.1 A selection major algal culture collections.

Algal culture collection	Website
Australian National Algae Culture Collection (ANACC)	www.csiro.au/ANACC
Culture Collection of Algae and Protozoa (CCAP)	www.ccap.ac.uk
Culture Collection of Algae at Goettingen (SAG)	www.epsag.uni-goettingen.de
Microbial Culture Collection at the National Institute of Environmental Studies (NIES)	mcc.nies.go.jp
National Center for Marine Algae and Microbiota (NCMA)	ncma.bigelow.org
The Culture Collection of Algae at the University of Texas at Austin (UTEX CCA)	utex.org

significantly to the characterization of microalgae for bioapplications [5]. However, not all microalgae can be used universally. There may be issues of ownership with agreements needed for access to samples and fair and equitable sharing of benefits arising from the use of microalga from a particular location, and some countries may impose strict import restrictions on microalgae from outside sources. Thus, the isolation, identification, and maintenance of native microalgae are an essential consideration in the development of microalgal industries.

Culture collections usually maintain microalgal strains in liquid or solid agar in Petri dishes or in nutrient media contained in small-scale culture vessels such as glass tubes and flasks or sterile disposable plastic tissue culture flasks. For autotrophic (photosynthetic) microalgae, cultures are usually maintained in constant environment rooms or cabinets with artificial lighting, usually with a photoperiod (light–dark cycle), simulating night and day (Figure 1.1a). Jeffrey and LeRoi recommended the use of a photoperiod for algal culture, although some algal production systems successfully use continuous light [6]. The choice of growth medium is very species specific and will depend on whether the microalga comes from a fresh, brackish, or seawater environment. Media contain macronutrients such as nitrogen and phosphorus, as well as micronutrients such as iron, zinc, cobalt, cadmium, and molybdenum, and vitamins such as B_1, B_{12}, and H (biotin). Media for diatom growth must contain silicon. Some media, such as f medium [7], have been used reliably for over 40 years to culture diverse microalgae. Several media have been developed that contain low levels of selenium (as selenite), as this is essential for some oceanic species (e.g., K medium [8]) or assists with long-term maintenance of fastidious microalgae such as dinoflagellates (e.g., GSe medium [9]). Details of methods for growing microalgae, including information on the different media available, are found in [10], as well as on the websites of some algal culture collections (Table 1.1).

There is an increasing move to cryopreserve strains in liquid nitrogen, either as an alternative to culturing in "fresh" media or as the sole form of preservation

Figure 1.1 (a) Microalgal cultures maintained in constant environment room. (Australian National Algae Culture Collection (ANACC).). (b) Raceway pond for *Dunaliella bardawil* production. Nature Beta Technologies Ltd., Eilat, Israel. (Courtesy of Dr Ami Ben-Amotz.). (c) Microalgal cultures grown in disposable plastic bags supported vertically in metal frames. Shellfish Culture Ltd., Tasmania. (Courtesy of Shellfish Culture Ltd.). (d) 60 L vertical annular column photobioreactors. Algal Culture Laboratory, CSIRO, Hobart, Australia. (Courtesy of Dion Frampton.). (e) 100 L stainless steel bioreactor. (CSIRO Fermentation Facility, Melbourne, Australia.)

for the few classes of microalgae that have proven robust enough for long-term preservation by this method [11]. Methods may need to be varied for different microalgae. Cryopreservation is a reliable form of maintenance for chlorophytes and cyanobacteria [12], but further testing of genetic stability is needed for other classes [13]. Some groups such as the diatoms where fresh cultures can gradually lose viability as cell size reduces with each vegetative cell division have eluded success [14] Preserving the genetic stability by cryopreservation is considered a key advantage to avoid potential mutations and genetic drift in "fresh" cultures [14].

While culture collections are a very valuable resource of microalgal biodiversity, there is much untapped natural biodiversity that offers biodiscovery for microalgae. For example, algal bloom species can be targeted for their good growth potential or robust genetics. Likewise, microalgae from extreme environments (e.g., hypersaline) offer opportunities for new strains and new bioproducts.

1.3 Microalgal Production Systems

Different algal cultivation techniques, from simple to complicated engineering, have been used to grow microalgae for bioproducts and other industrial applications in large-scale production systems. As well as considering growth technologies, the effectiveness of different growth systems depends on the growth characteristics of particular microalgal species, or even a particular strain; thus, cultivation conditions (temperature, light, salinity) must also be considered. Matching microalgal strain biology with growth technologies is crucial. For example, the open pond cultivation using hypersaline microalgae strains can reduce maintenance requirements, thereby preventing growth of other weed algae or grazers on the culture's stability. In contrast to the optimum use of fermentation technology needed for heterotrophic algae, low salinity (low chloride) media are required due to the corrosive effects on conventional fermenters during high temperature sterilization and constant exposure to high chloride levels during fermentation [15].

Most microalgae are phototrophic (autotrophic), using the sun's energy to carry out photosynthesis for growth. This process captures its chemical energy in biological molecules using carbon dioxide (CO_2), producing oxygen (O_2). Artificial illumination can be used to supplement, or instead of, solar energy. Alternatively, some microalgae are heterotrophic, that is, they use organic carbon (e.g., glucose, acetate, lactate, or glutamate) as an energy source. They can thus be grown in stainless steel fermenters using well-established fermenter technology with no light requirement. Some microalgae are capable of mixotrophic growth, which is a combination of both photoautotrophic and heterotrophic metabolism, either sequentially or simultaneously, depending on the light and nutritional status of the environment they inhabit [16, 17]. Unusually, the green alga *Chlorella vulgaris* can grow under heterotrophic, autotrophic, and mixotrophic conditions [18].

Microalgae typically divide vegetatively by binary fission, one cell becoming two cells. In batch cultivation, given sufficient nutrients and other conditions, the microalga cell number and biomass increase exponentially during the logarithmic growth phase, until one or more factors become limiting. Cells then enter the stationary phase, although a modest arithmetic growth can still occur. Finally, this leads to the death phase, with either limited growth factors or potential autoinhibitory substances [19]. Richmond and Hu detail the biological principles for culturing phototrophic microalgae [20], and those for growth in fermenters are described by [15].

1.3.1 Open Pond Cultivation

Open pond algal cultivations systems include naturally occurring ponds as well as low-technology constructions that are low cost and relatively easy to build and operate. Over the past decades, different types of ponds varying in size, shape, construction material, method of mixing, and inclination have been designed. Overall, other than natural ponds or lakes, there are three major types constructed: (i) inclined systems, where mixing is achieved through pumping and gravity flow; (ii) circular ponds, with agitation provided by a rotating arm;

and (iii) raceway ponds constructed as an endless loop, in which the culture is circulated by paddle wheels [21]. For commercial algal production, natural ponds and lakes, circular ponds with agitation, and raceways are successfully used. An example of long-term successful commercial production in open ponds is that of the green alga *Dunaliella salina* in Australia in artificial hypersaline "lakes" by Baden Aniline and Soda Factory (BASF), previously owned by Cognis Australia, the largest global producer of β-carotene. Inclined systems have not received much attention, although they have promise due to turbulent flow, very thin culture layers, high cell densities, and a higher surface area-to-volume ratio compared with other open systems [20, 21]. Although circular ponds require high energy input for mixing, they are widely used in Asia (Japan, Korea, and Indonesia) for mass production of *Chlorella* [22].

Raceway ponds are popular algal pond systems and are the preferred production system for many of the commercial operations, including Nature Beta Technologies Ltd., Eilat, Israel, a subsidiary of Nikken Sohonsha Co., Japan, that is cultivating and producing *Dunaliella bardawil* since 1985 using know-how and technology of the Weizmann Institute of Science (University in Rehovot, Israel) (Figure 1.1b). Excessive evaporation is a problem in these regions and a minimum depth of at least 15 cm is required in order to avoid a reduction in flow that can result in light limitation [23]. Raceway ponds are also termed high rate algal ponds (HRAP) that provide energy efficient and effective tertiary-level wastewater treatment and could produce sufficient algal biomass to be economically used as a feedstock for biofuels and other by-products, such as fertilizer [24]. See Section 1.10 for more details.

1.3.2 Plastic Bags and Tanks

With the development of the aquaculture industry and the need by hatcheries to rear larval and juvenile aquaculture animals came the need to produce microalgae as live feeds. While some hatcheries grow microalgae in multiples of relatively small-scale culture containers (e.g., 20 L carboys), it is more common for microalgal cultures to be grown in disposable plastic bags (200–1000 L is typical) that are either supported vertically in metal frames or lie horizontally (Figure 1.1c) usually using filtered seawater from the hatchery location with added nutrients.

1.3.3 Photobioreactors

Photobioreactors are bioreactors where phototrophic microalgae and other microorganisms and plant cells are grown under conditions that aim to ensure that algal growth can proceed without light limitation. They are usually considered to be closed systems, that is, in contrast to the low-technology systems considered earlier, they do not allow the direct exchange of gases such as CO_2 and O_2 or contaminants (other microorganisms, atmospheric particles, etc.) between the culture and the atmosphere [23]. The status of photobioreactor technologies has been reviewed previously (e.g., [21, 23]). While more expensive to operate than low-technology systems such as ponds and raceways, photobioreactors ensure high productivities along with quality control and low contamination rates.

Advantages of these systems and technical issues are discussed by [25]. It is easier to harvest the dense cultures obtainable using photobioreactors than is the case for dilute open pond cultures. Indeed, it is feasible to grow a dense culture that is already a slurry and therefore requires little effort to harvest. Closed systems allow efficient use of CO_2 and other gases such as flue gases for microalgal production. Moreover, photobioreactors can use both natural sunlight and artificial illumination. Many different designs have been developed (Figure 1.1d), but most are not amenable to scale up to the levels required for commercial production of microalgae. Considerable research undertaken by the University of Florence on photobioreactor production of the marine eustigmatophyte *Nannochloropsis* has resulted in commercial supply to Italian aquaculture industry [21].

