Energy from Microalgae: A Short History

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We are like dwarfs sitting on the shoulders of giants. We see more, and things that are more distant, than they did, not because our sight is superior or because we are taller than they, but because they raise us up, and by their great stature add to ours.

John of Salisbury – Bishop of Chartres (1159) 'Metalogicon'.

1 Introduction

The current extensive research and development activities on microalgae as commercial sources of renewable fuels and energy rely on the basic and applied research on biology, physiology, culture methods, culture systems etc. undertaken in the past. This chapter provides a brief overview of some of the major steps in the development of R&D on the mass culture algae for practical applications and commercial products, with a particular focus on microalgae as sources of renewable energy. It is almost impossible to cover all of the advances made, both small and large, over the last 140 years or so, but this chapter attempts to highlight the development and evolution of many of the key concepts and research in the field. The reader is also referred to the excellent review of the history of applied phycology written by Carl Soeder (1986).

Much of the development of large-scale microalgae production can be traced through the chapters of a small number of key books. In 1952 an Algae Mass Culture Symposium was held at Stanford University, California, USA, bringing together most of the workers in the field at that time. One important outcome of this symposium what the publication of "*Algae Culture. From Laboratory to Pilot Plant*" edited by J.S. Burlew (1953a). This small, but very important, volume brings together almost all of the work done including the first larger scale outdoor trials made to date in the USA, Germany, Japan and Israel.

It took another 27 years until the publication of the next major book in the field, a compilation of papers presented at as Symposium on the production and use of micro-algae biomass held in Israel in 1978, and which brings together many of the major developments in the field since the Burlew book's publication (Shelef and Soeder 1980). The findings of research in India as part of a joint Germany-India research effort are summarised in the books by Becker and Venkataraman (1982) and Venkataraman and Becker (1985). There was also a German-Egyptian research project (El-Fouly 1980). Clearly the interest in the commercial uses of microalgae was rapidly growing and in 1988 the first book focusing on algal biotechnology edited by Amos Richmond (1986) was published, soon to be followed by the book edited by Michael and Lesley Borowitzka (1988b) and by Becker (1994). Since then other books on this topic have been published (e.g., Richmond 2004), and also several books on particular species of interest (e.g., Avron and Ben-Amotz 1992; Vonshak 1997; Ben-Amotz et al. 2009) have been published.

2 The Pioneers

The culturing of microalgae in the laboratory is only about 140 years old, and the commercial farming of microalgae less than 60 years. Compare this with the thousands of years history of farming other plants.

Early attempts at culturing microalgae include those of Cohn (1850) who cultivated the chlorophyte *Haematococcus pluvialis* in situ, and Famintzin (1871) who cultured the green algae *Chlorococcum infusionum* and *Protococcus viridis* (now known as *Desmococcus olivaceus*) in a simple inorganic medium. Modern microalgae culture started with the culture experiments of Beijerinck with *Chlorella vulgaris* (Beijerinck 1890) and the apparent axenic culture of diatoms by Miquel (1892). Once algae could be cultured in the laboratory

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reliably, study of their nutritional requirements and their physiology were possible (e.g., Warburg 1919). Further improvements in laboratory culture, including the culture of axenic strains can be found in the book by Pringsheim (1947), and continuous culture was developed by Ketchum, Redfield and others (Ketchum and Redfield 1938; Myers and Clark 1944; Ketchum et al. 1949). All work on microalgae whether in the laboratory or in the algae production plant owes a great debt to these pioneers of phycology.

3 The Early Years (1940s & 1950s)

The idea that microalgae could be a source of renewable fuels also has a long history. Harder and von Witsch were the first to propose that microalgae such as diatoms might be suitable sources of lipids which could be used as food or to produce fuels (Harder and von Witsch 1942a, b) and later Milner (1951) also considered the possibility of photosynthetic production of oils using algae. In a detailed study, Aach (1952) found that *Chlorella pyrenoidosa* could accumulate up to 70% of dry weight as lipids (mainly neutral lipids) in stationary phase when nitrogen limited (Fig. 1.1). This study is also the first use of an internally lit photobioreactor which allowed an estimation of photosynthetic efficiency.

It was recognized that although microalgae could accumulate very high levels of lipids, the actual lipid productivity was low. As the need for liquid fuel alternatives also was no longer a problem post World War II the focus of research for the application of microalgae turned to these algae as a potential protein and food source (Spoehr and Milner 1948, 1949; Geoghegan 1951).

Work on larger-scale culture and the engineering requirements for algae production systems began at the Stanford Research Institute, USA in 1948–1950 (Cook 1950; Burlew 1953a, b), in Essen, Germany, where the utilization of CO_2 in waste gases from industry was a possibility (Gummert et al. 1953), and in Tokyo, Japan (Mituya et al. 1953) (Fig. 1.2). Smaller scale studies were also carried out by Imperial Chemical Industries Ltd in England by Geoghean (Geoghegan 1953) and Israel (Evenari et al. 1953). All of these studies used strains of *Chlorella*. The first significant outdoor pilot plant studies on the production of *Chlorella* were carried out in 1951 at Arthur D. Little Inc. in Cambridge, Massachusetts, USA (Anon 1953). This was a seminal study and the details and findings are worthy of summary here. Two types of 'closed' microalgae culture systems, which are now usually called 'closed photobioreactors', were developed and tested (see Fig. 1.2). The first consisted of thin walled (4 mm) polyethylene tubes which, when laid flat had a width of 1.22 m. Two parallel tubes were laid flat with a length of about 21 m with a U-shaped connection between the two tubes at one

