26 Lower Plants

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26.1 INTRODUCTION

The algae are an extremely diverse group of (mainly) photosynthetic organisms, ranging in size from approximately 1 µm for some picoplanktonic species to over 30 m in length for some of the large kelps. They are mainly aquatic, growing in fresh to hypersaline waters, but they are also found in soils and on many surfaces such as rocks, trees, and buildings. As photoautotrophs, algae use light energy to fix carbon dioxide (CO₂) into sugars during photosynthesis, and these are then further metabolized. Algae are being explored and developed as a source of a range of renewable, CO₂-friendly energy sources such as biodiesel, bioethanol, hydrogen, and methane (Chynoweth et al. 1987; Sheehan et al. 1998; Miyura 2002; Benemann 2004; Chisti 2007; Brennan and Owende 2010).

This chapter aims to provide a detailed overview of biofuels and energy production from algae, both the seaweeds and the microalgae.

26.2 METHANE

The extremely large biomass of seaweeds available in many parts of the world has the potential to be used as a source of renewable biofuel, either by fermentation to produce methane or through the production of ethanol from the sugars in the biomass. Renewable fuel production by the pyrolysis of macroalgae is also being considered (Ross et al. 2009).

The commercial use of seaweeds, especially the brown algae such as Laminaria (Saccharina) and Macrocystis and red algae such as Kappaphycus and Gracilaria, is a well established and very large industry. The industry uses wild stocks and cultivated biomass to produce biomass for use as foods and the production of hydrocolloids such as agar, carrageenan, and laminaran (Zemke-White and Ohno 1999; McHugh 2003). The annual harvest of wild seaweeds is estimated at approximately 1,000,000 t wet weight, and the amount produced by aquaculture at approximately 15,000,000 t wet weight (FAO 2006).
Methane production from macroalgal biomass, especially using the kelps *Macrocystis* and *Saccharina (Laminaria)*, the green alga *Ulva*, and the rhodophytes *Hypnea* and *Gracilaria*, has been studied by several authors (Hansson 1983; Habig et al. 1984; Schramm and Lehnberg 1984; Østergaard et al. 1993) and in the 1970s and 1980s extensive research was carried out on methane production, mainly from *Macrocystis pyrifera*, by the Marine Biomass Program in the United States (Flowers and Bird 1990; Chynoweth 2002).

The best anaerobic digestion systems for macroalgal biomass appear to be vertical flow reactors, especially when operated as an upflow solids reactor in which feed is added to the bottom of the reactor and effluent removed from the top of a nonmixed vessel. This system produces approximately 0.35 m$^3$ methane/kg volatile solids added at loading rates of 3.2 kg/m$^3$ per day (Chynoweth et al. 1987). The efficiency of the process can be improved using a two-stage system. In the first stage, digester marine algal hydrolysis and acidification occurs, but not conversion of volatile acids to methane. In the second stage, digester methanogenic bacteria convert the volatile acids to methane (Chynoweth et al. 1987). In the digestion of *Macrocystis* biomass, the methane yields are highly correlated with the mannitol and algin content of the biomass, with mannitol yielding approximately 75% more methane than algin. Similarly, methane yield in the red alga *Gracilaria* is closely correlated with the carbohydrate content or protein and carbohydrate content (Habig et al. 1984). On the other hand, the brown alga *Sargassum* is a poor feedstock, apparently because of the low mannitol content and an unidentified “fiber-like” component (Flowers and Bird 1990). More recently, trials on methane production using *Laminaria digitata* in Europe (Morand et al. 1991) and beach-cast *Laminaria* and *Ulva* in Japan (Koike et al. 2005) have been conducted. In the latter test, the maximum methane yield was 22 m$^3$/t biomass.

The anaerobic digestion of microalgal biomass to produce methane has also been examined by several workers since the original study of Golueke et al. (1957). Algae harvested from wastewater treatment ponds (Chen 1987; Chen and Oswald 1998; Yen and Brune 2007) and unialgal laboratory cultures of *Chlorella*, *Dunaliella*, *Tetraselmis*, *Scenedesmus*, and *Spirulina* (Asinari Di San Marzano et al. 1982; Samson and Leduy 1982; Sanchez and Travieso 1993; Munoz et al. 2005) have been used as feed biomass. These studies have reported methane yields of 0.09–0.45 L/g volatile solids. High temperatures (>40°C) enhance methane conversion. De Schlamphelaire and Verstaete (2009) have developed a closed-loop system combining an algal growth unit for biomass production, an aerobic digestion unit to convert the biomass to biogas (methane), and a microbial fuel cell to polish the effluent from the digester. This system resulted in a power plant with a potential capacity of 9 kW/ha of solar reactor.

