Algal Biotechnology

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1 Introduction

The term 'biotechnology' was first used in 1917 by Erecky (1917) who defined biotechnology as: '*all lines of work by which products are produced from raw materials with the aid of living organisms*'. Since the 1970s there have been many definitions of 'biotechnology' (Bud 1991), but the most widely accepted is that biotechnology is the use of living systems and organisms to develop or make useful products, or "*any technological application that uses biological systems*, *living organisms or derivatives thereof*, *to make or modify products or processes for specific use*" (definition used in the UN Convention on Biological Diversity). Algal biotechnology can therefore be defined as: "*the technological application of algae* (*both microalgae and macroalgae*) *or their derivatives to make or modify products or processes for specific use*". This definition encompasses the commercial-scale farming of seaweeds and microalgae for biomass as well as the production of specific compounds, the use of algae in wastewater treatment, the use of algal enzymes for specific purposes, the use of algal products in products such as functional foods etc. Although not specifically stated, the above definition also implies a practical and eventual commercial outcome. It does not refer to the technology *per se*, but rather the application of that technology or technologies. The term 'specific use' implies that there is a user – which in business terms means that there must be, ultimately, a customer and a market.

The phylogenetic diversity of the organisms we call 'algae' which include both prokaryotic and eukaryotic organisms (Adl et al. 2005; Borowitzka 2012) is reflected in the extensive range of products which can be produced from algae, as well as the

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variety of processes in which algae, or products derived from them, can be utilised. For convenience algal biotechnology is generally divided on the basis of the size of the algae under consideration into the biotechnology of the multicellular, large macro-algae or seaweeds, and the biotechnology of the microscopic and predominantly unicellular micro-algae. In this chapter I will be focussing almost exclusively on the microalgae, however the great importance of seaweeds to algal biotechnology must be recognised.

Currently, the quantity and value of wild harvested and farmed seaweeds and their products greatly exceeds that of the microalgae. Statistics available from the United Nations Food and Agriculture Organisation (FAO) show that in 2011 20 million t wet weight (= about 2 million t dry weight) of seaweeds were produced by aquaculture with a value of US$ 5.88×10^9. Seaweeds such as the brown algae *Saccharina japonica* and *Undaria pinnatifida* and the red algae *Kappaphycus* spp, *Gracilaria* spp and *Chondrus crispus* are mainly used to produce hydrocolloids such as alginate, agar, agarose, carrageenan and fucoidan (Bixler and Porse 2011), whereas other species such as the red alga *Pyropia* (*Porphyra*) spp and the green algae *Ulva* and *Caulerpa* are mainly used as food (Zemke-White and Ohno 1999). The cultivation methods used are described by Sahoo and Yarish (2005). Several elite strains have been bred using classical plant breeding methods (Zhang et al. 2011a, b) and tissue culture and molecular techniques are now widely being used in the breeding of elite cultivars and for propagation of the elite seed stock for cultivation (Reddy et al. 2008; Liu et al. 2011; Xu et al. 2012; Robinson et al. 2013).

2 Microalgae

Microalgal biotechnology started with pioneering studies on the large-scale culture of microalgae in Japan, Germany and the USA in the 1950s (Soeder 1986; Borowitzka 2013b). In the 1970s the first commercial production of *Chlorella* commenced in Japan and Taiwan (Soong 1980), and *Spirulina* harvesting started in Mexico in Lake Texcoco (Durand-Chastel 1980). Commercial culture of *Spirulina* commenced in the 1980s in the USA (Belay et al. 1994). The first microalga used for the production of a high-value fine chemical was the halophilic green alga, *Dunaliella salina*, cultured to produce β-carotene with commercial production commencing in the 1980s in Israel, Australia and the USA (Ben-Amotz 2004; Borowitzka 2005a). This was followed by the establishment of production of astaxanthin from *Haematococcus pluvialis* as a source of astaxanthin in the USA and Israel in the 1990s (Cysewski and Lorenz 2004), and the development of a commercial process to produce the long-chain polyunsaturated fatty acid, docosahexaenoic acid, by heterotrophic cultivation of the stramenopiles *Crypthecodinium cohnii* (dinoflagellate) and *Schizochytrium* sp. (thraustochytrid) in the USA (Kyle et al. 1992; Barclay et al. 2005). Table 1 summarises the main microalgae species currently produced commercially and the products derived from them. However, this table does not include the very large amounts of algae grown as feed in the aquaculture of molluscs,