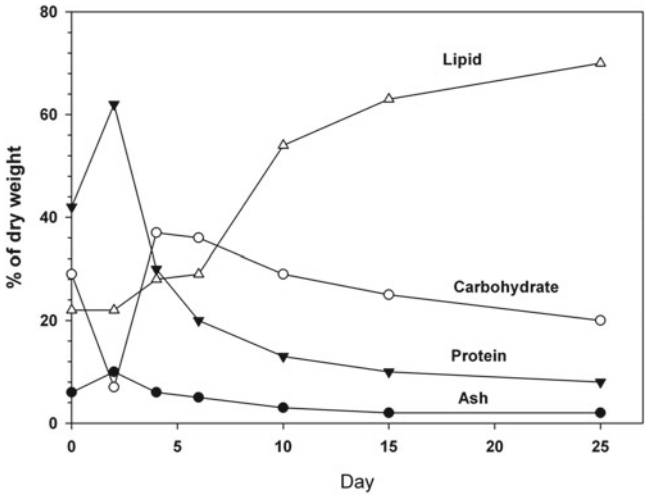

Fig. 1.1 Changes in proximate composition of *Chlorella pyrenoidosa* in batch culture showing accumulation of lipid as nitrogen is depleted (Redrawn from data in Aach 1952)

end. The total culture area was approximately 56 m^2 and the volume was 3,785–4,542 L. A heat exchanger was also installed. Circulation was by means of a centrifugal pump achieving flow rates of about 9 cm s^{-1} at a culture depth of 6.4 cm. The second unit designed to allow greater flow rates was straight, had no U-bend and an actual flow channel width of ~0.38 m and a length of 21.6 m, and achieved flow rates of about 30 cm s^{-1}. In both systems filtered air enriched with 5% CO_2 was provided and pH was maintained at about pH 6 by the periodic addition of dilute nitric acid. For inoculum production 10 vertical aerated (air + 5% CO_2) Pyrex columns (13.2 cm diameter × 1.8 m high) were used.

The first culture unit was operated for a total of 105 days in semi-continuous mode with daily harvests and with and without medium recycling after harvest between July and October (i.e. late summer). The average productivity over this period was about 6 g m^{-2} day^{-1} with productivities of up to 11 g m^{-2} day^{-1} achieved over shorter periods. The second culture system was operated from October to December, and daily productivities reached as high as 13 g m^{-2} day^{-1}. However, productivity was generally much lower due to declining weather conditions over this period (including snow!). This important study showed that reasonably long-term larger-scale outdoor culture of microalgae and recycling of the medium was possible, but it also highlighted the several, now well known, problems which can be experienced with 'closed' photobioreactors. For example, (i) cooling was necessary to maintain the culture below 27°C; (ii) contamination by other algae (especially *Chlorococcum*) and protozoa could not be eliminated; and (iii) adequate flow rates were essential to prevent the algae settling and sticking of the algae to the reactor walls could be a problem.