Microalgae generally have a high nitrogen (protein) content and therefore a low carbon-to-nitrogen ratio (C/N). This affects the performance of the anaerobic digester and can result in a significant release of ammonia during anaerobic digestion (Golueke et al. 1957; Samson and Leduy 1986); however, methanogenic bacteria can acclimate to high concentrations of ammonium (Koster and Lettinga 1984). Co-digestion with a high C/N material such as waste paper can result in a significant increase in methane production (Yen and Brune 2007).

The anaerobic digestion of marine microalgae also requires the use of salt-adapted microorganisms, which can tolerate the high salinities (Chen et al. 2008). Methane can also be produced from microalgal biomass by hydrothermal gasification at high temperatures (~350–400°C) and pressure in the presence of a nickel catalyst to produce a synthetic natural gas (Minowa and Sawayama 1999; Haiduc et al. 2009).

Sialve et al. (2009) have suggested that anaerobic digestion of microalgal biomass is the optimal strategy, on an energy-balance basis, for the energetic recovery from microalgal biomass. Furthermore, the nutrient-rich effluent of the digestion potentially can be recycled into new algal growth medium (Phang et al. 2000).

### 26.3 ETHANOL AND BUTANOL

The sugars and carbohydrates of algae may be fermented to produce ethanol or possibly butanol, both of which can be blended with petrol to produce a renewable transport fuel. For example, the brown
kelp *Saccharina latissima* (*Laminaria saccharina*) contains approximately 25% mannitol and 30% laminaran, a linear polysaccharide of \((1\rightarrow3)\)-\(\beta\)-d-glucopyranose, with the chains terminated by \(\alpha\)-mannitol. The bacterium *Zymobacter palmae* has been shown to be able to ferment the mannitol to ethanol (Horn et al. 2000a), and the yeast *Pichia angophorae* can use the mannitol and the laminaran; however, the ethanol yields are still low and the process requires further optimization (Horn et al. 2000b). Laminaran can also be fermented by the yeast *Saccharomyces cerevisiae* when used in combination with the enzyme laminarase (Adams et al. 2008). Recently, a process for ethanol production from seaweeds has been patented (Kim et al. 2008). The sugars produced by microalgae can, of course, also be fermented to produce ethanol (Nakas et al. 1983; Ueda et al. 1996). Microalgae such as *Chlorella* have a high starch content (~30-40% of dry weight) and an up to 65% ethanol conversion efficiency has been reported (Anonymous 1995; Hirano et al. 1997). Ueno et al. (1998) have also produced ethanol from the marine green alga *Chlorococcum littorale* in a dark fermentation process. Ethanol-producing cyanobacteria have also been developed (Fu and Dexter 2007; Lee 2008), and the ethanol can be recovered from the airspace above the medium in which the algae grow (Lee 2008; Woods et al. 2008).

Butanol is produced by anaerobic fermentation using solventogenic clostridia bacteria, such as *Clostridium acetobutylicum* and *C. beijerinckii*, and other bacteria, such as *Butyribacterium methylophoricum* and *Hyperthermus butylicus* (Dürre 2007). The clostridia secrete a wide range of enzymes that break down polymeric carbohydrates to various monosaccharides that are then taken up by the cells and metabolized. The current state of production of biobutanol has recently been reviewed by Ezeji et al. (2007). Butanol has several advantages over ethanol as a transport fuel in that it relatively less polar than ethanol and more similar to gasoline, making it easier to blend with gasoline. Algae are clearly a potential source of renewable biomass for biobutanol production; however, the only published study so far is on the production of butanol from algae (together with ethanol and 1,3-propanediol) is using glycerol-producing algae, such as *Dunaliella* spp. (Nakas et al. 1983).

### 26.4 PYROLYSIS

Pyrolysis is a thermochemical decomposition process in the virtual absence of oxygen. The pyrolysis of biomass produces char, a crude “bio-oil” and a noncondensable gas, which contains hydrogen, methane, and higher hydrocarbons. In “fast pyrolysis” the biomass is rapidly heated (in ~5–10 s) to between 400 and 500°C. In “slow pyrolysis,” the biomass is heated slower to less than approximately 400°C (Grierson et al. 2009). Fast pyrolysis produces more bio-oil than slow pyrolysis. The application of pyrolysis to produce liquid fuel from microalgae was first proposed by Ginzburg (1993) using *Dunaliella* biomass. Pyrolysis of microalgal biomass from the green algae *Chlorella protothecoides* and *Cladophora fracta* and the cyanobacterium *Microcystis aeruginosa* have given oil yields of up to 57.9% of the biomass dry weight (Peng and Wu 2000; Miao and Wu 2004a; 2004b; Demirbas 2006). Slow pyrolysis trials, which gave good bio-oil yields, have also been carried out with a range of microalgae species (Grierson et al. 2009). However, pyrolysis oils will require upgrading because they are acidic, unstable, viscous, and contain solids and chemically dissolved water (Demirbas 2001; Chiaramonti et al. 2007).