Geographic location is an important consideration for deciding whether microalgae culture will be efficient and effective. A temperature of 15 °C or above is considered essential for sustained algal production. Outdoor productivities are affected by environmental variables such as low seasonal and nighttime temperatures, as well as variable irradiance [26]. Zittelli *et al*. demonstrated that a combination of natural and artificial illumination gave optimum productivities of photobioreactor-grown *Nannochloropsis* between December and May in the continental Mediterranean climate of Italy [27]. It is also important to select the proper strain within the genus *Nannochloropsis* to match with technology and environmental criteria [28].

1.3.4 Hybrid or Combination Growth Systems

The choice of growth systems is not necessarily restricted to a single technology. Various multiphase cultivation strategies have been considered. Richmond suggested combining a tubular reactor connected to an open raceway in order to maximize biomass production by optimizing environmental variables [29]. With this system, the raceway cultures were used during the hottest part of the day, while photobioreactors were used for high productivities when the environmental stress was lower. Multiphase cultivation strategies can be devised that ensure maximum production of biomass in one stage and maximum induction and accumulation of desired products in the other. This concept was successfully applied to outdoor cultures of *Dunaliella* for β-carotene production, as well as to photobioreactor cultures of *Haematococcus* for astaxanthin production [30].

Cost of production will obviously vary with the combination of strain growth characteristics and the technology used and whether algal growth is indoors using artificial illumination or outdoors using solar radiation, or a combination of both. Zhang *et al*. estimated the production costs for growing *Nannochloropsis* sp. in a flat-plate glass reactor [31]. They determined that a 2000 L reactor was sufficient for the industrial-scale production of microalgae fed to rotifers, which were in turn fed to 8 million seabream (*Sparus aurata*) fingerlings annually.

1.3.5 Fermentation Systems

There has been extensive research and development (R&D) on fermentation technologies for growing microorganisms such as bacteria, yeast, and fungi for

industrial and medical applications. Industrial-scale fermentation technology is therefore much more mature and established compared with phototrophic production systems (Figure 1.1e). However, the number of microalgae produced heterotrophically remains very small. In general, technological developments have not focused on the particular requirements of microalgae, and only a handful of microalgae have been shown to grow effectively under heterotrophic conditions. These include species that can produce bioproducts of interest, for example, species of the chlorophytes *Chlorella*, *Dunaliella*, and *Haematococcus*, and *Botryococcus braunii* [32]. Furthermore, some obligate heterotrophs, such as the thraustochytrids *Aurantiochytrium* spp. and *Schizochytrium* spp., the dinoflagellate *Crypthecodinium* sp., the diatoms *Nitzschia alba* and *N. laevis*, and the green alga *Prototheca zopfii*, are fast growing, with a specific growth rate $>0.09\,h^{-1}$ (doubling times of 7–15 h) [17].

Thraustochytrids are heterotrophic protists found ubiquitously in the marine environment and play an important role in the marine ecosystem [33]. Thraustochytrids are classified into the class Labyrinthulomycota and phylum Heterokonta, which includes the chromophyte algae such as brown algae and diatoms, within the kingdom Chromista based on molecular phylogenetic studies [34, 35]. Variously considered fungi and microalgae, their bioresource potential is great with their capacity to produce high amounts (>60% total fatty acids (FAs)) of high-value omega-3 long-chain (≥C20) polyunsaturated fatty acids (omega-3 LC-PUFA) (also termed LC omega-3 oils), including docosahexaenoic acid (DHA) (22:6ω3) and eicosapentaenoic acid (EPA) (20:5ω3) [36].

The fast growth rates and high lipid capabilities of heterotrophic microalgae have been exploited to produce bioproducts and biofuels at commercial scales. The disadvantage is that this requires a large feedstock of carbon compared with photoautotrophic production. The heterotrophic cultivation of *Chlorella protothecoides* can result in a higher lipid content (55%) than autotrophic cultivation under similar conditions [37]. Harel and Place considered that heterotrophic microalgal production has high potential for aquaculture feeds and examined the industrial potential, including production issues [38]. Another commercial microalgae producer uses the heterotrophic process to develop Algamune™, made from the unusual green alga *Euglena gracilis* that is rich in beta-glucans, naturally occurring polysaccharides that can be used as an alternative to antibiotics in animal feed [39].

To alleviate the drawback of needing organic carbon as an energy source, there is interest in the heterotrophic cultivation of microalgae using waste carbon sources to produce bioproducts. For example, carbon sources derived from agro-industrial wastes (e.g., waste molasses, empty palm fruit bunches, spent yeast from brewery, and coconut water) have been explored to produce high-value omega-3 oils containing biomass from thraustochytrids [18, 40–42]. Yan *et al*. demonstrated that the heterotrophic cultivation of *C. protothecoides* using waste molasses has the potential to provide significantly high biomass yields, for example, up to $70.9\,g\,L^{-1}$, with 57.6% oil content after 178 h of cultivation [43].

1.4 Uses of Microalgal Bioproducts

1.4.1 Food

Microalgae cultivation for human health and as a food supplement by indigenous populations has a long history. While mostly freshwater species such as the edible blue-green algae (cyanobacteria; see Chapter 21), including *Nostoc*, *Arthrospira* (previously in the genus *Spirulina*), and *Aphanizomenon*, they demonstrate the use of microalgae food for thousands of years [44]. The first commercial large-scale microalgal cultivation started in the early 1960s, in Japan, with cultures of *Chlorella* by Nihon Chlorella Co., Ltd [45]. As a dry product, *Chlorella* is composed of ~45% protein, 20% fat, 20% carbohydrate, 5% fiber, and 10% minerals and vitamins [46]. In the 1960s, health foods became available in the form of tablets, granules, and drinks, as well as food additives in Japan [47]. However, *Chlorella* has proved difficult to grow on a large scale, and digestibility problems due to the tough cell wall of these species have also arisen in nutritional studies. An interesting sideline to this story is the use of so-called Japanese Chlorella as a health food and for aquaculture. While superficially similar to genuine *Chlorella*, detailed studies and biochemical analyses showed that this was actually the marine eustigmatophyte of the genus *Nannochloropsis*, which is notable for having a high content of the omega-3 LC-PUFA EPA [48].

In the early 1970s, a harvesting and culturing facility for *Arthrospira* (*Spirulina*) was established in Mexico by Sosa Texcoco, S.A. [49]. *Arthrospira* is used in human nutrition because of its high protein content and excellent nutritive value. Several possible health-promoting effects have also been claimed, including the alleviation of hyperlipidemia, suppression of hypertension, protection against renal failure, growth promotion of intestinal *Lactobacillus*, and suppression of elevated serum glucose levels (see [49] for an overview). *Arthrospira* (*Spirulina*) production for nutraceuticals is a commercial success story [50]. Companies such as Cyanotech Corporation and Earthrise in the United States have established a market for nutraceuticals from *Spirulina* that is sold as a "superfood." When grown using naturally occurring deep-sea nutrients, these products can be accredited with organic status. However, most *Arthrospira* production occurs in China and India. The cyanobacterium *Aphanizomenon flos-aquae* contains ~60% protein by dry weight and is known to contain an array of digestible nutrients, including essential FAs such as linoleic acid (18:2ω6) and linolenic acid (18:3ω3). *A. flos-aquae* has been harvested commercially in Oregon, United States, from naturally occurring algal blooms as a source of nutraceuticals [51]. However, such harvesting of natural blooms must necessarily take account of potential toxins (cyanotoxins and saxitoxins) that some cyanobacteria can produce with careful testing and regulatory controls [51].

1.4.2 Feeds

With phytoplankton a major basis of the marine food web, microalgae are key dietary components for marine animals. Development of the aquaculture industry for molluscan shellfish, fish, shrimps, and other aquatic animals has been dependent on developing microalgae as live feeds, and there is increasing

interest in microalgal concentrates and feed components. The successful rearing of larval and juvenile life stages depends on the right mix of microalgal feed species, with the natural assemblage of phytoplankton in seawater being insufficient to support optimum growth of reared animals. As early as the 1950s, there were investigations into the relative food value of different microalgal species for molluscan aquaculture [52]. However, detailed biochemical profiling of microalgal feed species was not undertaken until much later, with analyses of growth, lipids, amino acids, sugars, and vitamin compositions providing analytical data for the basis of "good" feed species or combinations thereof [53]. Since the mid-1980s in Australasia, the Australian National Algae Supply Service (as part of the Australian National Algae Culture Collection (ANACC)) has supplied quality-controlled microalgal "starter cultures," demonstrating the value of culture collections in underpinning the aquaculture industry. Hatchery production of live microalgal feeds can be a significant cost component for the industry. Therefore, there is ongoing interest in preserved microalgae [54] or use of microalgal biomass or components thereof as feed additives (e.g., [55, 56]). There is still much development potential in this area.

1.4.3 Biofuels

Microalgae are a potentially good renewable source of biofuels, averting the fuel versus food use of agricultural land that is a feature of biofuel production from land plants. They have a high productivity and lipid content and can be cultivated in saline or brackish water on nonarable land that is not favorable for terrestrial plant growth or other applications. Microalgae are a potential source of biodiesel, aviation fuel, biogas, and alcohols, and the entire biomass can be converted into "green crude" or "biocrude." From 1978 to 1996, the US Department of Energy funded the Aquatic Species Program to develop biodiesel from algae. This focused on the production of biodiesel from high lipid content microalgae grown in ponds using waste CO_2 from coal-fired power plants [57]. However, the program was discontinued because of federal budget cutbacks and low oil prices, making production of algae for biofuels an uneconomic endeavor. The estimated cost of algal oil production was in the range 40–60 USD per barrel compared with 20 USD per barrel for crude oil in 1995 [58]. Sun *et al*. demonstrated that algal oil production costs 10.87–13.32 USD per gallon to produce, that is, 460–560 USD per barrel, while the current crude oil price of ~41 USD per barrel gives a 10-fold economic gap between production cost and the cost needed to be competitive with crude oil [59]. Despite this, since the mid-1990s there has been considerable interest in the potential of microalgae as a source of biofuel. However up until the present, various studies have again not yet achieved any major advances in productivity and therefore in cost [60–64].