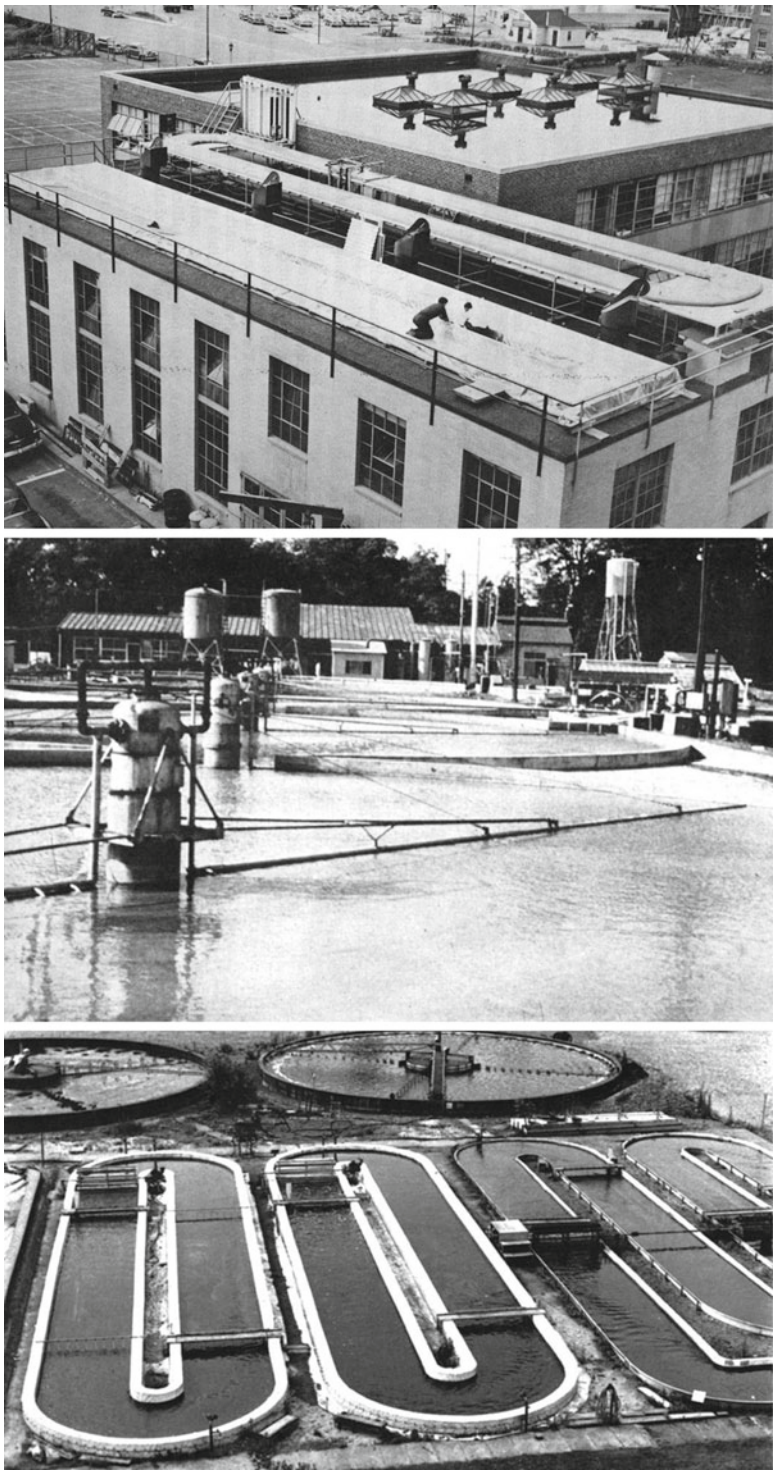

Fig. 1.2 Early large-scale algae culture systems. *Top*: Tube-type reactors on the roof of the building at Cambridge, Massachusetts, USA in 1951. The first unit in the *background* is in operation and the second unit in the *foreground* is under construction. The *glass columns* used to generate the inoculum can be seen at the *left* (From Anon 1953). *Middle*: Circular algae ponds at the Japanese Microalgae Research Institute at Kunitachi-machi, Tokyo (From Krauss 1962). *Bottom*: The outdoor algae ponds at the Gesellschaft für Strahlen- und Umweltforschung, Dortmund, Germany. The raceway ponds in the *foreground* are 20 m long and the circular ponds in the *background* have a diameter of 16 m (From Soeder 1976)

A German study (Gummert et al. 1953) carried out at the same time compared large-scale culture of *Chlorella pyrenoidosa* in 100 and 200 L tanks (15–21 cm deep) in a glasshouse with plastic lined, inclined trenches (9 m long, 70 cm wide, 20–24 cm deep at the low ends). The slope of the trenches was 6 mm m^{-1} and they were filled with 600 L culture with a culture depth of 9–15 cm. the tanks and the trenches were aerated with 1% CO_2 in air. The cultures were operated in semi-continuous mode for up to 10 days with the medium being recycled. As with the US pilot plant, contamination of the cultures with other algae and protozoa were issues at times, and it was recognized that the level of contamination

was greatly influence by climatic conditions as these affected the growth of the *Chlorella*. Some control of cyanobacterial contaminants was achieved by reducing the calcium concentration in the medium. It was also found that *Scenedesmus* (originally a contaminant) appeared to be more resistant to protozoan grazing, possibly because the *Scenedesmus* cells are larger than those of *Chlorella*.

In Wageningen, the Netherlands, larger-scale outdoor microalgae started in 1951 in 1 m^2 concrete tanks at a depth of 30 cm (Wassink et al. 1953), while in Russia large-scale outdoor cultivation of algae began in 1957 (see Gromov 1967 for review). Work in applied phycology began in Florence, Italy, in 1956, with a small pilot plant established in 1957 (Florenzano 1958).

These studies, as well as ongoing work in Japan (Sasa et al. 1955; Morimura et al. 1955; Kanizawa et al. 1958), using what we would now call both 'open' and 'closed' culture systems or photobioreactors, were the first steps from the laboratory towards eventual commercial microalgae production and identified most of the key issues still facing any attempts at commercial-scale microalgae production. Sasa et al. (1955) also were the first to do a detailed study of the seasonal variation in algae productivity over a whole 12 months period using a range of strains with different temperature tolerances. They demonstrated that, for year round culture, the species must have a wide temperature tolerance.

Another new application of microalgae, the use of algae in wastewater treatment, was proposed by Oswald and Gotaas (1957) following from the work of Oswald et al. (1953) on the oxygen-supplying role algal photosynthesis plays in sewage oxidation ponds. The option of generation energy from methane produced by fermenting the algal biomass obtained was also recognised (Golueke et al. 1957). Interestingly very little work has been done on microalgal biomass fermentation rather than methane production from seaweeds since then (c.f., Uziel 1978; Matsunaga and Izumida 1984; Chen 1987), and only now is this important topic again receiving attention.

At the same time as the above larger-scale culture experiments were taking place, important fundamental advances were being made in our understanding of algae light capture and photosynthesis. The study of photosynthesis and the efficiency of light utilization has been, and continues to be, central to attempts to optimize the productivity of algae cultures and is essential if the high-productivity cultures required for algae biofuels production are to be achieved. Kok (1948) demonstrated that about eight quanta are used per molecule of O_2 evolved although others (e.g., Pirt 1986) have suggested that fewer quanta are required. At Berkeley, USA, the pathway of carbon fixation in photosynthesis was also being elucidated by Calvin and Benson with the first paper of many published in 1948 (Calvin and Benson 1948) and including the discovery of the key role of ribulose 1,5-bisphosphate carboxylase (Quayale et al. 1954). The observation that alternating periods of light and dark enhanced the efficiency of light utilisation by algae (e.g., Emerson and Arnold 1932; Ricke and Gaffron 1943) – a phenomenon now generally known as the 'flashing-light effect' – led to further studies by Kok (1953, 1956) and Phillips and Myers (1954). The importance of the flashing-light effect and the duration of the light/dark cycles, and how these might be utilized in improving the productivity of algae cultures remains a topic of research and discussion (e.g., Laws 1986; Grobbelaar 1989, 1994; Grobbelaar et al. 1996; Nedbal et al. 1996; Janssen et al. 1999).

Another important discovery was made by Pratt who demonstrated that laboratory cultures of *Chlorella* could produce an autoinhibitor affecting both growth and photosynthesis (Pratt and Fong 1940; Pratt 1943). The problem of autoinhibition in high density cultures was later recognized (Javamardian and Palsson 1991) and the question of whether autoinhibitory substances reduce growth when culture medium is recycled is a current unresolved issue (Ikawa et al. 1997; Rodolfi et al. 2003).