Pyrolysis of dried biomass of the coccolithophores, *Emiliania huxleyi* and *Gephyrocapsa oceanica*, at 300°C produced a high yield of liquid-saturated hydrocarbons, the major components of which were normal alkanes in a series ranging from \(nC_{11}\) to \(nC_{35}\) (Wu et al. 1999a), and increasing temperature to 400°C resulted a decrease in the liquid saturates and an increase in hydrocarbon gases, mainly methane (Wu et al. 1999b). Coccolithophorid algae seem to be particularly well suited to pyrolysis as they have a high lipid and hydrocarbon content including long-chain (\(C_{37}-C_{39}\)) alkenones and alkenoates (Volkman et al. 1995; Bell and Pond 1996; Pond and Harris 1996). There is also good evidence that the yield of hydrocarbon gases is dependent on the lipid content of the biomass (Wu et al. 1996).
26.5 LIPIDS AND BIODIESEL

Microalgae are seen as an important future source of renewable biodiesel. The potential advantages of microalgae as sources of liquid biofuels compared to other oleaginous or sugar producing plants such as canola, oil palms, jatropha, sugarcane, and corn is that they

- Have markedly higher annual productivities than land plants per unit land area when grown in intensive culture,
- Can be grown using saline water (at salinities up to NaCl saturation), thus not competing with food crops for scarce freshwater resources, and
- Can be grown on land unsuitable for agriculture.

Compared with fossil diesel, algae-derived biodiesel has also been shown to have a substantial positive greenhouse gas and energy balance in preliminary modeling (Campbell et al. 2009).

The recognition that algae are potential sources of lipids for the production of biodiesel is not new. Many species of microalgae contain high levels of lipids in the range of 20–60% of dry weight, although contents greater than about 30% are generally only found in nutrient-depleted stationary-phase cultures (Borowitzka 1988; Griffiths and Harrison 2009; Huerliman et al. 2010) (Table 26.1). The lipid composition (i.e., fatty acid composition, saturated/polyunsaturated fatty acid ratio, proportion of phospholipids, etc.) of algae varies between taxa and, to some degree, with growth conditions.

The bulk of microalgal lipids are $C_{14}$ to $C_{22}$ chain length esters of glycerol and fatty acids. Triglycerides are the most common storage lipids, and these may constitute up to approximately 80% of the total lipids in nutrient-starved nongrowing cells (Tornabene et al. 1983). These storage lipids are usually located as droplets in the cytoplasm. The other major algal lipids are sulfoquinovosyl diglyceride, monogalactosyl diglyceride, digalactosyl diglyceride, lecithin, phosphatidyl glycerol, and phosphatidyl inositol as the main membrane lipids (Guschina and Harwood 2006).

The lipids of the different algal taxa vary in the composition of the main fatty acids (Table 26.2), and these differences in the composition of the lipids affect the efficiency of the conversion process to biodiesel and the properties of the biodiesel. Biodiesel is produced from algal lipids by esterifying free fatty acids or transesterifying triacylglycerol fatty acids using an alcohol, usually

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**TABLE 26.1**

Range of Lipid Contents Reported for Microalgae

<table>
<thead>
<tr>
<th>Algal Class</th>
<th>Total Lipids (% dry weight)</th>
<th>Percent of Total Lipid</th>
<th>Hydrocarbons (% dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Neutral Lipids</td>
<td>Glycolipids</td>
</tr>
<tr>
<td>Cyanophyceae</td>
<td>2–23</td>
<td>11–68</td>
<td>12–41</td>
</tr>
<tr>
<td>Rhodophyceae</td>
<td></td>
<td>41–58</td>
<td>42–59</td>
</tr>
<tr>
<td>Cryptophyceae</td>
<td>3–17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinophyceae</td>
<td>5–36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillariophyceae</td>
<td>1–39</td>
<td>14–60</td>
<td>13–44</td>
</tr>
<tr>
<td>Heterokontophyta</td>
<td>12–72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyceae</td>
<td>1–70</td>
<td>21.66</td>
<td>6.62</td>
</tr>
</tbody>
</table>