Table 1 Current species of the major microalgae species produced commercially and their products

Species	Product(s)	Main production locations
Chlorella spp	Biomass, extracts for human and animal nutrition	Taiwan, China, Germany
S*pirulina* (*Arthrospira*) spp	Biomass, phycocyanin	China, Taiwan, India, USA, Thailand
Dunaliella salina	β-carotene	Australia, Israel
Haematococcus pluvialis	Astaxanthin	Israel, USA, China
Crypthecodinium cohnii	Docosahexaenoic acid	USA

crustaceans and fish (Borowitzka 1997; Neori 2011), nor the application of microalgae in other processes such as wastewater treatment (Green et al. 1996; Craggs et al. 2013; Sutherland et al. 2014) and use as fertilizer, or for the production of extracts used for a variety of applications such as in cosmetics, functional foods, and in animal husbandry (Gellenbeck 2012; Schwenzfeier et al. 2013; Kotrbáček et al. 2015). Microalgae are also being considered for the bioremediation of carbon dioxide (Moheimani et al. 2012).

3 Production of High Value Chemicals

Currently, the main high value products produced by microalgal biotechnology are the carotenoids β-carotene and astaxanthin, and the long chain polyunsaturated fatty acids docosahexaenoic acid and eicosapentaenoic acid. Smaller quantities of other products such as the phycobilin pigment, phycocyanin, are also produced (Borowitzka 2013d).

3.1 Carotenoids

The carotenoids which are accumulated in high amounts are secondary metabolites, and this means that they are mainly formed once the cells are no longer in the exponential phase of growth; i.e. they algae are either growing slowly or not at all. This presents a challenge in the development of an effective production process as the algae have a low carotenoid content under the conditions where growth is fastest, and accumulate the carotenoids only when growth is slowed or stopped. This means that either a two-step process must be used, with biomass generation in the first step, and product accumulation in the cells in the second step (Ben-Amotz 1995), or a culture regime at which neither growth nor product accumulation is maximal, but the productivity of product formation is highest (Borowitzka et al. 1984).

The halophilic green unicellular alga *Dunaliella salina* (sometimes also referred to as *D. bardawil* – Borowitzka and Siva 2007) is the best natural source of

β-carotene with the alga reported to contain up to 14 % of dry weight as β-carotene (Aasen et al. 1969). The main commercial production of *Dunaliella* β-carotene occurs in Australia at Hutt Lagoon in Western Australia and Whyalla in South Australia, with smaller production at Eilat in Israel (Borowitzka 2010). The two production plants in Australia use very large, simple, unmixed shallow ponds of a total area greater than 740 ha at each plant, whereas in Israel much smaller paddle-wheel driven raceway ponds are used. *Dunaliella salina* grows best at very high salinities between 20 and 35 % (w/v) NaCl. The β-carotene is accumulated in lipid droplets in the chloroplast and, unlike the synthetic β-carotene which is only in the form of the all-*trans* isomer, the *Dunaliella* β-carotene occurs both as the all-*trans* and the 9-*cis* isomers (Ben-Amotz et al. 1988). The maximum cell β-carotene content which can be achieved by *D. salina* is mainly a function of the salinity the algae is grown at, with higher salinities resulting in higher carotenoid contents (Borowitzka et al. 1990). The rate at which the β-carotene is formed is a function of the growth irradiance with higher irradiances leading to faster β-carotene accumulation (Borowitzka 2013a).