Hydrogen production by algae in the light was also first demonstrated in 1942 (Gaffron and Rubin 1942), but the possibility of using microalgae hydrogen for energy was not considered until later.

Another important development at this time was the study of the sexuality and genetics of *Chlamydomonas* by Ralph Lewin (1949, 1951, 1953, 1954) which built on the earlier studies of Pascher (1916, 1918), Moewus (see excellent summary of Moewus' work by Gowans 1976), and Lerche (1937), paving the way for future genetic manipulation of microalgae (see review by Radakovits et al. 2010).

4 The 1960s and 1970s

By the beginning of the 1960s the understanding of many aspects of photosynthesis, microalgal biology, physiology and nutrition had come a long way (see for example: Hutner and Provasoli 1964).

Although the initial phase of work on microalgae mass culture in the USA had largely ceased by the mid-1950s it was revitalised at the beginning of the 1960s by William (Bill) Oswald and colleagues at the University of California, Berkeley who focused on the large-scale culture of algae for biomass production and for wastewater treatment (Oswald et al. 1957; Oswald and Golueke 1960). In the early 1960s a 2,700 m^2 (about 10^6 L capacity) meandering pond was constructed at Richmond, California (Oswald 1969a, b). The research carried out here eventually led to the construction of large-scale wastewater treatment ponds at several locations in California and which are still in operation (Oswald 1988). In 1971, John H. Ryther and colleagues at Woods Hole

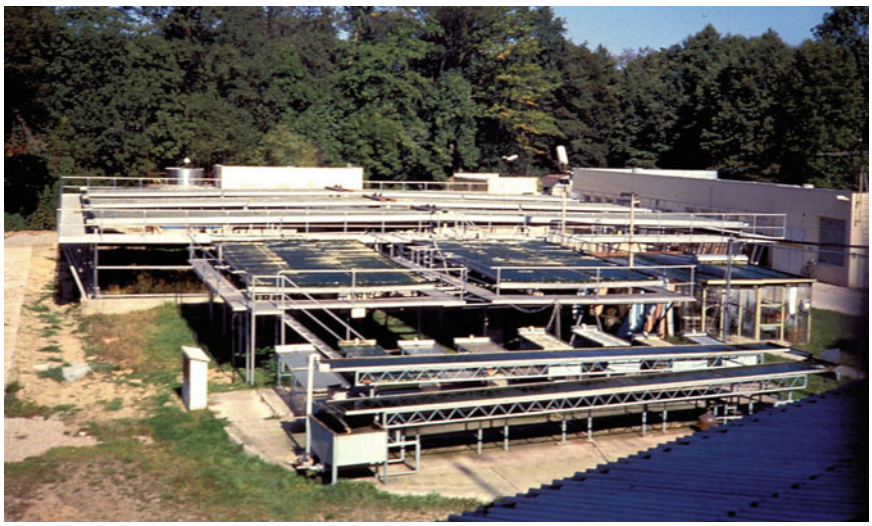

Fig. 1.3 The sloped cascade systems of the Academy of Sciences of the Czech Republic, Institute of Microbiology, Department of Phototrophic Microorganisms, at the Opatovicky mlyn near Trebon, Czech Republic

Oceanographic Institution, Massachusetts, USA, began work on the marine counterpart of Oswald's work starting with two small 4 m^2 (2,000 L) circular ponds (Goldman and Stanley 1974) and culminating in outdoor experiments with six 150 m^2 (35,000 L) ponds which were mixed by small pumps (Goldman and Ryther 1976; D'Elia et al. 1977; Goldman 1979). These studies, amongst other things, led to important advances in the understanding of nutrient requirements of the algae and limitations to growth, the effects of temperature and species succession in open ponds.

Work in Germany started in the 1950s continued at the Kohlenbiologische Forschungsstation in Dortmund where an extensive facility with four 80 m^2 paddle-wheel mixed raceway ponds and two 200 m^2 circular ponds similar to the Japanese design were constructed (Fig. 1.2) for studies of the freshwater algae *Scenedesmus* and *Coelastrum* (Stengel 1970; Soeder 1976, 1977). The Dortmund group also set up several algae research stations in collaboration with foreign governments in Thailand, Peru and India where the climate was better for algae culture than in Germany (Soeder 1976; Heussler 1980).

In 1960 the Laboratory for Microalgal Culture was established in Trebon, Czechoslovakia. In order to maximize productivity and yields, they developed a shallow sloping, extremely well mixed, culture system designed for the efficient utilization of light. The first unit built in 1960 had 12 m^2 of total surface area, and by the end of 1963 two 50 m^2 and one 900 m^2 units has been constructed (Setlik et al. 1967, 1970) and these, with some modifications, are still operational today (Doucha and Livansky 1995; Doucha et al. 2005) (Fig. 1.3). Similar 50 m^2 units were also built in Tyliez, Poland in 1966 and in Rupite, Rumania in 1968 (Vendlova 1969).

In Israel, the work of A.M. Mayer at the Hebrew University also continued in the 1960s with experiments in a 2,000 L, 1 m deep, tank which had a transparent side for better light supply to the algae (Mayer et al. 1964). Work on algae wastewater treatment was commenced by Shelef in the early 1970s with the construction of a 300 m^2 pond in Jerusalem modeled on Oswald's design (Shelef et al. 1973). In 1974 Amos Richmond commenced work on microalgae at the Beersheva campus of the Institute of Desert Research in 1974 with a number of 1 m^2 mini-ponds (Richmond 1976), later continuing the work at the Sede Boquer campus. Since then, the group a Sede Boquer has expanded and has made major contributions to the development of commercial scale algae culture, especially for *Spirulina, Porphyridium* and *Haematococcus* (e.g., Vonshak et al. 1982; Richmond 1988; Boussiba et al. 1997; Arad and Richmond 2004), and also towards our understanding of the limiting factors to outdoor microalgae production (e.g., Richmond et al. 1980; Richmond and Grobbelaar 1986; Hu et al. 1998b).