a High value for B. braunii.
b High value for the coccolithophorid algae (Prymnesiophyceae).
methanol or ethanol (Demirbas 2003). Methanol is more reactive than ethanol, and the fatty acid methyl esters (FAMEs) produced are more volatile than the fatty acid ethyl esters (FAEEs) produced when ethanol is used. Methanol is also cheaper; however, methanol is produced from nonrenewable fossil fuel feedstocks whereas ethanol can be produced from renewable feedstocks (sugars). The transesterification processes are catalyzed with alkalis, such as NaOH, KOH, or sodium metoxide. Alternatively, acid-catalyzed transesterification with simultaneous esterification of free fatty acids can be carried out using sulfuric, hydrochloric, phosphoric, or sulfonic acid (Meher et al. 2006). However, acid-catalyzed transesterification has a slower reaction rate than alkali-catalyzed transesterification, and the acids are more corrosive, thus making the process more expensive. Other methods under development are lipase-enzyme-catalyzed transesterification (Ranganathan et al. 2008; Robles-Medina et al. 2009), noncatalytic conversion by transesterification and esterification under supercritical alcohol conditions (Kudsiana and Saka 2001), and the use of metal-oxide base catalysts at high pressure and temperature (McNeff et al. 2008). A range of solid heterogeneous catalyst processes that are potentially more effective than current methods are also under development (Helwani et al. 2010).

### TABLE 26.2
Summary of Principal Fatty Acids for the Major Classes of Microalgae

<table>
<thead>
<tr>
<th>Class</th>
<th>Major Fatty Acids</th>
<th>Representative Genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanophyceae</td>
<td>14:0, 16:0, 16:1(n-7), 18:1, 18:2(n-3), 18:3(n-3)</td>
<td>Oscillatoria, Spirulina</td>
</tr>
<tr>
<td>Rhodophyceae</td>
<td>16:0, 20:4(n-6), 20:5(n-3)</td>
<td>Porphyridium, Rhodella</td>
</tr>
<tr>
<td>Cryptophyceae</td>
<td>14:0, 16:0, 16:1(n-7), 18:3(n-3), 18:4(n-3), 20:5(n-3)</td>
<td>Chroomonas, Rhodomonas</td>
</tr>
<tr>
<td>Bacillariophyceae</td>
<td>14:0, 16:0, 16:1(n-7), 16:3(n-4), 20:5(n-3)</td>
<td>Chaetoceros, Navicula, Phaeodactylum, Skeletonema</td>
</tr>
<tr>
<td>Raphidophyceae</td>
<td>14:0; 18:3(n-3), 18:4(n-3), 18:5(n-3), 22:6(n-3)</td>
<td>Emiliania, Gephyrocapsa, Pleurochrysis</td>
</tr>
<tr>
<td>Pavlovophyceae</td>
<td>14:0, 16:0, 16:1(n-7), 20:5(n-3)</td>
<td>Pavlova</td>
</tr>
<tr>
<td>Prymnesiophyceae</td>
<td>14:0, 18:3(n-3), 18:4(n-3), 18:5(n-3), 22:6(n-3)</td>
<td>Emiliania, Gephyrocapsa, Pleurochrysis</td>
</tr>
<tr>
<td>Eustigmatophyceae</td>
<td>14:0, 16:0, 16:1(n-7), 20:5(n-3)</td>
<td>Nannochloropsis</td>
</tr>
<tr>
<td>Xanophyceae</td>
<td>14:0, 16:0, 16:1(n-9), 16:1(n-7), 18:1, 20:5(n-3)</td>
<td>Monodus</td>
</tr>
<tr>
<td>Dinophyceae</td>
<td>[14:0], 16:0, [18:0], [18:1(n-9)], 18:5(n-3), [22:2], [22:6(n-3)]</td>
<td>Alexandrium, Amphilidinium, Coolia; Gymnodinium, Heterocapsa</td>
</tr>
<tr>
<td>Prasinophyceae</td>
<td>16:0, 16:4(n-3), 18:1(n-9), 18:3(n-3), 18:3(n-4), 20:5(n-3), [22:6(n-3)]</td>
<td>Isochrysis, Tetraselmis</td>
</tr>
<tr>
<td>Chlorophyceae</td>
<td>16:0, 16:4(n-3), 18:1, 18:3(n-3), [20:4(n-6)]</td>
<td>Botryococcus, Chlorella, Dunaliella, Oocysts</td>
</tr>
</tbody>
</table>


*a* Only reported for Parietochloris incisa (Bigogno C., et al. Phytochemistry 60, 497–503, 2002.)