Interest in and demand for a renewable source of transportation fuels from microalgae is also driven by other environmental and social factors, for example, government incentives and the opportunity to reduce greenhouse gas (GHG) emissions, as well as the rise of new regional business enterprises. Of relevance for photosynthetic microalgae is the capacity to exploit waste CO_2 resources, for example, exhaust fumes from coal power plants, and to fix CO_2 from the

atmosphere. The energy efficiency of microalgae has prompted interest in their use for GHG reductions by capturing CO_2 and power plant flue gases [65]. It is estimated that replacing just 10% of Australia's mineral diesel with microalgae-derived biodiesel would bring about a reduction of nearly 4 million tonnes of CO_2 emissions from fossil fuels [66]. This can be in conjunction with wastewater treatments or coproduction of high-value coproducts [26]. In order for microalgae to economically supply future demand of biofuel, all of the biomass produced will need to be used for potential bioproducts such as omega-3 oils, proteins, carbohydrates, carotenoid pigments, industrial enzymes, and exopolysaccharides (EPS). It is envisioned that a sustainable future for algal biofuels involves the coproduction of high-value bioproducts, in addition to biofuels, in a biorefinery approach where a number of by-products from the microalgal biomass become saleable commodities to offset the cost of production [64].

A potential alternative approach is the conversion of whole-cell microalgal biomass from hydrothermal liquefaction into hydrocarbon feedstocks for biocrude [67]. Laboratory-scale studies demonstrated the promise of the technology, but the commercial success of large-scale production has been elusive due to the significant economic gap between the cost of production and the commodity price of petroleum-based fuels.

Microalgal strain selection and optimization of growth, harvesting, and oil production using existing technologies remain key issues [68]. Life-cycle assessment is a useful tool to understand environmental implications such as GHG emissions and energy balance of microalgae-derived biofuel production [69]. The analysis can also identify improvements in cultivation conditions, in particular the cultivation system energy and nutrient inputs and microalgae yield, which are critical for developing a sustainable production system [70].

1.4.4 Neutral Lipids: Hydrocarbons

Some microalgae produce high quantities of hydrocarbons that have biofuel potential. Studies have demonstrated that the green alga *Botryococcus braunii* can accumulate and secrete a high level of lipid that is mostly hydrocarbon (>76% of the algal dry weight) growing in its natural environment [71, 72]. However, very slow growth has hampered efforts to industrialize *Botryococcus*. Nonetheless, it remains a goal in the development algal biofuels [73, 74].

1.5 Chemotaxonomy: Setting the Stage for Selecting Biofuel Microalgae by Taxonomic Group

FA composition (see Chapter 6) is an important consideration for assessing the suitability of microalgae-derived oil for biofuels. Other chemotaxonomic characterizations, for example, sterols, hydrocarbon, alkyl diols, and other parameters (dry weight of algae per volume of medium, cetane number, and yield), are useful tools for identifying algal groups and strains that are potential candidates for biodiesel production. While there may be interspecies and even

interstrain variation at the higher taxonomic groups, there are characteristics that are representative of that group. FAs with fewer double bonds (indicating the degree of unsaturation) and shorter chain length (number of carbons $<C_{20}$) are more desirable for biodiesel production due to their oxidative and thermal stability. Oxidation results in the formation of undesirable products, for example, alcohols that reduce the flash point of biodiesel, aldehydes that cause rancidity, and short-chain FAs that are corrosive to engine components [75, 76]. Furthermore, saturated FAs typically have higher solidification temperatures and therefore cannot be used at lower temperatures.

Having a high level of long-chain and highly unsaturated PUFAs (with four or more double bonds in some microalgal oils) is a negative feature for a biodiesel candidate and limits the number of microalgal species that can be used [28]. This can be overcome if the PUFAs are subsequently removed by various separation technologies in a biorefinery approach, generating a high-value by-product of biodiesel production. The cetane number can be used to measure "biodiesel quality potential" of an algal culture's potential to produce relatively high levels of FAs under the culture conditions employed. Cetane number is inversely proportional to the FA methyl ester (FAME) chain length and to number of double bonds and increases with FA carbon chain length and increasing saturation (fewer double bonds) [77, 78]. The current standard for diesel sold in Australia requires a minimum cetane number of 46, while European diesel has a minimum cetane number of 51. Cetane number affects combustion irregularity, as it relates to the ignition delay time of a fuel upon injection into the combustion chamber. An adequate cetane number for a specified engine ensures improved ignition, fuel combustion, reduced noise, and white smoke that contains carbon monoxide (CO), nitrogen oxide (NO_x), and hydrocarbon (HC) emissions [79].

The composition and characteristics of different classes of microalgae from laboratory screening are given in Table 1.2, data that informs selection of candidate microalgae for different oil applications. This screening of diverse microalgae maintained by the ANACC was done from 60 mL flask cultures grown for 2 weeks. While at small scale of test cultures, chemotaxonomy can be used as a tool for the rapid detection of strains producing favorable quantities and qualities of oil. Combined with manipulation of culture conditions and with suitable culture management practices, oil content and composition can be further manipulated.

1.6 Manipulating Microalgal Lipid Composition with Culture Growth Phase and Conditions

Selecting microalgae for lipids is not limited to lipid quality and quantity, as other parameters are also important. By manipulating the culture conditions, for example, temperature, irradiance, and nutrient availability, microalgal oil content and composition can be optimized for productivity and for particular applications. In general, lipid accumulation in photosynthetic microalgal cells is triggered by nutrient limitation. However, PUFA synthesis takes place in cells

Table 1.2 Summary of features based on fatty acid composition of the chemotaxonomic microalgal groups, using screening data compiled from >40 species held in the Australian National Algae Culture Collection.

Taxonomic group (Class)	Chemotaxonomic group	Chemotaxonomic features with respect to PUFA[a)]	Biomass productivity (dw g m^3 d^{-1})	Total FAME (g 100 g^{-1} dw)	Cetane number
Cyanophyceae	Cyanobacteria	Lack C_{20} or C_{22} PUFA; favorable fatty acid composition for biodiesel but typically low lipid levels	3.3–38.1	1.0–5.4	46–60
Chlorophyceae	Green algae with only ω-type desaturases	Lack 18:3ω6, 18:4ω3, or longer; good biodiesel candidates	4.1–23.2	3.7–10.1	40–44
	Green algae with Δ6	Have 16:3ω6, 16:4ω3, 18:4ω3, but not longer nor more unsaturated	5.8–31.8	3.3–13.9	34–49
	Green algae with Δ 6 and Δ 5	Have small amounts of C_{20} and some produce C_{22} PUFA (<1%)	11.9–24.4	8.1–15.1	36–44
	Botryococcus	High hydrocarbon levels; small amounts of C_{20} and C_{22} PUFA (<3%); lipid mostly hydrocarbon; very low fatty acid content	465.7	1.2	33
	Haematococcus	Has 16:4ω3 and 18:4ω3; may have C_{20} PUFA; astaxanthin production as a valuable coproduct	28.3–30.9	10.0–14.7	40–46
Prasinophyceae	Prasinophytes (or may be EPA-producing chlorophytes)	Has larger amounts of C_{20} or C_{22} PUFA (>5%); C_{20} usually low if high 18:5ω3; usually has high 18:4ω3	9.5–33.1	3.3–5.9	35–44
Euglenophyceae	Euglenophytes	Like flagellates[b)](high 18:4ω3, C_{20} and C_{22} PUFA)	9.0–52.6	5.4	30
Bacillariophyceae	Diatoms	High C_{20}, low C_{18} and C_{22} PUFA	3.3–21.1	2.0–18.3	37–58
Cryptophyceae	Cryptomonads	High 18:4ω3, C_{20} and C_{22} PUFA	12.7–17.4	5.6–8.4	26–31
Dinophyceae	Dinoflagellates	High 18:4ω3, C_{20} and C_{22} PUFA (C_{20} usually low if high 18:5ω3)	2.4–12.9	0.6–4.8	20–47
Raphidophyceae	Raphidophytes	High 18:4ω3 and C_{20} PUFA	9.5	9.8	36
Haptophyceae	Haptophytes	High 18:4ω3, C_{20} and C_{22} PUFA (C_{20} usually low if high 18:5ω3)	6.4–15.4	5.8–13.7	22–36
Chrysophyceae	Chrysophytes (but may be haptophytes)	High 18:4ω3, C_{20} and C_{22} PUFA (C_{20} usually low if high 18:5ω3)	2.3–10	1.6	41
Rhodophyceae	Rhodophytes	High C_{20} PUFA lack C_{22} PUFA	11.2	2.7	37
Eustigmatophyceae	Eustigmatophyte	High C_{20} PUFA lack C_{22} PUFA	2.9–18.4	5.1–11.0	31–50

a) PUFA, polyunsaturated fatty acid.
b) Cryptophytes, dinoflagellates, and some prasinophytes.

undergoing balanced growth (during the logarithmic phase), and the total lipid content increases as the biomass increases [80]. Culturing microalgae until they reach late stationary phase optimizes the potential for biodiesel lipids, due to the increase in yield (lipid accumulation) and cetane number. On the other hand, the higher proportion of PUFAs earlier in the growth cycle is better for PUFA production. This highlights the importance of optimizing the culture conditions, including choice of growth technologies and growth parameters, in order to optimize the biomass and oil yield, as well as the quantity and quality of the biodiesel derived from these microalgal lipids.

1.7 High-Value Lipids: Long-Chain Polyunsaturated Fatty Acids

Dietary consumption of essential long-chain polyunsaturated FAs (LC-PUFAs), in particular omega-3 FAs like DHA (22:6ω3), EPA (20:5ω3), and arachidonic acid (AA) (20:4ω6), has many demonstrated benefits in human health (see Chapter 6). Studies have shown that the consumption of LC-PUFAs helps prevent the risk of cardiovascular diseases, neural disorders, arthritis, asthma, and skin diseases [81–84]. As well, DHA and EPA are essential in feeds for aquaculture-reared fish, both for animal development and for acceptable lipid profiles for market.