In France research on the mass culture of microalgae began at the Institute Petrole with research on culturing *Spirulina* (Clement et al. 1967; Clement 1975). At Oran in Tunisia and Antibes, France, several culture units between 5 and 700 m^2 were constructed. The culture units consisted of two adjacent horizontal channels, 10–20 cm deep, connected to a deep trough at each end. Circulation is by an airlift (using CO_2-enriched air) at opposing ends of the system so that the liquid is lifted up at one end of each through and flows down to the other end.

The first large-scale outdoor trials of growing *Dunaliella salina* were conducted in the Ukraine in the 1960s (Massyuk 1966, 1973; Massyuk and Abdula 1969)

Some work on large-scale outdoor tank culture of *Phaeodactylum tricornutum* was also undertaken in the UK (Ansell et al. 1963).

The state-of-the-art and a critical assessment of the possibility of using algae for energy towards the end of the 1970s was succinctly summarised by Oswald and Benemann (1977).

Fig. 1.4 Commercial *Chlorella* production farm near Taipei, Taiwan

Fig. 1.5 Early commercial *Spirulina* production ponds near Bangkok, Thailand

Importantly, the commercial production of microalgae, mainly for use as nutritional supplements and nutraceuticals, also started also in the 1960s (see below).

5 Commercial Production of Microalgae

Although microalgae have been harvested from natural populations (*Spirulina* and *Nostoc* spp) for food for hundreds of years in Mexico, Africa and Asia (Farrar 1966; Johnston 1970; Ciferri 1983), the 'farming' of microalgae was a very new development following from the early studies summarised above.

Unlike the early interest and studies on the mass culture of microalgae in the USA and Germany which had somewhat of a 'start-stop' pattern, those in Japan continued uninterrupted (Tamiya 1957; Krauss 1962) (Fig. 1.2) and eventually led to the development of a *Chlorella* industry in Japan and Taiwan in the early 1960s for use a heath food and nutritional supplements and expanded to China and other countries in Asia in the 1970s. Here the algae are grown in open pond systems, especially the circular centre-pivot systems (Fig. 1.4) or the closed circulation systems developed at the Tokugawa Institute for Biological Research, Tokyo, with mixotrophic culture using either acetate or glucose being common (Stengel 1970; Tsukuda et al. 1977; Soong 1980; Kawaguchi 1980). Harvesting is by centrifugation, followed by spray drying and breaking of the cells by bead mills or similar. This industry has been very successful and current annual production of *Chlorella* in Asia is about 5,000 t dry biomass, with a wholesale price of between US$20–30 kg^{-1}.

The first *Spirulina* (now known as *Arthrospira*) production plant was established in the early 1970s on Lake Texcoco near Mexico City, Mexico (Durand-Chastel 1980). This plant was however not really a controlled production system, but rather a managed harvest of the natural *Spirulina* population in the lake. This plant ceased operation in 1995. Other *Spirulina* production plants using raceway pond cultivation systems where developed in the early 1980s in the USA (e.g. Earthrise Nutritional LLC in California, and Cyanotech Corp in Kona, Hawaii) (Belay et al. 1994; Belay 1997). These two plants produced about 1,000 t $year^{-1}$ dry *Spirulina* biomass once fully operational. *Spirulina* plants were also established in Thailand (Tanticharoen et al. 1993; Bunnag et al. 1998; Shimamatsu 2004) (Fig. 1.5), and in the late 1990s *Spirulina*

production became established in China and rapidly grew with world production now estimated to be in excess of 5,000 t year^{-1} (Lee 1997; Li 1997; see also Fig. 1 in Borowitzka 1999).

The next microalgae to reach commercialization was the halophilic green alga, *Dunaliella salina,* as a source of β-carotene, with production plants being established in the early-mid 1980s in Israel, the USA and Australia. Much detail of the scientific journey from the laboratory to commercialization of *D. salina* in Australia has been published (Borowitzka and Borowitzka 1981, 1988a, b, 1989, 1990; Borowitzka et al. 1984, 1985; Moulton et al. 1987; Curtain et al. 1987; Schlipalius 1991; Borowitzka 1991, 1992, 1994). The two *D. salina* plants on Australia use extensive culture in very large (individual ponds up to 400 ha each and with a total pond area for each plant in excess of 700 ha), shallow unmixed ponds (Borowitzka 2005). Although this type of culture process means that productivity is much lower than in raceway ponds, low land costs, an extremely efficient low cost harvesting process, and an optimum climate for *D. salina* means that the Australian plants produce the algal biomass at a very low cost. On the other hand, the Israeli *D. salina* plant uses raceway ponds (Ben-Amotz and Avron 1990; Ben-Amotz 2004). Today production by the Australian and Israeli plants is estimated to be >1,000 t year^{-1} *Dunaliella* biomass and the extracted β-carotene sells for about US$600–3,000 kg^{-1} depending on formulation, mainly for use in the pharmaceutical and nutraceutical industries. Dried and stabilised whole algal biomass is also sold as use as a pigmenter in prawn feed (Boonyaratpalin et al. 2001).