Only the major fatty acids making up approximately 75% of the total fatty acids are listed. Note fatty acids in brackets are observed in only some species.
Lipid extracts of many algae have been reported to contain relatively high levels of free fatty acids. These can lead to the formation of soaps in alkali-based transesterification, which reduces yield and increases the level of downstream processing and water use required to remove these soaps. Acid-based catalysis simultaneously esterifies the free fatty acids and transesterifies the triacylglycerols. This is well demonstrated in a study comparing acid- and alkali-catalyzed transesterification of lipids from the diatom Chaetoceros muelleri (Nagle and Lemke 1990). Using hydrochloric acid-methanol they achieved a maximum 4% FAME yield, whereas using NaOH as catalyst the yield was only 1.65%. Robles-Medina et al. (2009) have proposed a two-stage process to overcome this problem. In the first stage, acid catalysts are used to convert the free fatty acids to methyl esters, and in the second stage, an alkali-catalyzed process is used to convert the remaining triacylglycerols to methyl esters. However, the high free fatty acid content may be an artifact due to the activity of endogenous lipases during the lipid extraction process. If, when harvested, cells of the diatom Skeletonema costatum were immediately treated with boiling water to inactivate the lipases before lipid extraction by the Bligh and Dyer (1959) method; no free fatty acids could be detected (Berge et al. 1995).

Combined extraction and esterification is also possible. Belarbi et al. (2000) used a slurry (82% water by weight) of either the diatom Phaeodactylum tricornutum or the green alga Monodus subterraneus and transesterified these with methanol and acetyl chloride by heating in a boiling water bath for 120 min at 2.5 atm. They obtained a yield of 77.5% FAMEs.

The properties of the biodiesel are mainly determined by the component fatty acids of the algal lipids used to produce them (Knothe 2005). Of particular interest are the cloud point (the temperature at which the fuel becomes cloudy because of solidification), the pour point (the temperature at which the fuel stops flowing), and the cetane index (related to the ignition delay time and combustion quality of the fuel). Oils with a high content of unsaturated fatty acids result in a biodiesel that is less viscous and has a greater cloud point and pour point, making it more suitable for use in colder climates. However, this biodiesel is more prone to oxidation and has a lower cetane index. Oils with a high proportion of long-chain fatty acids (>C18) have a higher cetane index. The oxidative stability of the biodiesel is strongly affected by the position of double bonds in the saturated fatty acids. For example, esters of linoleic acid (double bonds at Δ9 and Δ12) oxidize more slowly than esters of linolenic acid (double bonds at Δ9, Δ12, and Δ15) (Frankel 1998). Unsaturation may also decrease the lubricity of the fuel and may contribute to gum formation in the engine.

The European standards for biodiesel for vehicle use (EN14214) and for heating oil (EN 14213) limit the content of FAMEs with four or more double bonds to a maximum of 1 mol % (Knothe 2006). Many oleaginous microalgae, especially the diatoms, cryptomonads, haptophytes, and eustigmatophytes (Brown et al. 1997) have a high content of highly unsaturated fatty acids such as eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3), which means that their lipids are likely to meet the European standards without further treatment such as partial catalytic hydrogenation of the oils (Dijkstra 2006). Much of the research to date has focused on microalgae species with a high content of long-chain polyunsaturated fatty acids for pharmaceutical and nutritional applications (Molina Grima et al. 1999; Kawachi et al. 2002; Tonon et al. 2002), but the great and still largely unexplored diversity of the microalgae does provide the opportunity to seek species and strains with reduced levels of polyunsaturated fatty acids as sources of lipids for the production of biodiesel.

The lipid content of microalgae also varies between species as well as with the growth stage and growth conditions (Borowitzka 1988). Many microalgae increase their lipid content when nutrient limited, especially nitrogen limited (Griffiths and Harrison 2009; Rodolfi et al. 2009). This increased lipid content is mainly due to an increase in triacylglycerols (TAGs), which act as storage lipids (Borowitzka 1988; Roessler 1990). Diatoms also increase their lipid content under silicon limitation (Coombs et al. 1967; Taguchi et al. 1987). In some microalgae species such as Monodus subterraneus, Isochrysis galbana, Pavlova lutheri, P. tricornutum, and Chaetoceros spp., phosphate limitation also leads to an increase in the TAG content (Khozin-Goldberg and Cohen...
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2006). On the other hand, other species of algae such as *Dunaliella tertiolecta*, *Tetraselmis* sp., *Nannochloris atomus*, *Biddulphia aurita*, and *Syeneda ulna* have a reduced lipid content when nutrient limited (Shifrin and Chisholm 1981; Siron et al. 1989; Reitan et al. 1994). In the green alga *Chlorella vulgaris*, iron supplementation has also been shown to increase the neutral lipid content (Liu et al. 2008). The addition of CO\(_2\) or bicarbonate to the cultures may also increase the lipid content of some (e.g., Muradyan et al. 2004; Chiu et al. 2009; Guihéneuf et al. 2009; Widjaja et al. 2009) but not all species of algae (Raghavan et al. 2008). However, because algal biomass productivity is generally stimulated by CO\(_2\) addition, the lipid productivity may still be enhanced even if the lipid content per cell is not. A more detailed treatment of the effects of nutrition and environmental factors on algal lipid content and the fatty acid profile may be found in Borowitzka (1988), Hu et al. (2008), and Harwood and Guschina (2009).