Microalgae are the fundamental source of omega-3 LC-PUFAs in the marine ecosystem. However, fish oil is currently the primary source for producing omega-3 oils for dietary health and feed applications. Fish accumulate omega-3 FAs by consuming microalgae or other marine organisms in the food web. The production of fish meal and oil (i.e., including for use in nutraceutical fish oil capsules and aquaculture feeds) is dependent on wild fisheries. However, there is growing concern about the health of ocean fish stocks, the ecological effects of industrial fishing, and the levels of pollutants in some oils. The increasing global population, coupled with expanding demands for these health-benefiting oils by the aquaculture, nutraceutical, and pharmaceutical sectors, will place further pressure on the fish oil supply.

The LC-PUFA composition of microalgal classes is shown in Table 1.2, and key LC-PUFAs are shown on a class basis in Figure 1.2. While there is stability within classes, the relative proportions of desired FAs can be manipulated by changing the growth conditions [80]. Unusual FAs have been identified, including C_{18}–C_{22} trans ω3 PUFA from the Northern and Southern Hemisphere in the haptophyte *Imantonia rotunda* [85], indicating that there is still much to be learned about the biosynthetic pathways for FA synthesis in marine microalgae.

To synthesize LC-PUFA ($\geq C_{20}$), most marine microalgae use the conventional desaturation and elongation biosynthetic pathway, while some use the polyketide synthase (PKS) pathway [86, 87]. The PKS pathway does not require multiple desaturase and elongase enzymes, but instead employs a PKS gene cluster for the synthesis of LC-PUFA under anaerobic conditions [86]. The thraustochytrid *Schizochytrium* might use both pathways for PUFA biosynthesis, depending on substrate availability [88]. The omega-type (ω-type) FA desaturases and

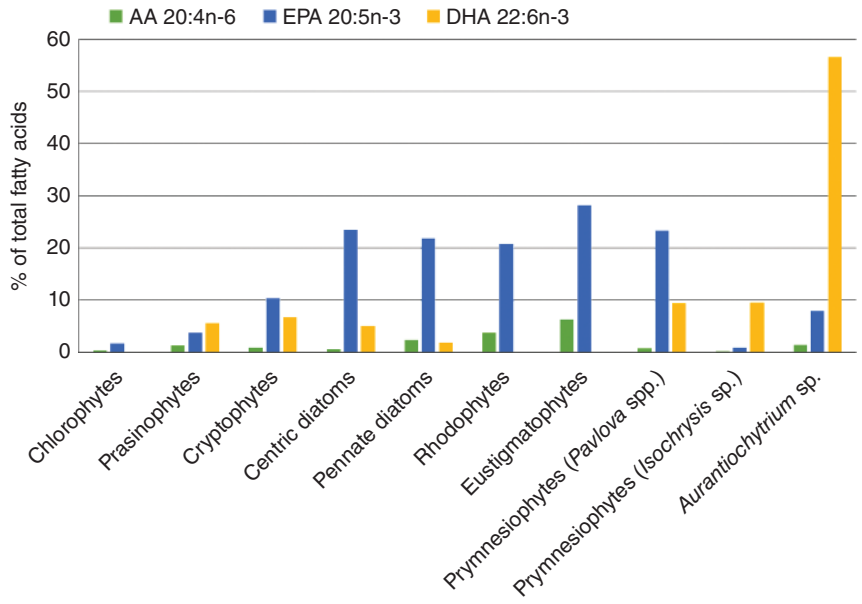

Figure 1.2 Average percentage compositions of the long-chain polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (DHA; 22:6n-3), eicosapentaenoic acid (EPA; 20:5n-3), and arachidonic acid (AA; 20:4n-6) of different microalgal classes. (Data compiled from >40 species held in the Australian National Algae Culture Collection.)

delta-type (Δ-type) desaturase are key enzymes that are generally responsible for increasing the number of double bonds on unsaturated FAs. The ω-type desaturases are enzymes that introduce a methylene-interrupted double bond (third carbon along) on the ω side (i.e., toward the methyl end of the FA molecule) of an existing double bond in an FA. Unlike animals, most photosynthetic organisms typically have ω-type desaturases. The Δ-type desaturases introduce on the Δ side (toward the ester/carboxyl end) of an existing double bond in a FA. Most organisms (excluding some groups examined here) possess examples of Δ-type desaturase enzymes. As detailed for biofuels, oils high in saturated and monounsaturated are preferred for biodiesel production, whereas microalgae with these enzymes are a positive feature for the production of high levels of long-chain and highly unsaturated PUFAs (Table 1.2).

The thraustochytrid *Thraustochytrium*, the green eustigmatophyte *Nannochloropsis*, the diatoms *Attheya* and *Pseudo-nitzschia*, and the red alga *Rhodomonas* are good producers of LC omega-3 oils, especially DHA and EPA [36, 48, 89]. Already a small player in the supply chain for these valuable oils, microalgae could be an increasing contributor to future renewable sources of omega-3 LC-PUFA-rich oil for the nutraceutical industry. Martek Biosciences (Columbia and Boulder, United States; the latter operation formerly known as OmegaTech), now part of the Royal Dutch State Mines (DSM), is growing microalgae heterotrophically (using glucose and yeast extract) in conventional fermenters to produce health-benefiting LC omega-3 FAs [15, 90, 91]. Martek

Biosciences cultures the diatom *N. alba* (high in EPA), the heterotrophic thraustochytrids, and the dinoflagellate *Crypthecodinium cohnii* (high in DHA) for their production of LC omega-3 oils [15].

1.8 High-Value Lipids: Carotenoid Pigments

Microalgae are an excellent source of natural carotenoid and chlorophyll pigments [92] (see Chapter 4). Over 600 carotenoids are known and many of these are produced by microalgae. Sophisticated high-performance liquid chromatography (HPLC) methods using reverse-phase C18 columns to separate both carotenoids and chlorophylls in a single analysis have been developed [93, 94]. This has enabled the availability of extensive databases of the characteristic pigment compositions of the different algal classes [6, 92, 95].

A distinction is made between primary and secondary carotenoids. Primary xanthophylls (i.e., oxygenated carotenoids) are structural and functional components of the cellular photosynthetic apparatus and are hence essential for cell survival. Secondary xanthophylls are produced in large quantities after exposure to specific environmental stimuli (carotenogenesis), for example, high light, oxygenated species, and so on [96]. Figure 1.3 gives examples of pigment profiles of different microalgal classes. Within a single algal class, there can be compositional variations between genera, species, and even strains, which need to be considered when searching for and selecting strains for pigment production. However, as a preliminary screening device, the algal class is generally a good guide to which pigments will predominate.

An example of microalgal pigment applications is the requirement for pigments in the diet of aquaculture-reared salmonids to ensure they attain commercially desirable pink-colored flesh [97]. Synthetic pigments have traditionally been used, but natural sources of astaxanthin are now available. For example, the green microalga *Haematococcus pluvialis* has a particularly high content of astaxanthin (1–3% of the dry weight) and is now grown commercially as a carotenoid source for use in aquaculture, in poultry feed, and as an antioxidant [97]. There is a striking difference in the pigment composition of *Haematococcus* between the different life stages of vegetative cells and the resting stage: vegetative cells can contain significant amounts of lutein and are low in astaxanthin, while the aplanospores contained high levels of astaxanthin. See Table 1.3 for a full pigment composition of *Haematococcus* and [99] for more information on the regulation of pigment synthesis.

BASF is now a major supplier of β-carotene isolated from the halotolerant green alga *D. salina* grown in 400 ha hypersaline "lakes" at Hutt Lagoon in Western Australia and at Whyalla in South Australia. At Whyalla, the company processes up to one million liters of brine per hour. This pigment has a multitude of uses in food products and as a source of pigmentation in farmed prawns [100].

While there are already established markets for some microalgal carotenoids, for example, β-carotene, there is potential for other pigments in new applications or as an alternative source for established applications. Lutein, a carotenoid

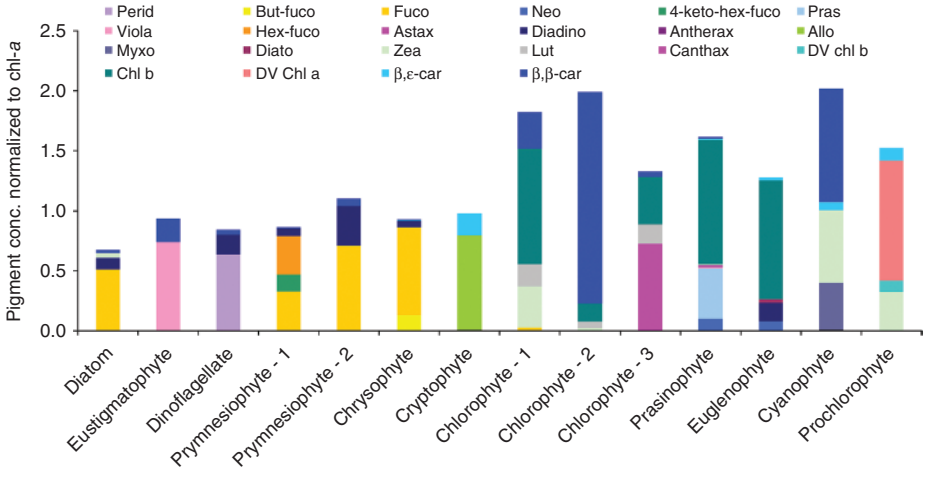

Figure 1.3 Pigment profiles of different microalgal classes from strain representatives held in the Australian National Algae Culture Collection. Within one class, there can be variation between genera, species, and even strains. Pigment key: But-fuco, 19′-butanoyloxyfucoxanthin; Fuco, fucoxanthin; Neo, 9′-*cis*-neoxanthin; 4-keto-hex-fuco, 4-keto-19′-hexanoyloxyfucoxanthin; Pras, prasinoxanthin; Viola, violaxanthin; Hex-fuco,19′-hexanoyloxyfucoxanthin; Astax, astaxanthin; Diadino, diadinoxanthin; Antherax, antheraxanthin; Allo, alloxanthin; Myxo, myxoxanthophyll; Diato, diatoxanthin; Zea, zeaxanthin; Lut, lutein; Canthan, canthaxanthin; DV chl *b*, divinyl chlorophyll *b*; Chl *b*, monovinyl chlorophyll *b*; DV chl *a*, divinyl chlorophyll *a*; β,ε-car, β,ε-carotene; β,β-car, β,β-carotene.