In the late 1990s commercial production of the freshwater green alga *Haematococcus pluvialis* as a source of the carotenoid astaxanthin started at Cyanotech in Hawaii (Cysewski and Lorenz 2004). The culture system here is a combination of 'closed' tower reactors and raceway ponds. *Haematococcus* production by several other small producers commenced in Hawaii in subsequent years using a wide range of, mainly, 'closed' culture systems (e.g. Olaizola 2000). More recently, a large production plant in Israel, using a 2-stage culture process with combination of plate rectors and a large outdoor tubular photobioreactors has been established (Fig. 1.6). The astaxanthin from *Haematococcus* is mainly sold as a nutraceutical and antioxidant. The astaxanthin-containing algae are too expensive to use as a colouring agent in the farming of salmonids despite the algal biomass being a very effective pigmenter (Sommer et al. 1992). The heterotrophic production of *Crypthecodinium cohnii* as a source of eicosapentaenoic acid also commenced in the USA in the 1990s (Kyle et al. 1992; Barclay et al. 1994). In Germany, *Chlorella* is produced at Klötze in what is the world's largest tubular photobioreactor system (~700 m^3 volume, ~500 km of glass tube length) (Moore 2001).

The other important, and often overlooked, commercial production of microalgae is the production of microalgae as food for larval fish, mollusks and crustaceans and also in the grow-out diet of bivalve mollusks (Borowitzka 1997; Zmora and Richmond 2004; Neori 2011). Both in quantity and with respect to production cost, these algae are the most abundant and valuable produced. The high production cost is due to a combination of the species being grown and the relatively small-scale of the individual culture facilities.

Fig. 1.6 Commercial *Haematococcus pluvialis* production plant of Algatechnologies Ltd. in Israel (Courtesy Professor Sammy Boussiba)

Much can be learned from the experience of the commercial producers, however due to commercial sensitivity relatively little detailed information is publicly available.

6 The "Algae Species Programme" (USA)

The potential of algae as sources of energy was not completely forgotten and in 1960 Oswald and Golueke (1960) proposed the fermentation of microalgae biomass to produce methane as a source of energy. In 1980 the US Department of Energy began the 'Aquatic Species Programme (ASP)'. This initiative aimed to develop algae as sources of oils liquid fuels which would be able to compete with fossil fuels. Some earlier reports by Benemann and coworkers (Benemann et al. 1977, 1978) had suggested that this was possible.

The history of this programme and the main findings are summarised in detail by Sheehan et al. (1998) and a number of recommendations for future research are made. The reader is referred to this comprehensive report and only the major conclusions will be discussed here.

Sheehan et al. (1998) note in the conclusion to their report that 'perhaps the most significant observation is that the conditions that promote high productivity and rapid growth (nutrient sufficiency) and the conditions that induce lipid accumulation (nutrient limitation) are mutually exclusive. Further research will be needed to overcome this barrier, probably in the area of genetic manipulation of algal strains

to increase photosynthetic efficiency or to increase constitutive levels of lipid synthesis in algal strains'. With respect to photosynthetic efficiency they suggest that one approach is that photosynthetic productivity and light utilization could be maximized in microalgae by reducing the size of the light-harvesting antenna through mutation or genetic engineering as proposed by Neidhardt et al. (1998). This approach has been shown possible at the laboratory level (Melis et al. 1999).

They also point out that 'the ideal organism(s) for a biofuels production facility will likely be different for each location, particularly for growth in outdoor ponds. The best approach will likely be to screen for highly productive, oleaginous strains at selected sites, optimize growth conditions for large-scale culture, and optimize productivity and lipid production through genetic manipulation or biochemical manipulation of the timing of lipid accumulation in the selected strains. It is also likely that more than one strain will be used at a site, to maximize productivity at different times of the year.'

The ASP programme demonstrated that some species of microalgae could be cultivated reliably on a large scale for relatively long periods. These outdoor open pond studies showed that there were no fundamental engineering and economic issues that would limit the technical feasibility of microalgae culture, either in terms of net energy inputs, nutrient (e.g., CO_2) utilization, water requirements, harvesting technologies, or general system designs. However, although the productivities, in terms of total biomass and algal lipids (oils) achieved were high, they were still well below the theoretical potential, and (importantly) the requirements for economical viability. The authors of the report also note that the outdoor testing showed that most of the algae selected and tested in the laboratory could were not robust in the field and that, in fact, the best approach to successful cultivation of a consistent species of algae was to allow a contaminant native to the area to take over the ponds!.

Sheehan et al. (1998) also concluded that: 'the only plausible near- to mid-term application of microalgae biofuels production is integrated with wastewater treatment. In such cases the economic and resource constraints are relaxed, allowing for such processes to be considered with well below maximal productivities'. It remains to be seen whether this proves possible.

7 The RITE Biological CO_2 Fixation Programme (Japan)

In 1990 the Japanese Ministry of International Trade and Industry (MITI) through the New Energy and Industrial Technology Developments Organisation (NEDO) launched an innovative R&D programme including projects at the Research Institute of Innovative Technology for the Earth (RITE) to develop effective and clean methods of biological fixation of CO_2 based on the effective integration of photosynthesis functions of microorganisms (Michiki 1995). Although this initiative was not concerned with energy production, it is important within the context of the development of large-scale microalgae production. The RITE project had five major themes:

1. Highly-efficient photosynthesizing bacteria and microalgae with high CO_2 fixation capability;
2. Development of high-density, large-volume photosynthesis in culture systems (photobioreactors) for CO_2 fixation;
3. Development of technology to collect and utilise solar irradiance at the maximum efficiency;
4. The technology to produce useful substances and energy from microalgae;
5. The technology of the total system for CO_2 fixation and utilization at the maximum efficiency.

Unlike the US SERI programme there is no single source of information of the outcomes of the RITE programme and below I attempt to summarise some of the findings as published in the scientific literature.