In Australia *Dunaliella* β-carotene production is a continuous culture production process. The growth salinity used is a compromise between the optimum salinity for β-carotene productivity (~22 % NaCl) and a salinity at which potential problems with predatory protozoa and/or the brine shrimps *Artemia* and *Parartemia* can be avoided (salinities >25 % NaCl) as well as the ability to supply sufficient seawater to replace water lost by evaporation from the large ponds (Borowitzka and Borowitzka 1989). The very large ponds, which are about 30–40 cm deep on average, are predominantly mixed by wind and convection currents resulting in low biomass productivity. However, the close-to-optimum climatic conditions mean that the biomass has a high β-carotene content and production occurs over the whole year. This, combined with the low land costs and an extremely efficient proprietary harvesting and β-carotene extraction methods, plus the economics of scale, means that this is the lowest cost microalgae production in the world. The products produced include a range of concentrations of β-carotene either in solution or as crystal suspensions in oil for use in the food, pharmaceutical and nutraceutical industries, as well as dried whole-cell β-carotene-rich *D. salina* biomass which can be used in aquaculture as a pigmenter for prawns or as a feed supplement for cattle and other animals.

Astaxanthin is produced using the freshwater green alga *Haematococcus pluvialis*, and high astaxanthin contents of up to 8 % of dry weight are found in the non-motile aplanospore stage of this alga. The astaxanthin is accumulated in lipid droplets in the cytoplasm of the aplanospores both in the form of free and esterified (3*S*,3′*S*) astaxanthin (Grung et al. 1992; Collins et al. 2011). Unlike *D. salina*, *Haematococcus* must be grown in a two-stage batch mode. In the first stage the *Haematococcus* biomass is generated by culturing the flagellated motile cells in nutrient-rich medium. In the second stage the cells are transferred to low-nutrient medium under high light conditions where they develop into the aplanospore stage and accumulate the astaxanthin. At Cyanotech in Hawaii, the first stage culture takes place in closed photobioreactors and this is then followed by the second stage in open raceway ponds (Olaizola 2000; Cysewski and Lorenz 2004). In Israel,

Algaetechnologies grows both stages in glass tubular photobioreactors outdoors. The current production plant has about 300 km of glass tubing on 10 ha of arid land. AstaReal in Sweden grow *Haematococcus* indoors in artificially lit reactors mixotrophically using organic carbon substrates such as acetate. *Haematococcus* can also be grown heterotrophically, but heterotrophically-grown cells do not accumulate large amounts of astaxanthin (Kobayashi et al. 1992; Orosa et al. 2001).

Haematococcus production is significantly more expensive than the production of *D. salina*, and therefore the *Haematococcus* astaxanthin is too expensive to be used as a pigmenter in the culture of salmonids, even though it is a very effective pigmenter (Sommer et al. 1992). The main markets therefore are for use as an antioxidant for human health.

Microalgae are also potential sources of other carotenoids such as zeaxanthin, lutein and canthaxanthin, however none of these has as yet been developed as a commercial product (Orosa et al. 2000; Blanco et al. 2007; Granado-Lorencio et al. 2009).

3.2 Fatty Acids

Over the last 30 or so years microalgae been intensively studied as potential sources of long-chain polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA) and several potential candidate species have been identified (Borowitzka 1988; Ratledge 2010). The only current commercial production is by the heterotrophic culture of the dinoflagellate *Crypthecodinium cohnii* to produce DHA for use in infant formula and other applications (Mendes et al. 2009). The fungi-like thraustochytrids, *Thraustochytrium* and *Schizochytrium* which are related to the dinoflagellates, are also used to produce both EPA and DHA heterotrophically (Barclay et al. 2010).

Many phototrophic algae have been proposed as potential sources of long-chain fatty acids. These include *Lobosphaera* (*Parietochloris*) *incisa* which has a high AA content (Bigogno et al. 2002), *Nannochloropsis* spp and *Phaeodactylum tricornutum* with a high EPA content (Chrismadha and Borowitzka 1994; Chini Zitelli et al. 1999), and *Isochrysis* sp. T. Iso (= *Tisochrysis lutea*) with a high DHA content (Tzovenis et al. 1997), however the productivity of these fatty acids is much lower than in the fungi-like organisms grown heterotrophically. It remains to be seen whether these photoautotrophic algae can be developed into commercially viable sources of these long-chain polyunsaturated fatty acids.