Table 1.3 Pigment composition of vegetative cells and resting-stage aplanospores of *Haematococcus*.

Pigment	Vegetative cells (%)	Aplanospores (%)
Neoxanthin	13.0	ND
Violaxanthin	14.5	ND
Lutein-5,6-epoxide	5.2	ND
Lutein	53.8	1.0
β,β-Carotene	13.5	3.0
Canthaxanthin	ND	2.0
Adonirubin	ND	2.0
Echinenone	ND	3.0
Astaxanthin (unesterified)	ND	1.0
Astaxanthin (monoester)	ND	49.0
Astaxanthin (diester)	ND	39.0

ND, not detected.
Source: Adapted from [98].

pigment from green algae, is receiving much attention for preventing age-related macular degeneration of the eyes. Research has focused on screening for microalgae that produce high contents of lutein, as well as identifying conditions that optimize lutein production [101]. Different procedures for recovering lutein from microalgal biomass are also being investigated [102]. The fast-growing *C. protothecoides* (a chlorophyte) is of interest as a promising organism for the commercial production of lutein by heterotrophic fermentation [101]. Wei *et al*. showed that adding reactive oxygen species (ROS) could increase yields of carotenoid up to 31.4 mg L^{-1} and biomass yields up to 15.9 g L^{-1} [103].

Fucoxanthin has an identified role in combating obesity. While there are macroalgal sources [104], microalgae offer an attractive alternative with potentially novel applications. For example, early research is demonstrating that fucoxanthin from *Odontella aurita* has interesting antiproliferative activity in well-differentiated pathologic cells [105].

1.9 High-Value Bioproducts: Polysaccharides

Microalgae living in aquatic ecosystems commonly secrete extracellular polymeric substances. These can be formed as capsular material that closely surrounds the microbial cell, or they may be released into the surrounding environment as dispersed slime, with no obvious association with any particular cell [106–108]. A large proportion (40–95%) of this polymeric material is composed of EPS, and it may also include proteins, nucleic acids, and lipids [109]. EPS form a highly hydrated matrix [110] and provide a layer of protection to cells against toxic compounds [111, 112] or digestion by other organisms [113]. Microbial EPS also improve the bioavailability of trace nutrients, including iron, to phytoplankton in iron-limited regions of the world's ocean [114]. As well, EPS play an important role in preventing cellular desiccation in thraustochytrids [115].

There is growing interest in isolating and identifying new microbial polysaccharides with a biotechnological potential. Polysaccharides produced by microorganisms have a range of uses, for example, as gelling, flocculent, and thickening agents in the food and beverage, oil, cosmetic, paper, paint, adhesive, and textile industries [116, 117]. Clinical applications include pharmaceuticals, cancer therapy, drug delivery, promotion of bone healing, and wound dressings for patients with burns, chronic ulcers, or extensive tissue loss [118–122]. As high energy compounds, EPS are also suitable for fermentation into bioethanol as a biofuel [123]. Alternatively, EPS may be a high-value coproduct formed in low amounts during the production of microbial biofuel [124]. EPS from labyrinthulomycetes exhibit broad-spectrum antiviral activities against viruses, for example, the enterovirus, retrovirus, adenovirus, and cytomegalovirus [125].

1.10 Wastewater Bioremediation and Bioproducts

Using waste streams as a nutrient source for microalgae production has the potential not only to reduce industrial GHG emissions (including power

plants) but also to reduce substantially the cost of commercial production of biofuels and commodity chemicals. Early R&D [126] has been followed by the recent recognition of sustainability and cost benefits, leading to a resurgent interest in wastewater bioremediation, combined with bioproduct production. Considerable R&D is still required for economic algal biofuels and other products, although the near-term opportunity is in wastewater treatment, particularly municipal wastewaters [127]. By adding CO_2 to such wastewater treatment ponds, it would be possible to grow enough algae to remove all N and P nutrients, thus achieving an improved level of treatment of the wastewaters while also generating algal biomass with a potentially high oil content. The CO_2 required for the process could be provided by the organic matter in the wastewater itself. In this scenario, algal wastewater treatment, especially if municipal, agricultural, and industrial wastes are used, combined with biofuel production, has the potential to reduce GHG emissions by ~1% [127]. Such systems could be further developed to recycle available nutrients and waters, greatly increasing biofuel outputs. HRAP can be developed as a low-cost efficient wastewater treatment technology have much higher treatment performance and algal productivity and could provide sufficient algal biomass to be economically used as a biofuel feedstock [24, 128]. Model scenarios to convert algal biomass grown using wastewater in HRAPs have demonstrated the potential value in a large urban wastewater treatment plant (Melbourne Water, Australia) for profitable bioproduct and biofuel production [129].

1.11 Other Bioapplications and the Potential for Bioengineering

Microalgae can be used to produce other high-value bioproducts, such as skin care products and cosmetics. There has been interest in bioactive compounds from microalgae for decades [50], but only a few products have reached market. Microalgal oil from the thraustochytrid *Ulkenia* sp. is used in the skin care serum "Blue Therapy Serum-In-Oil Night" marketed by Biotherm L'Oreal [130]. The Algenist GENIUS Ultimate Anti-Aging products (formerly owned by TerraVia) contain *C. protothecoides* oil and *H. pluvialis* extract [131].

As well, there is a recent increasing trend toward cultivating microalgae for medical applications. Diatom (*Thalassiosira pseudonana*)-derived nanoporous biosilica was used to deliver chemotherapeutic drugs to cancer cells [132] (see Chapter 27). This reduces costs and toxic chemicals used to manufacture the nanoporous silica-based materials as drug delivery vehicles. The thraustochytrid *Schizochytrium* sp. is being used to produce recombinant antigens in a readily usable form for vaccination against influenza [133].

The thraustochytrid *Aurantiochytrium*, as well as the green alga *B. braunii*, are known to accumulate relatively high amounts of squalene in cells [72, 134]. Squalene is a lipid intermediate in the biosynthesis of cholesterol and other steroids. As a natural antioxidant, it has been suggested that it could effectively reduce the incidence of coronary heart disease and cancer [135]. Coherent anti-Stokes

Raman scattering (CARS) microscopy can be used to monitor squalene accumulation in cells of the thraustochytrid *Aurantiochytrium mangrovei* in real time [136]. It can also screen for special cells, in a nondestructive and nonperturbative manner, that have a high capability to accumulate squalene. The study shows the relevance of emerging technologies for potential product development.

While there is vast untapped natural biodiversity in microalgae, current research in genomic and metabolic engineering is enhancing growth through photosynthesis [137] and improving the production of target bioactive compounds by enhancing or modifying biosynthetic pathways [138, 139]. As well, via microalgal gene discovery, their genes have been used to extend PUFA synthesis pathways in land plants to produce omega-3 LC-PUFA [140–144]. These new niche crops provide an alternative source to fish oil, thus contributing to the sustainability and protection of wild fish resources.

1.12 Conclusions

The potential for bioproducts from microalgae has gained increased interest due to growing concerns about rising global population, future availability, and overuse of food and fossil fuels, as well as environmental degradation and pollution. In addition to producing lipids for biofuel production, microalgae are capable of synthesizing a range of high-value bioproducts, for example, proteins, enzymes, PUFA, carotenoid pigments, and EPS. Combining production of high-value coproducts with biofuel production is desirable when it adds greater value to the production process and improves process economics. Such a biorefinery approach can also integrate environmental applications such as GHG capture and wastewater bioremediation. The biorefinery approach will accelerate the development of technologies to large-scale commercial viability, which will allow economic sustainability for future commodity production. The twenty-first century has the potential to be the time when "microalgae come of age." They have historically been used as a food and medicine by humans and have an essential place in the aquatic food web. Advances have now allowed the production of a wide range of bioactive compounds of considerable interest and value to humanity. Microalgae are thus increasingly attractive in a world with restricted renewable resources. There is room to further explore the largely untapped global microalgal biodiversity. Genetic improvements to microalgal strains and technological advances in microalgal bioproduction systems will be significant features of a more sustainable world.

Acknowledgments

The authors thank the Commonwealth Scientific and Industrial Research Organisation (CSIRO) National Facilities and Collections, Intelligent Processing Transformational Capability Platform (IP TCP), and Oceans and Atmosphere

Flagship for their support; Lesley Clementson for pigment data of different microalgal classes in Figure 1.3; Graeme Dunstan for fatty acid compositions and summary of the chemotaxonomic microalgal groups in Table 1.2; Shellfish Culture Ltd., Australia; and Dr Ami Ben-Amotz for supplying photos in Figure 1.1.

References

1 Guiry, M.D. and Guiry, G.M. (2016) Algaebase, http://www.algaebase.org: National University of Ireland (21 August 2017).
2 Guiry, M.D. (2012) How many species of algae are there? *J. Phycol.*, **48**, 1057–1063.
3 Van den Hoek, C., Mann, D.G., and Jahns, H.M. (eds) (1995) *Algae. An Introduction to Phycology*, University Press, Cambridge.
4 Walters, C. (2006) The origin of petroleum, in *Practical Advances in Petroleum Processing* (eds C. Hsu and P. Robinson), Springer, New York, pp. 79–101.
5 Slocombe, S.P., Zhang, Q., Ross, M. *et al*. (2015) Unlocking nature's treasure-chest: screening for oleaginous algae. *Sci. Rep.*, **5**, 1–17. doi: 10.1038/srep09844
6 Jeffrey, S. and LeRoi, J. (1997) *Simple Procedures for Growing Scor Reference Microalgal Cultures*, UNESCO Publishing, Paris.
7 Guillard, R.R. and Ryther, J.H. (1962) Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula Confervacea* (Cleve) Gran. *Can. J. Microbiol.*, **8**, 229–239.
8 Keller, M.D., Selvin, R.C., Claus, W. *et al*. (1987) Media for the culture of oceanic ultraphytoplankton. *J. Phycol.*, **23**, 633–638.
9 Blackburn, S.I., Bolch, C.J., Haskard, K.A., *et al*. (2001) Reproductive compatibility among four global populations of the toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae). *Phycologia*, **40**, 78–87.
10 Andersen, R.A. (ed.) (2005) *Algal Culturing Techniques*, 1st edn, Academic Press, Elsevier Amsterdam.
11 Andersen, R.A. and Sexton, J.P. (2009) The importance of algae and culture collections, with comments on marine algal cryopreservation. *Phycologia*, **48**, 152–153.
12 Mori, F., Erata, M., and Watanabe, M.M. (2002) Cryopreservation of cyanobacteria and green algae in the NIES-collection. *Microbiol. Cult. Coll.*, **18**, 45–55.
13 Müller, J., Day, J.G., Harding, K. *et al*. (2007) Assessing genetic stability of a range of terrestrial microalgae after cryopreservation using amplified fragment length polymorphism (AFLP). *Am. J. Bot.*, **94**, 799–808.
14 Day, J.G. and Brand, J.J. (2005). Cryopreservation methods for maintaining microalgal cultures, in *Algal Culturing Techniques*, (ed. R.A. Andersen). Academic Press, New York, pp. 165–187.