The initial algae isolation and screening programme focused on high-CO_2–tolerant strains, strains which were acid and high temperature tolerant and strains with a high level of polysaccharide production (Hanagata et al. 1992; Kurano et al. 1995; Murakami and Inkenouchi 1997). The marine green alga *Chlorococcum littorale* was found to grow well at high CO_2 concentrations (Kodama et al. 1993; Chihara et al. 1994), whereas the rhodophyte *Galdieria partita* grew well at high temperature (50 °C) and acid pH (pH 1) and could tolerate 50 ppm SO_2 (Kurano et al. 1995; Uemura et al. 1997). The marine prasinophyte, *Prasinococcus capsulatus,* was the best strain isolated for extracellular polysaccharide production (Miyashita et al. 1993).

The focus of the culture systems was on closed photobioreactors with or without a solar collector to transmit light into the photobioreactor and included flat plate photobioreactors, internally lit stirred photobioreactors, and a dome-shaped photobioreactor (Usui and Ikenouchi 1997; Nanba and Kawata 1998; Zhang et al. 1999). Almost all of the studies were on a small lab-scale.

Using high cell density cultures (~80 g L^{-1}) in a flat panel photobioreactor high rates of CO_2 fixation of 200.4 g CO_2 m^{-2} day^{-1} could be achieved with *C. littorale* (Hu et al. 1998a). *C. littorale* could also produce ethanol by dark fermentation under anaerobic conditions (Ueno et al. 1998). Detailed studies on the physiology and biochemistry of this high-CO_2 tolerant alga were also carried out (e.g., Pesheva et al. 1994; Satoh et al. 2001) as were studies of some of the other species identified in the original screening programme (Suzuki et al. 1994; Uemura et al. 1997).

Some small-scale pond studies were also carried out near Sendai by Mitsubishi Heavy Industries and several electric utilities, in particular Tohoku Electric Co. Culture experiments were in small 2 m^2 raceway ponds using *Phaeodactylum tricornutum* and *Nannochloropsis salina* obtained from the NREL culture collection, and later with strains of *Tetraselmis* that spontaneously appeared and dominated the cultures (Negoro et al. 1993; Hamasaki et al. 1994; Matsumoto et al. 1995). The green alga, *Tetraselmis,* could be cultivated for the whole year with a annual mean productivity of about 11 g m^{-2} day^{-1}, whereas the cultures of the other two species were unstable.

These studies showed that microalgae could be grown on untreated CO_2-containing flue gas from power stations (Negoro et al. 1991, 1992), an important finding both for CO_2-bioremediation and for future work on growing microalgae for biofuels using power station flue gas as a CO_2 source.

8 Other Work

8.1 Botryococcus

While the studies in the USA focussed mainly of algae growing is saline water, the discovery in Australia that the green alga, *Botryococcus braunii*, produces long-chain hydrocarbons (Wake and Hillen 1980; Wake 1984) led to extensive studies of this species, especially in Europe. *Botryococcus braunii* is unusual in that it produces high levels of long-chain hydrocarbons (botryococcenes) and related ether lipids which have great similarity to fossil oils (Moldowan and Seifert 1980) and which could be a source of renewable fuels (Casadevall et al. 1985). These hydrocarbons are mainly accumulated in the extracellular matrix (Largeau et al. 1980; Bachofen 1982; Wake 1983) leading to the attractive concept of non-destructive extraction of the hydrocarbons. This alga has been studied extensively since the 1980s (see review by Metzger and Largeau 2005), but its slow growth has so far means that it is an unlikely candidate for commercial biofuels production.

8.2 Hydrogen

The discovery of Gaffron and coworkers (Gaffron 1939; Gaffron and Rubin 1942) that unicellular green algae were able to produce H_2 gas upon illumination was seen initially as a biological curiosity. In the 1970s biological production of H_2 became the subject of extensive applied research in the USA, Japan and Europe (Mitsui and Kumazawa 1977; Zaborski 1988), research which continues (Miyake et al. 2001). The concepts and developments have been extensively reviewed (Benemann 2000, 2009; Melis and Happe 2001).

8.3 Closed Photobioreactors

Work on closed photobioreactors, which has started with the work in the USA again gained impetus in the 1980s. In France Claude Gudin and Daniel Chaumont at the Centre d'Etudes Nucléares de Cararache constructed a tubular photobioreactor made of 64 mm diameter polyethylene tubes each 20 m long and with a total length of 1,500 m. They used a double layer of tubes with the culture in the upper layer of tubes. Temperature control was by placing the tubes in a pool of water and either floating or submerging the tubes by adjusting the amount of air in the lower tubes (Gudin 1976). The is pilot plant had five identical units, 20 m^2 in area with a total volume of 6.5 m^3 (Gudin and Chaumont 1983; Chaumont et al. 1988). This pilot plant operated from 1986 to 1989 and achieved productivities of 20–25 g m^{-2} day^{-1} with *Porphyridium cruentum*. In the UK at Kings College, London, Pirt and coworkers (Pirt et al. 1983) developed a tubular photobioreactor consisting of 52, 1 cm diameter, 1 m long glass tubes connected with silicone rubber U-bends to form a vertical loop, which they used to culture *Chlorella*.

Helical photobioreactors, consisting of flexible tubes wound around an upright cylindrical structure were first used in the laboratory by Davis et al. (1953) to grow *Chlorella*. A flattened version of this basic design and made of glass tubes was later used by Setlik et al. (1967), Jüttner (Jüttner et al. 1971; Jüttner 1982) and Krüger and Eloff (1981). Furthermore, Jüttner et al. (1971) developed an automated turbidostat system for continuous production of microalgae based on the principles outlined earlier by Myers and Clark (1944) and Senger and Wolf (1964). This basic tubular photobioreactor concept was developed further and improved for large-scale production and patented by Robinson and Morrison (1992). In their reactor, which they called the 'Biocoil', a number of bands of tubes were wrapped around an open cylinder for support and the bands of tubes are connected to a common manifold to equalise pressure within the tubes and reduce O_2 build-up due to shorter tube lengths, allowing the reactor to be scaled up to volumes of up to 2 m^3. At various times large (~ 1,000 L) reactors were operated in the UK (Luton and Dorking) (Fig. 1.7) and Melbourne, Australia, growing *Spirulina*, and in Perth, Australia, growing a range of microalgae including *Tetraselmis* and *Isochrysis* in continuous cultures for up to 12 months (Borowitzka, unpubl. results).