3.3 Other Products and Processes

Microalgae are also grown to produce stable isotope-labelled compounds for use in medical diagnostics and for research (Radmer 1996) or for the production of bioplastics (Samantaray and Mallick 2012). Phycobilin pigments are also produced

from cyanobacteria and the red unicell *Porphyridium* for use as fluorescent markers in immunology and also in cosmetics (Arad et al. 1996; Eriksen 2008). There is also the potential to use microalgae to carry out chemical transformations as a potential step in the production of useful compounds (Rao et al. 1999; Tripathi et al. 2002) or for the breakdown of xenobiotics such as pesticides or hormones (Pollio et al. 1996; Ghasemi et al. 2011).

Microalgae also produce many novel bioactive compounds (Borowitzka 1995) and the range of bioactivities reported from extracts of microalgae in the last 50–60 years includes antioxidant (Goiris et al. 2012; Klein et al. 2012), antibiotic (Kellam and Walker 1989; Nagai et al. 1995; Volk and Furkert 2006), antiviral (Hayashi et al. 1996; Boyd et al. 2009), anticancer (Gerwick et al. 1994; Morlière et al. 1998; Schwatsmann et al. 2001), anti-inflammatory (Baker 1984; Raposo et al. 2013), antihypertensive (Yamaguchi et al. 1989; Samarakoon et al. 2013) and other activities. Many microalgae, especially the dinoflagellates and the cyanobacteria, also produce potent toxins which have some applications in research and as lead compounds for the development of drugs. However, despite decades of research there are only a few drugs from marine organisms currently approved or in clinical trials, and all originate from organisms other than the microalgae (Mayer et al. 2010). However, some of these compounds appear to have a microalgal (mainly cyanobacterial) origin (Gerwick and Moore 2012). An example of this is dolastatin 10, originally isolated from the sea hare *Dolabella auricularia* (Pettit et al. 1987), but which was later found to originate from the cyanobacterium *Symploca* sp., a component of the diet of the sea hare (Luesch et al. 2001). Dolastatin 10 is the basis for the FDA approved drug Brentuximab Vendotin (Katz et al. 2011) for use in Hodgkin's lymphoma and anaplastic large cell lymphoma.

4 Biofuels

Interest in the use of microalgae as a source of sustainable renewable fuels and bioenergy has burgeoned again in the last decade and is again the focus of extensive research and development. It presents particular problems for algal biotechnology, mainly because of the very low value of the product (e.g. biodiesel) and the extremely large scale of production required (Borowitzka and Moheimani 2013b). The concept of using algae to produce biofuels such as biodiesel is not new (Harder and von Witsch 1942), and a major research effort occurred in the early 1990s, especially in the USA and in Japan (see Sheehan et al. 1998; Borowitzka 2013b for summaries), and now even greater efforts are under way. Although technically feasible, the major barrier to commercialisation remains in the fact that production of algal biofuels still is far too expensive (Stephens et al. 2010; Sun et al. 2011) and major improvements are needed in all steps of the process from algae culture to harvesting and fuel production (Fon Sing et al. 2013). The perceived advantages of

algae over terrestrial energy crops are that species exist which can be grown on a saline water source. Furthermore, although higher plant energy crops and algae both require fertilizer for maximum productivity, the effective utilisation of the applied fertilizer is much better for algae as they are grown in enclosed containers such as ponds or photobioreactors. However, large scale production of biofuels from algae will require large amounts of nutrients (N & P) and thus will compete for these with the fertilizer requirements of agriculture, even is wastewater is used. The key issues and requirements for environmentally and commercially sustainable microalgal biofuel production have been recently reviewed by Borowitzka and Moheimani (2013b).