### 26.6 HYDROCARBON PRODUCERS

The hydrocarbon content of most microalgae is quite low (Borowitzka 1988), with the marked exception of the colonial green alga *Botryococcus braunii*, which is found in freshwater, brackish lakes, and other water bodies in temperate and tropical zones and which has a very high content of hydrocarbons and ether lipids (Metzger and Largeau 2005). There are three chemical “races” of *Botryococcus*: (1) the A-race that produces essentially n-alkadiene and triene hydrocarbons, odd-carbon-numbered from C\(_{23}\) to C\(_{33}\); (2) the B-race, which produces C\(_{30}\)-C\(_{37}\) triterpenoid hydrocarbons, the botryococenes, and C\(_{34}\) methylated squalenes; and (3) the L-race, which produces a single tetraterpenoid hydrocarbon, lycopadiene. Hydrocarbon contents range from 0.4 to 61% of dry weight for A-race strains, approximately 9–40% for B-race strains, and 0.1–8% for L-race strains (Metzger and Largeau 2005). In the Berkeley strain of *B. braunii*, approximately 7% of the botryococenes, mainly C\(_{30}\) and C\(_{32}\) botryococenes, are located in the cells, whereas the external colonial matrix contains more than 99% of the C\(_{31}\) and C\(_{34}\) compounds as well as lower-chain-length botryococenes (Wolf et al. 1985). *B. braunii* also contains “normal” lipids and sterols (Metzger and Largeau 1999).

Hydrocarbon productivity is greatest during the exponential growth phase and does not occur in nitrogen- and phosphorus-deficient media (Largeau et al. 1980; Casadevall et al. 1985; Dayanandra et al. 2007). The hydrocarbon productivity can be enhanced by bubbling the culture with CO\(_2\)-enriched air (Casadevall et al. 1985; Ranga Rao et al. 2007). A day/night cycle rather than continuous light also seems to favor hydrocarbon production (Dayanandra et al. 2007). Trials of growing this alga outdoors in tubular photobioreactors up to a volume of 200 L have been carried out (Gudin and Chaumont 1983). *Botryococcus* also grows well on secondarily treated piggery wastewater (An et al. 2003). Recovery of the hydrocarbons can be by solvent extraction (Metzger and Largeau 1999) or by extraction using supercritical CO\(_2\) (Mendes et al. 1994). Frenz et al. (1989a, 1989b) used a novel process in which they extracted the hydrocarbons by a short contact of the wet biomass with a nontoxic solvent such as hexane without reducing cell viability. This process recovered up to 70% of the total hydrocarbons.

The other group of microalgae that produces significant amounts of hydrocarbons and long-chain methyl and ethyl ketones (alkenones) and may have a high lipids content is the coccolithophorids (Fernandez et al. 1994; Bell and Pond 1996). At least one species, *Pleurochrysis carterae*, grows very well in outdoor raceway ponds (Moheimani and Borowitzka 2006, 2007) and is therefore also of interest as a potential source of renewable fuel.

### 26.7 LARGE-SCALE PRODUCTION OF MICROALGAE

Commercial-scale algae culture has been carried out all over the world for the last 30+ years. The two largest commercial algae production plants are at Hutt Lagoon, Western Australia and Whyalla, South Australia, growing the halophilic algae *Dunaliella salina* for the production of
\( \beta \)-carotene for the nutritional supplement industry (Borowitzka and Borowitzka 1989; Borowitzka and Hallegraeff 2007). *Dunaliella* is also commercially grown in Israel and India. The blue-green alga *Spirulina* (*Arthrospira*) is grown in the United States, China, Taiwan, Thailand, and India (Belay 1997; Hu 2004), the green alga *Chlorella* in Taiwan, Indonesia, Japan, and Germany (Lee 1997), and the green alga *Haematococcus* in the United States, Israel, and Sweden (Cysewski and Lorenz 2004). A number of other species such as *Chaetoceros*, *Isochrysis*, *Tetraselmis*, *Nannochloropsis*, and *Nitzschia* are also grown around the world as feed for aquaculture species (Borowitzka 1999b; Muller-Fuega 2004). Although some of these production plants are very large (e.g., the *Dunaliella* plants in Australia have an area >750 ha), they are still very small compared with the production plants needed for biodiesel production. For example, a raceway pond-based production plant producing microalgae with a 40% lipid content at an average daily productivity of 30 g/m² per day would need approximately 75.5 km² of ponds to produce 10,000 barrels (1 barrel = 158.987 L) of oil per day. Therefore, the extremely large scale required for biofuel production using microalgae presents a new and significant challenge (Borowitzka and Moheimani 2011; Fon Sing et al. 2011).