Flat plate-type photobioreactors also have a long history starting with the rocking tray of Milner (Davis et al. 1953) growing *Chlorella*, and later with designs by Anderson and Eakin (1985) growing *P. cruentum*, and Samson and LeDuy (1985) growing *Arthrospira* (*Spirulina*) *maxima.* Other, more sophisticated flat panel photobioreactors were developed in France (Ramos de Ortega and Roux 1986) and Italy (Tredici et al. 1991; Tredici and Materassi 1992) and later in Germany

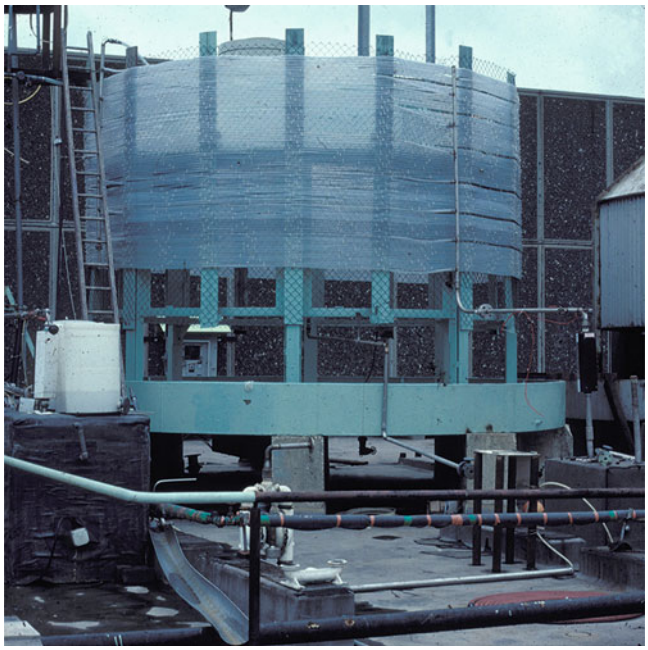

Fig. 1.7 The first pilot scale helical tubular photobioreactor (Biocoil) in Dorking, United Kingdom, located on the roof of a brewery in 1983. CO_2 from the brewing process was used to enhance the growth of *Spirulina*

(Pulz 2001) and Israel (Hu et al. 1996). Many versions of closed photobioreactors have been patented, especially in the last few decades (e.g., Ichimura and Ozono 1976; Selke 1976; Hills 1984; Huntley et al. 1991; Delente et al. 1992; Kobayashi 1997; Skill 1998; Buchholz 1999), and more details on the principal types of closed photobioreactors and their development can be found in the review by Tredici (2004) and Chapter 7 of this volume.

8.4 Downstream Processing

Although extensive work on the culture of microalgae was done up to the early 1980s relatively little work was done on the harvesting, dewatering and further processing of the algal biomass. Burlew (1953b) considered centrifugation or gravity settling, possibly followed by spray drying bur recognised that the costs of these processes at the large-scale had not yet been considered. Froth flotation as a method of harvesting was proposed by Levin et al. (1962), and the first studies of harvesting methods from sewage grown algae was that of Golueke and Oswald (1965) in the USA, and Caldwell, Connell Engineers (1976) in Australia, and the state-of-the art was reviewed by Benemann et al. (1980) and Shelef et al. (1984). It was recognised fairly early that the economics of commercial utilisation of microalgae was depended significantly on the cost of harvesting and dewatering (e.g., Soeder 1978). Of particular interest are also the detailed studies of the relative costs of different harvesting methods by Mohn and coworkers (Mohn 1980, 1988; Mohn and Cordero-Contreras 1990) based on detailed comparisons of different harvesting methods at Dortmund and Jülich in Germany.

9 Conclusion

It is impossible to do justice to all the work that has been done in the last 140 years on microalgae and the application of microalgae as sources of renewable energy. The above chapter is a short overview of the developments and highlights along the path of what we hope will be a commercially successful, renewable, and environmentally benign energy source for humanity in the future. Like most R&D efforts the path is full of bumps, interruptions, twists and turns and, at times, apparent dead ends. It is also the nature of applied research that much of the relevant information may not be found in the mainstream scientific literature, but in reports of contractors and consultants which may not be easily attainable. However, the effort to track down such reports can be very rewarding and very much can be learned from the extensive work that has been done.

The vagaries of research funding and political priorities have resulted in periodic intense bursts of research activity (e.g. the ASP programme in the USA and the RITE programme in Japan) and changes in the principal research focus (e.g. algae as sources of protein and nutrition, algae for high-value chemicals, algae for wastewater treatment, algae for biofuels, algae for CO_2 capture) and advances in basic biology and new research methods (e.g. studies on photosynthesis and the recent developments in molecular biology and the sequencing of algal genomes) have provided important new insights and opportunities. Much can be learnt from these past efforts, both the successes and the failures. Unfortunately recent papers on algae biofuels show that some people apparently have not learned from the experiences in the laboratory and in commercial microalgae production. However, most applied phycologists are very forward looking and inherently very optimistic, and they appreciate and recognise the important contributions made by their predecessors and build on these.

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