Although much of the effort has been on lipid-rich microalgae for the production of biodiesel, alternatives such as continuous hydrocarbon production using *Botryococcus braunii* are being explored (Moheimani et al. 2014; Griehl et al. 2014). An alternative to using the lipids and hydrocarbons to produce liquid fuels exist where the whole biomass is converted to a fuel feedstock by hydrothermal liquefaction (Eboibi et al. 2014). Hydrogen production using microalgae has also been suggested (Benemann 2000). However, most studies have been limited to small indoor laboratory studies with only a few outdoor studies and the process is far from being scalable and commercially viable (Dasgupta et al. 2010; Geier et al. 2012).

5 Food and Fertilizer

Microalgae have also often been considered as an important protein source especially well suited for developing countries (Soeder 1976). However, despite extensive research in the 1980s on cyanobacteria and green algae in India (Becker and Venkataraman 1982), Thailand (Payer et al. 1978), Peru (Castillo et al. 1980) and elsewhere, this has not become a reality except at village-scale in parts of India (Jeeji Bai 1998). As with microalgae for biofuels, it is the high cost of microalgal protein compared to other sources combined with the unconventional nature of the food that has limited its widespread application (Babu and Rajasekaran 1991).

On the other hand, the use of nitrogen-fixing cyanobacteria as a fertilizer, especially in rice cultivation, is practiced widely in Asia, especially in India and Vietnam (Kulasooriya 1998; Vaishampayan et al. 2001). Microalgae such as *Chlorella* spp. have also been used as soil conditioners to improve soil water retention and nutrient availability to plants (Metting 1988). The possibility of producing nitrogen fertilizer by the culture of N_2-fixing cyanobacteria is also of great interest (Benemann 1979; Moreno et al. 2003; Silva and de Jesus Silva 2013).

Aside from their use as biofertilizers, cyanobacteria and microalgae have been shown to produce plant growth-promoting phytohormones (Stirk et al. 2002; Hashtroudi et al. 2013).

6 Genetic Improvement

Genetic improvement of microalgae to produce strains with superior characteristics such as better light utilisation efficiency or enhanced product formation potentially can be carried out by various means such as:

Breeding
Mutagenesis
Genetic engineering

In those species of microalgae which undergo sexual reproduction there is the potential to use basic 'breeding' methods to enhance desirable traits. The best understood system for this approach is the green alga *Chlamydomonas* having strong + and – mating types allowing genetic manipulation through breeding (Lewin 1951; Pröschold et al. 2005). *Dunaliella salina* has a similar mating system, but as yet no strong mating types have been isolated (Ruinen 1938; Huismann and Borowitzka, unpublished results). However, for most microalgae their life-cycles and sexual reproduction (if any) are poorly understood or sexual reproduction has never been observed and no breeding systems exist. Furthermore, breeding, possibly combined with mutagenesis, is a slow process.

Mutagenesis has been used to produce carotenoid-overproducing strains or strains with other desirable characteristics for several microalgae species such as *D. salina* (Shaish et al. 1991; Jin et al. 2003), *H. pluvialis* (Tripathi et al. 2001; Chen et al. 2003), *Chlorella* spp. (Cordero et al. 2011) and others (Suzuki et al. 1999; Doan and Obbard 2012).

6.1 Genetic Engineering, Molecular Biology and the 'Omics'

Advances in molecular biology of the microalgae in the recent decades, accompanied by developments in metabolomics, genomics, transcriptomics and proteomics as well as in bioinformatics, are providing powerful toolkits which can be applied to the development of new and improved algal strains, products and processes. The genomes of a number of microalgae species have been fully sequenced [e.g. the diatoms *Thalassiosira pseudonana* (Armbrust et al. 2004) and *Phaeodactylum tricornutum* (Bowler et al. 2008), the chlorophyte *Ostreococcus tauri* (Derelle et al. 2006) and several species of the eustigmatophyte *Nannochloropsis* (Pan et al. 2011; Radakovits et al. 2012; Vieler et al. 2012; Jinkerson et al. 2013)], and the sequencing of other microalgae such as *Dunaliella* is in progress. Metabolomics and proteomics are assisting the annotation of the sequenced genomes and the assessment and refining of metabolic network models which will aid efforts to modify selected metabolic pathways (May et al. 2008; de Oliveira Dal'Molin et al. 2011). Similarly, studies of the transcriptomics of microalgae are providing insights into metabolic pathways and their regulation which will provide important aids to enhancing the

production of specific compounds in algae (Cadoret et al. 2012; Gao et al. 2012; Ioki et al. 2012; Kristof and Sheshadri 2012; Lv et al. 2013).