15 Barclay, W., Apt, K., and Dong, X.D. (2013) Commercial production of microalgae via fermentation, in *Handbook of Microalgal Culture* (eds A. Richmond and Q. Hu), John Wiley & Sons, Ltd, Ltd, pp. 134–145.
16 Chojnacka, K. and Marquez-Rocha, F.J. (2004) Kinetic and stoichiometric relationships of the energy and carbon metabolism in the culture of microalgae. *Biotechnology*, **3**, 21–34.
17 Bumbak, F., Cook, S., Zachleder, V. *et al*. (2011) Best practices in heterotrophic high-cell-density microalgal processes: achievements, potential and possible limitations. *Appl. Microbiol. Biotechnol.*, **91**, 31–46.
18 Liang, Y., Sarkany, N., and Cui, Y. (2009) Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. *Biotechnol. Lett.*, **31**, 1043–1049.
19 Fogg, G.E. (1965) *Algal Cultures and Phytoplankton Ecology*, University of Wisconsin Press, Madison.
20 Richmond, A. and Hu, Q. (2013) *Handbook of Microalgal Culture: Applied Phycology and Biotechnology*, John Wiley & Sons, Ltd.
21 Tredici, M.R. (2004) Mass production of microalgae: photobioreactors, in *Handbook of Microalgal Culture: Biotechnology and applied Phycology* (ed. A. Richmond), vol. **1**, John Wiley & Sons, Ltd., pp. 178–214.
22 Lee, Y.-K. (2001) Microalgal mass culture systems and methods: their limitation and potential. *J. Appl. Phycol.*, **13**, 307–315.
23 Tredici, M. (1999) *Photobioreactors*, John Wiley & Sons, Ltd, New York.
24 Craggs, R.J., Lundquist, T.J., and Benemann, J.R. (2013) Wastewater treatment and algal biofuel production, in *Algae for Biofuels and Energy* (eds A.M. Borowitzka and R.N. Moheimani), Springer, Netherlands, pp. 153–163.
25 Grobbelaar, J.U. (2009) Factors governing algal growth in photobioreactors: the "open" versus "closed" debate. *J. Appl. Phycol.*, **21**, 489–492.
26 Van Harmelen, T. and Oonk, H. (2006) Microalgae biofixation processes: applications and potential contributions to greenhouse gas mitigation options. *TNO Built Environment and Geosciences, Apeldoorn, The Netherlands*: 56.
27 Zittelli, G.C., Rodolfi, L., and Tredici, M.R. (2003) Mass cultivation of *Nannochloropsis* sp. in annular reactors. *J. Appl. Phycol.*, **15**, 107–114.
28 Rodolfi, L., Chini, Z.G., Bassi, N. *et al*. (2009) Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnol. Bioeng.*, **102**, 100–112.
29 Richmond, A. (1987) The challenge confronting industrial microagriculture: high photosynthetic efficiency in large-scale reactors. Proceeding of the Twelfth International Seaweed Symposium (eds M.A. Ragan and C.J. Bird), Springer, Netherlands.
30 Hu, Q. (2004) Environmental effects on cell composition, in *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, 1st edn (ed. A. Richmond), John Wiley & Sons, Ltd., pp. 83–94.
31 Zhang, C.W., Zmora, O., Kopel, R. *et al*. (2001) An industrial-size flat plate glass reactor for mass production of *Nannochloropsis* sp. (Eustigmatophyceae). *Aquaculture*, **195**, 35–49.

32 Lee, Y.K. (2004) Algal nutrition-heterotrophic carbon nutrition, in *Handbook of Microalgal Culture: Biotechnology and Applied Phycology* (ed. A. Richmond), John Wiley & Sons, Ltd., Oxford, pp. 116–124.

33 Raghukumar, S. (2002) Ecology of the marine protists, the labyrinthulomycetes (thraustochytrids and labyrinthulids). *Eur. J. Protistol.*, **38**, 127–145.

34 Honda, D., Yokochi, T., Nakahara, T. *et al*. (1999) Molecular phylogeny of labyrinthulids and thraustochytrids based on the sequencing of 18 s ribosomal rna gene. *J. Eukaryot. Microbiol.*, **46**, 637–647.

35 Leander, C.A., Porter, D., and Leander, B.S. (2004) Comparative morphology and molecular phylogeny of aplanochytrids (labyrinthulomycota). *Eur. J. Protistol.*, **40**, 317–328.

36 Lewis, T.E., Nichols, P.D., and McMeekin, T.A. (1999) The biological potential of thraustochytrids. *Mar. Biotechnol.*, **1**, 580–587.

37 Miao, X. and Wu, Q. (2006) Biodiesel production from heterotrophic microalgal oil. *Bioresour. Technol.*, **97**, 841–846.

38 Harel, M. and Place, A.R. (2004) Heterotrophic production of marine algae for aquaculture, in *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, 1st edn (ed. A. Richmond), John Wiley & Sons, Ltd, pp. 513–524.

39 Algal Scientific C (2016) Algamune: The World's First Beta Glucan Commercially Produced from Algae, https://www.businesswire.com/news/home/20150331005311/en/Algal-Scientific-Secures-7-Million-Funding-Accelerate

40 Kim, K., Jung, K.E., Ryu, B.-G. *et al*. (2012) A novel fed-batch process based on the biology of *Aurantiochytrium* sp. KRS101 for the production of biodiesel and docosahexaenoic acid. *Bioresour. Technol.*, **135**, 269–274.

41 Ryu, B.-G., Kim, K., Kim, J. *et al*. (2012) Use of organic waste from the brewery industry for high-density cultivation of the docosahexaenoic acid-rich microalga, *Aurantiochytrium* sp. KRS101. *Bioresour. Technol.*, **129**, 351–359.

42 Lee Chang, K.J., Paul, H., Nichols, P.D. *et al*. (2015) Australian thraustochytrids: potential production of dietary long-chain omega-3 oils using crude glycerol. *J. Funct. Foods*, **19**, 810–820.

43 Yan, D., Lu, Y., Chen, Y.-F. *et al*. (2011) Waste molasses alone displaces glucose-based medium for microalgal fermentation towards cost-saving biodiesel production. *Bioresour. Technol.*, **102**, 6487–6493.

44 Jensen, G.S., Ginsberg, D.I., and Drapeau, C. (2001) Blue-green algae as an immuno-enhancer and biomodulator. *JANA*, **3**, 24–30.

45 Richmond, A. (2008) *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, John Wiley & Sons, Ltd.

46 Belasco, W. (1997) Algae burgers for a hungry world? The rise and fall of *Chlorella* cuisine. *Technol. Cult.*, **38**, 608–634.

47 Yamaguchi, K. (1996) Recent advances in microalgal bioscience in Japan, with special reference to utilization of biomass and metabolites: a review. *J. Appl. Phycol.*, **8**, 487–502.

48 Volkman, J.K., Brown, M.R., Dunstan, G.A. *et al*. (1993) The biochemical composition of marine microalgae from the class eustigmatophyceae. *J. Phycol.*, **29**, 69–78.

49 Spolaore, P., Joannis-Cassan, C., Duran, E. *et al*. (2006) Commercial applications of microalgae. *Soci. Biotechnol.*, **101**, 87–96.

50 Barclay, W. and Apt, K. (2013) Strategies for bioprospecting microalgae for potential commercial applications, in *Handbook of Microalgal Culture* (eds A. Richmond and Q. Hu), John Wiley & Sons, Ltd, Ltd, pp. 69–79.

51 Carmichael, W.W., Drapeau, C., and Anderson, D.M. (2000) Harvesting of *Aphanizomenon flos-aquae* Ralfs ex Born. & Flah. var. *flos-aquae* (Cyanobacteria) from Klamath Lake for human dietary use. *J. Appl. Phycol.*, **12**, 585–595.

52 Davis, H. and Guillard, R. (1958) Relative value of ten genera of microorganisms as foods for oyster and clam larvae. US. Fish Wildlife Serv. *Fish. Bull*, **136**, 293–304.

53 Brown, M., Jeffrey, S., Volkman, J. *et al*. (1997) Nutritional properties of microalgae for mariculture. *Aquaculture*, **151**, 315–331.

54 Knuckey, R.M., Brown, M.R., Robert, R. *et al*. (2006) Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. *Aquacult. Eng.*, **35**, 300–313.

55 Bharathiraja, B., Chakravarthy, M., Kumar, R.R. *et al*. (2015) Aquatic biomass (algae) as a future feed stock for bio-refineries: a review on cultivation, processing and products. *Renewable Sustainable Energy Rev.*, **47**, 634–653.

56 Maisashvili, A., Bryant, H., Richardson, J. *et al*. (2015) The values of whole algae and lipid extracted algae meal for aquaculture. *Algal Res.*, **9**, 133–142.

57 Sheehan, J., Dunahay, T., Benemann, J. *et al*. (1998) *Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae; Close-out Report*, National Renewable Energy Lab, Golden, CO, US Department of Energy, http://www.osti.gov/energycitations/servlets/purl/15003040-tW7nZs/native/ (21 Aug 2017)

58 Pienkos, P.T. and Darzins, A. (2009) The promise and challenges of microalgal-derived biofuels. *Biofuels, Bioprod. Biorefin.*, **3**, 431–440.