The production of biofuels from algae needs not only to be technically feasible, but also must be commercially viable and environmentally sustainable. The actual production costs of these commercial producers are difficult to obtain; however, the best estimates are given in Table 26.3. How do these costs compare with the production costs required to produce biodiesel? Current prices for comparative feedstock for biodiesel are palm oil (~$U.S. 0.40–0.50/kg) and canola/rape seed oil (~$U.S. 0.60/kg). Assuming that an alga’s oil content is approximately 40% of dry weight (achievable average), then to produce oil at $U.S. 0.50/kg, the algal biomass must be produced at a cost of less than approximately $U.S. 0.20/kg (Note: This includes harvesting costs, but it does not include lipid extraction costs). This presents a major challenge for algal oil production for biofuels. To achieve this cost, reliable high productivities all year round of a biomass with a high lipid content (at least 30–40% of dry weight) are essential. Furthermore, culture and harvesting systems have to be constructed and operated at very low cost.

To achieve high biomass productivities, the following key conditions should be met:

- Maximal available sunshine (i.e., as little cloud cover as possible), and
- As little rainfall as possible (because rain means clouds, thus reducing light available, and for better control of salinity).

### TABLE 26.3

**Estimated Production Costs of Commercially Grown Microalgae**

<table>
<thead>
<tr>
<th>Alga</th>
<th>Culture System</th>
<th>Place</th>
<th>Estimated Production Cost for Dry Biomass in Australian Dollars per Kilogram</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dunaliella salina</em></td>
<td>Extensive (~270 ha) open ponds</td>
<td>Australia</td>
<td>$6</td>
</tr>
<tr>
<td><em>Chlorella spp.</em></td>
<td>Open ponds</td>
<td>Taiwan</td>
<td>$17</td>
</tr>
<tr>
<td></td>
<td>Open ponds (shallow cascade system)</td>
<td>Australia (pilot plant operation)</td>
<td>$15</td>
</tr>
<tr>
<td><em>Spirulina</em></td>
<td>Open raceway ponds</td>
<td>United States</td>
<td>$15–20</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>Tubular photobioreactor</td>
<td>Israel</td>
<td>&gt;$50</td>
</tr>
<tr>
<td>Several species for aquaculture</td>
<td>Mainly closed reactors</td>
<td>Australia, United States, Europe</td>
<td>$50 to &gt;$1000*</td>
</tr>
</tbody>
</table>

*a Some of the high costs can be attributed to the small scale of the operations.
For environmental sustainability, the algae should be grown in saline medium to minimize the need for freshwater.

The lowest cost culture system for microalgae production at this time is the raceway. The best annual average productivity for long-term (>6 months) continuous culture in a raceway reported in the literature is approximately 20 g ash-free dry weight/m² per day for the coccolithophorid alga *Pleurochrysis caterae* in Perth, Western Australia (Moheimani and Borowitzka 2006). In current commercial-scale operations, this is not achieved. In summer, very high productivities of 41–47 g dry weight/m² per day have been achieved. The lipid content of this alga is approximately 30–35% of dry weight in log-phase growing cultures. However, it should be noted that the growth conditions or the lipid productivity for this alga have not, as yet, been optimized. To achieve such productivity, the density of the algal culture needs to be controlled to optimize the available light reaching the cells, and for most species CO₂ needs to be added (Borowitzka 1998). The system should also be operated as a continuous culture. This means the algal strain used and the operating regime must be able to exclude, or at least control, the growth of contaminating species and predators such as protozoa. This is possible for quite a number of species such as *D. salina* (because of the very high salinity), *Spirulina* spp. (because of the high alkalinity), *P. tricornutum*, and *Chlorella* spp. (because of high growth rate), *Pleurochrysis caterae* (possibly because of the production of acrylic acid by the alga) and a range of other fast-growing marine algae such as *Nannochloropsis*, *Tetraselmis*, *Nannochloris*, *Pheodactylum*, and *Chlorococcum* (Ben Amotz, personal communication).

For the production of algal lipids to produce biodiesel, it is not the biomass productivity but the lipid productivity that is important (Griffiths and Harrison 2009). Most algae achieve their maximal lipid (oil) content in the stationary phase of growth; however, continuous culture for maximal productivity means that the cells should always be in the log phase of growth. This requires algal strains that have a high lipid content while actively growing so as to have a high lipid productivity. Lipid productivity is a function of the algal productivity and the lipid content. The effect of productivity and cell lipid content on lipid productivity is shown in Figure 26.1. At the potentially achievable scenario of a productivity of 30 g dry weight/m² per day with a lipid content of 40% of the biomass, 12 kg oil/ha per day are produced.