Genetic engineering to produce transgenic cyanobacteria is well developed and, thanks to their similarity to other prokaryotes such as *Escherichia coli*, many methods for genetic manipulation of these organisms so that a wide range of transgenic cyanobacteria have been created since the 1970s (Heidorn et al. 2011; Ruffing 2011). The development of molecular genetic techniques for the in vivo analysis of gene function and regulation, the manipulation of endogenous genes, and the introduction and expression of foreign genes in eukaryotic algae is in a much earlier stage of development and so far is limited to a small number of species (Cadoret et al. 2012; Rasala et al. 2013). The best developed 'molecular toolkits' are for the green algae *Chlamydomonas reinhardtii* and *Volvox carterae*, and the diatom *Phaeodactylum tricornutum*. However, DNA transformation has also been achieved for more commercially important algae species such as *Haematococcus* (Steinbrenner and Sandmann 2006), *Dunaliella* (Wang et al. 2007; Li et al. 2008). The potential of these molecular techniques was first demonstrated by the conversion of the obligate photoautotrophic diatom *P. tricornutum* into a heterotroph through the introduction of a human glucose transporter gene (Zaslavskaia et al. 2001).

By far the best studied alga is *Chlamydomonas reinhardtii* with nuclear transformation first being achieved in the late 1980s, soon followed by chloroplast transformation (Kindle and Sodeinde 1994; Purton 2007). There now exist a range of transformation techniques, selectable markers and reporter genes, sophisticated vectors for foreign gene expression and for silencing of target genes by RNA interference (RNAi), and DNA tagging techniques for identifying novel genes, promoters, and enhancer elements for this alga. The use of *C. reinhardtii* as a 'cell factory' for producing proteins, including therapeutic proteins is being studied intensively but only transgene expression in the chloroplast has led to protein accumulation to economically viable levels (Rasala and Mayfield 2010). Although transgene expression from the nuclear genome offers several advantages over chloroplast expression, such as glycosylation and other post-translational modifications and heterologous protein-targeting to sub-cellular locations or for secretion (León-Bañares et al. 2004), the molecular mechanism(s) for the poor transgene expression from the nuclear genome are not yet completely understood. However, some progress is being made, and recently Rasala et al. (2012) a xyalanase-excreting strain of *C. reinhardtii*.

Molecular methods are also being developed to improve the yield of particular products of commercial interest such as carotenoids or lipids.

In order to achieve commercial production and use of transgenic algae and their products much research and development is still required. Furthermore, the potential environmental or other risks of large-scale production still need to be evaluated (Flynn et al. 2013). The current regulations concerning the use of transgenic plants were not designed for organisms such as algae which have markedly different reproductive and dispersion mechanisms compared to higher plants.

7 Algae Production Systems

The first step in the commercial application of algae is the cultivation of the algae to produce the desired biomass at the required scale and cost. For microalgae the available cultivation systems can be broadly classified as 'open' and 'closed' systems (Borowitzka 1999). In open systems the algae cultures are directly exposed to the environment, whereas in closed systems the cultures are contained within a more or less sealed, and usually transparent, container. Currently almost all commercial microalgae production is in open systems, with the paddle wheel mixed raceway pond being the most common. The other main types of open systems are the extremely large, unmixed shallow ponds used for the cultivation of *D. salina* in Australia, the central pivot ponds used for the cultivation of *Chlorella* in Asia, the very shallow sloping cascade ponds in the Czech Republic for the cultivation of *Chlorella*, and the deep tank systems used in the production of microalgae for aquaculture feed. More details of open systems can be found in recent reviews (Borowitzka 2005b; Borowitzka and Moheimani 2013a). The reason for the popularity of these systems is that the cost of producing algal biomass in such systems is considerably lower than cultivation in closed photobioreactors. Indeed, the major challenge to the development of any new commercial products or processes using microalgae is the ability to produce the algal biomass at a low cost. This has recently led to a re-evaluation of the design of raceway ponds to improve the efficiency of water circulation and thus reduce operating costs (Chiaramonti et al. 2013; Liffman et al. 2013). Commercially viable production also requires the culture to be reliable for long periods with a high productivity.