59 Sun, A., Davis, R., Starbuck, M. *et al*. (2011) Comparative cost analysis of algal oil production for biofuels. *Energy*, **36**, 5169–5179.

60 Sheehan, J., Camobreco, V., Duffield, J. *et al*. (1998) *Life Cycle Inventory of Biodiesel and Petroleum Diesel for Use in an Urban Bus*. Final Report, National Renewable Energy Lab, Golden, CO.

61 Benemann, J.R. (2008) *Opportunities and Challenges in Algae Biofuels production*, http://www.fao.org/uploads/media/algae_positionpaper.pdf (21 Aug 2017)

62 Lundquist, T.J., Woertz, I.C., Quinn, N. *et al*. (2010) A realistic technology and engineering assessment of algae biofuel production. *Energy Biosci. Inst.*, 1–178.

63 Chen, J., Wang, Y., Benemann, J.R. *et al*. (2016) Microalgal industry in China: challenges and prospects. *J. Appl. Phycol.*, **28**, 715–725.

64 US DOE (U.S. Department of Energy), (2016) *National Algal Biofuels Technology Review*. Office of Energy Efficiency and Renewable Energy, Bioenergy Technologies Office, http://energy.gov/sites/prod/files/2016/06/f33/national_algal_biofuels_technology_review.pdf. (27 March 2016)

65 Hsueh, H.T., Chu, H., and Yu, S.T. (2007) A batch study on the bio-fixation of carbon dioxide in the absorbed solution from a chemical wet scrubber by hot spring and marine algae. *Chemosphere*, **66**, 878–886.

66 Rann, M. (2009) *Microalgal Biodiesel – A Renewable Future*, http://www.renewablessa.sa.gov.au/files/bioalgae.pdf. (23 January 2016)

67 Eboibi, B., Lewis, D.M., Ashman, P.J. *et al*. (2015) Influence of process conditions on pretreatment of microalgae for protein extraction and production of biocrude during hydrothermal liquefaction of pretreated *Tetraselmis* sp. *RSC Adv.*, **5**, 20193–20207.

68 Klein-Marcuschamer, D., Chisti, Y., Benemann, J.R. *et al*. (2013) A matter of detail: assessing the true potential of microalgal biofuels. *Biotechnol. Bioeng.*, **110**, 2317–2322.

69 Clarens, A. and Colosi, L. (2013) Life cycle assessment of algae-to-energy systems, in *Advanced Biofuels and Bioproducts* (ed. J. Lee), Springer, New York, pp. 759–778.

70 Lee Chang, K.J., Rye, L., Dunstan, G.A. *et al*. (2014) Life cycle assessment: heterotrophic cultivation of thraustochytrids for biodiesel production. *J. Appl. Phycol.*, **27** (2), 639–647.

71 Maxwell, J.R., Douglas, A.G., Eglinton, G. *et al*. (1968) The Botryococcenes – hydrocarbons of novel structure from the alga *Botryococcus braunii*, Kützing. *Phytochemistry*, **7**, 2157–2171.

72 Tanoi, T., Kawachi, M., and Watanabe, M.M. (2011) Effects of carbon source on growth and morphology of *Botryococcus braunii*. *J. Appl. Phycol.*, **23**, 25–33.

73 Metzger, P. and Largeau, C. (2005) *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. *Appl. Microbiol. Biotechnol.*, **66**, 486–496.

74 Watanabe, M.M. and Tanabe, Y. (2013) Biology and industrial potential of *Botryococcus braunii*, in *Handbook of Microalgal Culture* (eds A. Richmond and Q. Hu), John Wiley & Sons, Ltd, Ltd, pp. 369–387.

75 Monyem, A., Canakci, M., and Van Gerpen, J.H. (2000) Investigation of biodiesel thermal stability under simulated in-use conditions. *Appl. Eng. Agric.*, **16**, 373–378.

76 Knothe, G. (2007) Some aspects of biodiesel oxidative stability. *Fuel Process. Technol.*, **88**, 669–677.

77 Klopfenstein, W.E. (1982) Estimation of cetane index for esters of fatty acids. *J. Am. Oil Chem. Soc.*, **59**, 531–533.

78 Gerpen, J.V. (2005) Biodiesel processing and production. *Fuel Process. Technol.*, **86**, 1097–1107.

79 Wallington, T., Kaiser, E., and Farrell, J. (2006) Automotive fuels and internal combustion engines: a chemical perspective. *Chem. Soc. Rev.*, **35**, 335–347.

80 Dunstan, G., Volkman, J., Barrett, S. *et al*. (1993) Changes in the lipid composition and maximisation of the polyunsaturated fatty acid content of three microalgae grown in mass culture. *J. Appl. Phycol.*, **5**, 71–83.

81 Takahata, K., Monobe, K., Tada, M. *et al*. (1998) The benefits and risks of n-3 polyunsaturated fatty acids. *Biosci. Biotechnol., Biochem.*, **62**, 2079–2085.

82 Tapiero, H., Ba, G.N., Couvreur, P. *et al*. (2002) Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. *Biomed. Pharmacother.*, **56**, 215–222.

83 Horrocks, L. and Farooqui, A. (2004) Docosahexaenoic acid in the diet: its importance in maintenance and restoration of neural membrane function. *Prostaglandins Leukot Essent Fatty Acids*, **70**, 361–372.

84 Kris-Etherton, P.M., Hecker, K.D., and Binkoski, A.E. (2004) Polyunsaturated fatty acids and cardiovascular health. *Nutr. Rev.*, **62**, 414–426.

85 Lee Chang, K.J., Dunstan, G.A., Mansour, M.P. *et al*. (2016), A novel series of C_{18}–C_{22} *trans* ω3 PUFA from Northern and Southern Hemisphere strains of the marine haptophyte *Imantonia rotunda*. *J. Appl. Phycol.*, **28**, 3363–3370.

86 Metz, J.G., Roessler, P., Facciotti, D. *et al*. (2001) Production of polyunsaturated fatty acids by polyketide synthases in both prokaryotes and eukaryotes. *Science*, **293**, 290–293.

87 Huang, J., Aki, T., Yokochi, T. *et al*. (2003) Grouping newly isolated docosahexaenoic acid-producing thraustochytrids based on their polyunsaturated fatty acid profiles and comparative analysis of 18S rRNA genes. *Mar. Biotechnol.*, **5**, 450–457.

88 Lippmeier, J., Crawford, K., Owen, C. *et al*. (2009) Characterization of both polyunsaturated fatty acid biosynthetic pathways in *Schizochytrium* sp. *Lipids*, **44**, 621–630.

89 Abedi, E. and Sahari, M.A. (2014) Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. *Food Sci. Nutr.*, **2**, 443–463.

90 Barclay, W., Meager, K., and Abril, J. (1994) Heterotrophic production of long chain omegah-3 fatty acids utilizing algae and algae-like microorganisms. *J. Appl. Phycol.*, **6**, 123–129.

91 Khozin-Goldberg, I., Leu, S., and Boussiba, S. (2016) Microalgae as a source for VLC-PUFA production, in *Lipids in Plant and Algae Development* (eds Y. Nakamura and Y. Li-Beisson), Springer International Publishing, Switzerland, pp. 471–510.

92 Wright, S.W. and Jeffrey, S.W. (2005) Pigment markers for phytoplankton production, in *Marine Organic Matter: Biomarkers, Isotopes and DNA* (ed. J.K. Volkman), Springer, Berlin Heidelberg, pp. 71–104.

93 Wright, S. (1991) Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Mar. Ecol. Prog. Ser.*, **77**, 183–196.

94 Van Heukelem, L. and Thomas, C.S. (2001) Computer-assisted high-performance liquid chromatography method development with

applications to the isolation and analysis of phytoplankton pigments. *J. Chromatogr. A*, **910**, 31–49.

95 Wright, S.W. and Jeffrey, S.W. (1987) Fucoxanthin pigment markers of marine phytoplankton analysed by HPLC and HPTLC. *Mar. Ecol. Prog. Ser.*, **38**, 259–266.

96 Jin, E., Polle, J.E., Lee, H.-K. *et al*. (2003) Xanthophylls in microalgae: from biosynthesis to biotechnological mass production and application. *J. Microbiol. Biotechnol.*, **13**, 165–174.

97 Lorenz, R.T. and Cysewski, G.R. (2000) Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. *Trends Biotechnol.*, **18**, 160–167.

98 Harker, M. and Young, A.J. (1995) Inhibition of astaxanthin synthesis in the green-alga *Haematococcus pluvialis*. *Eur. J. Phycol.*, **30**, 179–187.

99 Jeffrey, S.W., Egeland, E., and Enfield, N. (2009) Pigments of green and red forms of *Dunaliella*, and related chlorophytes, in *The Alga* Dunaliella*: Biodiversity, Physiology, Genomics and Biotechnology* (eds A. Ben-Amotz, J.E.W. Polle, and D.V.S. Rao), Science Publishers, Enfield, pp. 111–145.

100 Borowitzka, M.A. (1999) Commercial production of microalgae: ponds, tanks, tubes and fermenters. *J. Biotechnol.*, **70**, 313–321.

101 Shi, X.M. and Chen, F. (2002) High-yield production of lutein by the green microalga *Chlorella protothecoides* in heterotrophic fed-batch culture. *Biotechnol. Progr.*, **18**, 723–727.

102 Cerón, M.C., Campos, I., Sanchez, J.F. *et al*. (2008) Recovery of lutein from microalgae biomass: development of a process for *Scenedesmus almeriensis* biomass. *J. Agric. Food. Chem.*, **56**, 11761–11766.

103 Wei, D., Chen, F., Chen, G. *et al*. (2008) Enhanced production of lutein in heterotrophic *Chlorella protothecoides* by oxidative stress. *Sci. China, Ser. C Life Sci.*, **51**, 1088–1093.

104 Mori, K., Ooi, T., Hiraoka, M. *et al*. (2004) Fucoxanthin and its metabolites in edible brown algae cultivated in deep seawater. *Mar. Drugs*, **2**, 63–72.

105 Moreau, D., Tomasoni, C., Jacquot, C. *et al*. (2006) Cultivated microalgae and the carotenoid fucoxanthin from *Odontella aurita* as potent anti-proliferative agents in bronchopulmonary and epithelial cell lines. *Environ. Toxicol. Pharmacol.*, **22**, 97–103.

106 Sutherland, I.W. (1982) Biosynthesis of microbial exopolysaccharides, in *Advances in Microbial Physiology* (eds A.H. Rose and J.G. Morris), Academic Press, Londan, pp. 79–150.

107 Decho, A.W. (1990) Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. *Oceanogr. Mar. Biol. Annu. Rev.*, **28**, 73–153.

108 Wotton, R.S. (2004) The utiquity and many roles of exopolymers (EPS) in aquatic systems. *Sci. Mar.*, **68**, 13–21.

109 Flemming, H.C. and Wingender, J. (2001) Relevance of microbial extracellular polymeric substances (EPSS) – Part I: Structural and ecological aspects. *Water Sci. Technol.*, **43**, 1–8.

110 Flemming, H.C., Wingender, J., Moritz, R. *et al*. (1999) Physico-chemical properties of biofilms - a short review, in *Biofilms in the Aquatic Environment* (eds K. Cw, C. Dow, A. Godfree, and D. Holt), Royal Society of Chemistry, Cambridge, pp. 1–12.

111 Bitton, G. and Freihofer, V. (1977) Influence of extracellular polysaccharides on the toxicity of copper and cadmium toward *Klebsiella aerogenes*. *Microb. Ecol.*, **4**, 119–125.

112 Jeanthon, C. and Prieur, D. (1990) Susceptibility to heavy metals and characterization of heterotrophic bacteria isolated from two hydrothermal vent polychaete annelids, *Alvinella pompejana* and *Alvinella caudata*. *Appl. Environ. Microbiol.*, **56**, 3308–3314.

113 Caron, D.A. (1987) Grazing of attached bacteria by heterotrophic microflagellates. *Microb. Ecol.*, **13**, 203–218.

114 Hassler, C.S., Schoemann, V., Nichols, C.M. *et al*. (2011) Saccharides enhance iron bioavailability to southern ocean phytoplankton. *Proc. Natl. Acad. Sci. U.S.A.*, **108**, 1076–1081.

115 Jain, R., Raghukumar, S., Tharanathan, R. *et al*. (2005) Extracellular polysaccharide production by thraustochytrid protists. *Mar. Biotechnol.*, **7**, 184–192.

116 Sutherland, I.W. (1972) Bacterial exopolysaccharides, in *Advances in Microbial Physiology* (eds A.H. Rose and D.W. Tempest), Academic Press, London, pp. 143–213.

117 Sandford, P.A. (1984) Biotechnology of marine polysaccharide, in *Biotechnology of Marine Polysaccharide* (eds R.R. Colwell, E.R. Pariser, and A.J. Sinksey), McGraw-Hill, New York, pp. 454–516.

118 Labare, M.P., Guthrie, K., and Weiner, R.M. (1989) Polysaccharide exopolymer adhesives from periphytic marine bacteria. *J. Adhes. Sci. Technol.*, **3**, 213–223.

119 Weiner, R.M. (1997) Biopolymers from marine prokaryotes. *Trends Biotechnol.*, **15**, 390–394.

120 Sutherland, I.W. (1998) Novel and established applications of microbial polysaccharides. *Trends Biotechnol.*, **16**, 41–46.

121 Guezennec, J. (2002) Deep-sea hydrothermal vents: a new source of innovative bacterial exopolysaccharides of biotechnological interest? *J. Ind. Microbiol. Biotechnol.*, **29**, 204–208.

122 Zanchetta, P., Lagarde, N., and Guezennec, J. (2003) A new bone-healing material: a hyaluronic acid-like bacterial exopolysaccharide. *Calcif. Tissue Int.*, **72**, 74–79.

123 González López, C.V., Acién Fernández, F.G., Fernández Sevilla, J.M. *et al*. (2009) Utilization of the cyanobacteria *Anabaena* sp. ATCC 33047 in CO_2 removal processes. *Bioresour. Technol.*, **100**, 5904–5910.

124 Donot, F., Fontana, A., Baccou, J. *et al*. (2012) Microbial exopolysaccharides: main examples of synthesis, excretion, genetics and extraction. *Carbohydr. Polym.*, **87**, 951–962.

125 Raghukumar, S., Madhavan, H.N., and Malathi, J. (2014) *Extracellular Polysaccharides from Labyrinthulomycetes with Broad-Spectrum Antiviral Activities*, http://www.google.com/patents/WO2014045191A2?cl=en (21 Aug 2017)

126 Benemann, J.R. and Oswald, W.J. (1996) *Systems and Economic Analysis of Microalgae Ponds for Conversion of CO_2 to Biomass. Technical Report*, California University, Berkeley, CA. Department of Civil Engineering. http://www.osti.gov/scitech/servlets/purl/493389. (21 Aug 2017)

127 Benemann, J. (2013) Microalgae for biofuels and animal feeds. *Energies*, **6**, 5869–5886.

128 Mehrabadi, A., Craggs, R., and Farid, M.M. (2015) Wastewater treatment high rate algal ponds (WWT HRAP) for low-cost biofuel production. *Bioresour. Technol.*, **184**, 202–214.

129 Batten, D., Beer, T., Freischmidt, G. *et al*. (2013) Using wastewater and high-rate algal ponds for nutrient removal and the production of bioenergy and biofuels. *Water Sci. Technol.*, **67**, 915–924.

130 Biotherm L'Oréal (2013) *Blue Therapy Serum-in-Oil: the New Anti-Aging by Biotherm*, http://www.loreal.ca/en-ca/media/news/2013/oct/blue-therapy-serum-in-oil-the-new-anti-aging-by-biotherm (21 Aug 2017)

131 Algenist (2016) *Algenist Branded Skin and Personal Care Products- GENIUS Collection*.

132 Delalat, B., Sheppard, V.C., Rasi, G.S. *et al*. (2015) Targeted drug delivery using genetically engineered diatom biosilica. *Nat. Commun.*, **6**, 1–11.

133 Bayne, A.C.V., Boltz, D., Owen, C. *et al*. (2013) Vaccination against influenza with recombinant hemagglutinin expressed by *Schizochytrium* sp. confers protective immunity. *PLoS One*, **8**, e61790.

134 Nakazawa, A., Kokubun, Y., Matsuura, H. *et al*. (2014) TLC screening of thraustochytrid strains for squalene production. *J. Appl. Phycol.*, **26**, 29–41.

135 Smith, T.J. (2000) Squalene: potential chemopreventive agent. *Expert Opin. Invest. Drugs*, **9**, 1841–1848.

136 Ishitsuka, K., Koide, M., Yoshida, M. *et al*. (2017) Identification of intracellular squalene in living algae, *Aurantiochytrium mangrovei* with hyper-spectral coherent anti-Stokes Raman microscopy using a sub-nanosecond supercontinuum laser source. *J. Raman Spectrosc.*, **48**, 8–15.

137 Work, V.H., D'Adamo, S., Radakovits, R. *et al*. (2012) Improving photosynthesis and metabolic networks for the competitive production of phototroph-derived biofuels. *Curr. Opin. Biotechnol.*, **23**, 290–297.

138 Radakovits, R., Eduafo, P.M., and Posewitz, M.C. (2011) Genetic engineering of fatty acid chain length in *Phaeodactylum tricornutum*. *Metab. Eng.*, **13**, 89–95.

139 Jinkerson, R.E., Radakovits, R., and Posewitz, M.C. (2013) Genomic insights from the oleaginous model alga *Nannochloropsis gaditana*. *Bioengineered*, **4**, 37–43.

140 Zhou, X.-R., Robert, S.S., Petrie, J.R. *et al*. (2007) Isolation and characterization of genes from the marine microalga *Pavlova salina* encoding three front-end desaturases involved in docosahexaenoic acid biosynthesis. *Phytochemistry*, **68**, 785–796.

141 Petrie, J.R., Liu, Q., Mackenzie, A.M. *et al*. (2010) Isolation and characterisation of a high-efficiency desaturase and elongases from microalgae for transgenic LC-PUFA production. *Mar. Biotechnol.*, **12**, 430–438.

142 Petrie, J.R., Shrestha, P., Mansour, M.P. *et al*. (2010) Metabolic engineering of omega-3 long-chain polyunsaturated fatty acids in plants using an acyl-coa δ6-desaturase with ω3-preference from the marine microalga *Micromonas pusilla*. *Metab. Eng.*, **12**, 233–240.

143 Petrie, J., Nichols, P., Devine, M. *et al*. (2013) Engineered oilseed crops with fish oil DHA levels. *INFORM*, **24**, 648–652.

144 Petrie, J.R., Shrestha, P., Belide, S. *et al.* (2014) Metabolic engineering *Camelina sativa* with fish oil-like levels of DHA. *PLoS One.*, **9** (1), e85061. doi: 10.1371/journal.pone.0095409.

About the Authors

Susan I. Blackburn is past Director and now Honorary Fellow to the Australian National Algae Culture Collection, ANACC in Commonwealth Scientific and Industrial Research Organisation (CSIRO)'s National Collections and Marine Infrastructure. She is also former President of the International Society for Applied Phycology, ISAP (Immediate Past President) and Director of the Network of Asia Oceania Algae Culture Collections. Her research expertise spans microalgal biodiversity, environmental issues including harmful algal blooms and bioapplications of microalgae, including bioproducts such as omega-3 oils, biofuels, and other biotechnology potential of microalgae.

Kim Jye Lee-Chang is a research scientist in CSIRO Oceans and Atmosphere, who is interested in marine biotechnology research for developing microalgae for biofuels and bioproducts such as omega-3 oils, as well as other biotechnology potential of algae. He was awarded CSIRO Office of the Chief Executive (OCE) postdoctoral fellowship in 2014 that was supported through the CSIRO Intelligent Processing Transformational Capability Platform. He completed his PhD in 2013: "Microalgae—A Renewable Source of Biofuels, Omega-3 Oils and Other Co-products." The project was a joint project with University of Tasmania and the CSIRO. Kim has identified new endemic strains of highly productive thraustochytrids and by optimizing growth conditions has been maximizing their potential for biofuels and omega-3 oils production.