Although a plethora of different designs and configurations of closed photobioreactors has been developed, the cost of production of algal biomass in these reactors is significantly higher than the cost of production in raceway ponds. The most common types of closed photobioreactors are various configurations of tubular photobioreactors and panel photobioreactors (Pulz 2001; Chini Zittelli et al. 2013). Tubular photobioreactors are used for the commercial production of *Haematococcu*s in Israel and *Chlorella* in Germany and the *Haematococcus* plant at Kibbuz Ketura, Israel, has over 300 km of glass tubing in the culture system. As yet there is no significant commercial production of microalgae using panel-type photobioreactors, although some of these designs are showing promise (e.g., Rodolfi et al. 2009). For the production of microalgae for aquaculture large bags of up to 10,000 L or large bubble-column reactors are often used. Other closed photobioreactor types include the dome-shaped photobioreactors (Sato et al. 2006) used in Hawaii and internally-lit vat-type photobioreactors both of which are used by Fuji Chemicals for the production of *H. pluvialis* and astaxanthin.

There is ongoing debate on the relative merits and disadvantages of 'open' *vs*. 'closed' culture systems (Grobbelaar 2009), but as yet closed photobioreactors have proven suitable only for extremely high value products because of the higher capital and operating costs. Open ponds, such as raceway ponds, have been shown to be suitable for the long-term (i.e. greater than 6 months) culture of a range of freshwater, marine and halophilic algae. In most cases these algae grow in a selective envi-

ronment such as high salinity (e.g., *D. salina*), high alkalinity (e.g., *Arthrospira*) or high nutrients (e.g., *Chlorella*, *Scenedesmus*, *Phaeodactylum*), but other species also have the capability of outcompeting any potential contaminating organisms (e.g. *Pleurochrysis carterae* – Moheimani and Borowitzka 2006; *Tetraselmis* sp – Fon Sing and Borowitzka 2015), and have been grown successfully outdoors for long periods. Similarly, not all algae species can be grown reliably and for long periods in closed photobioreactors where sensitivity to shear created by the circulation system and the predilection of many species to 'stick' to the photobioreactor walls limit the choice of species which can be cultured successfully. Closed photobioreactor systems also have a shorter light path which means that the algae can be grown at a higher cell density potentially reducing harvesting costs.

Irrespective of the culture system used, the reliable high-productivity culture of microalgae requires the selection of strains which are well suited to the environmental conditions, especially the temperature range experience over the whole year that they will be exposed to at the place where they are being grown (Borowitzka 2013c). Furthermore, since light is the energy source for all microalgae (except those grown heterotrophically) the efficient use of the available light is all important and is a critical factor in the design of the culture systems and the operational management of the cultures (Richmond 1996). There is a strong interaction between temperature and light and microalgae use the available light most efficiently when the temperature is optimal (Collins and Boylen 1982). As almost all large-scale cultures are also carbon limited the supply of additional C, usually as carbon dioxide but sometimes also as bicarbonate, is important to achieve high productivities (Moheimani 2013). An excellent overview of the main biological principles of the mass cultivation of photoautotrophic microalgae has recently been published by Richmond (2013).

8 Conclusion

Microalgal biotechnology is still in the relatively early stages of development. Although commercial production of microalgae and microalgal products has been carried out for over 40 years, new products and processes using a wider suite of species are being developed, as are new and improved cultivation systems, harvesting methods and in further downstream processing. New markets and applications are also being developed. Furthermore, the potential of molecular biology and the new 'omics' is still being explored and is also expected to become an important part of the future expansion and success of algal biotechnology.

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