The algae also need to have a wide temperature tolerance and grow well outdoors. In the high solar irradiation environments, where high average annual productivities are possible, temperatures in open ponds can reach up to approximately 35°C in summer, and they may cool down to less than 5°C at night in winter. Similarly, closed photobioreactors heat up rapidly during the day on sunny

![Figure 26.1](image-url) **Figure 26.1** Effect of productivity and cell lipid content (numbers at end of lines are percent lipid content) on lipid productivity. The fine line shows the potentially achievable target of an annual average productivity of 30 g dry weight/m² per day at a lipid content of 40%. 
days unless cooled, but they cool down rapidly at night unless heated, and cooling and heating require a significant energy input. The need to reduce the need for freshwater also means that saline water should be used to make up evaporative losses, and this, in turn, means that the algae should be able to grow well over an extended range of salinities.

Harvesting and dewatering also presents a major cost to any microalgae production process (Mohn and Cordero-Contreras 1990; Borowitzka 1999a; Molina Grima et al. 2003) and have been reviewed by Mohn (1988) and Molina Grima et al. (2004). The solids content of microalgal cultures in large-scale systems ranges from 0.1 to approximately 1 g/L, meaning that very large volumes of water have to be processed. Most of the microalgae of interest are also very small (<20 µm diameter) and have a density very similar to that of the medium they are growing in. Because the water also still contains significant quantities of nutrients, the growth medium must be recycled after harvesting of the algae for economic and environmental reasons. This excludes some types of harvesting. The cheapest harvesting method is filtration; however, most algae, other than filamentous species such as *Spirulina*, are too small for effective filtration. The next best method is settling followed by dewatering, but, where this is not possible, flocculation and flotation (or settling) could be used. The method of harvesting will depend on the species of alga cultured (Shelef et al. 1984; Mohn 1988) and may also be affected by the growth phase (Danquah et al. 2009).

Extraction of the lipids from microalgae will probably need to be by solvent extraction (Molina Grima et al. 1995; Lee et al. 2010).

### 26.8 OPEN POND CULTURE VERSUS CLOSED PHOTOBIOREACTORS

Closed photobioreactors are often cited as the solution for the production of microalgae for biodiesel (e.g., Chisti 2007; McCall 2008; Rodolfi et al. 2009), and combined closed photobioreactor/open pond systems also have been proposed (Huntley and Redalje 2007). However, despite more than 50 years of work on closed photobioreactors, they have as yet not been shown to be commercially viable for microalgal production, except for the very high-value alga *Haematococcus pluvialis* and *Chlorella* for the health-food market. The various designs of closed photobioreactors recently have been reviewed by Tredici (2004) and Carvalho et al. (2006). Although closed systems appear to be a solution for some of the problems encountered in open systems, such as the potential control of contaminants and greater control of environmental factors, they present other challenges and problems, and it has been our experience that many species of algae cannot be grown in closed systems. Closed reactors also require cooling during periods of high irradiance and are more difficult to scale up (Borowitzka 1996, 1999b). Table 26.4 compares several aspects of open and closed culture systems.

To date, productivities in large-scale closed systems (calculated on a grams-per-liter basis) are only approximately 2–3 times those of open systems. However, the capital costs of closed systems are at least 5–10 times higher and the operating costs, especially energy costs for circulating the algal culture and for temperature control, are also much higher. Furthermore, open raceway culture systems have the advantage of being a well-known, proven, and reliable technology. Details of open pond culture can be found in Borowitzka (2005).

### 26.9 THE FUTURE

The high activity in research and development on the development of new algae and on processes to produce renewable biofuels from algae will mean that the current economic and technological challenges will be overcome in time (Stephens et al. 2010b). In many laboratories, there is ongoing isolation and screening for better strains suited to large-scale cultivation, and improved lipid productivity will broaden the range of species available. There is also a search for valuable coproducts that may help to improve the economics of microalgal fuel production (Stephens et al. 2010a).
Genetic engineering also presents a new, and as yet little explored option for overcoming some of the limitations of growth and lipid production. One potential strategy that has been proposed is to engineer diatoms, and possibly other algae, to secrete some of their lipids (Ramachandra et al. 2009). For example, in corals the symbiotic dinoflagellates have been shown to apparently secrete lipid-like osmiophilic material (Crossland et al. 1980). The green alga *B. braunii* also accumulates hydrocarbons extracellularly.

Another possibility is to enhance the light utilization efficiency of the algae. For example, Melis et al. (1999) found that mutants of *D. salina* with small chlorophyll antenna size showed higher photosynthetic productivities and photon use efficiencies (see also Mitra and Melis 2008). A mutant of *Chlamydomonas reinhardtii* (tl1) with truncated light harvesting antenna size also had enhanced photosynthetic productivity in laboratory and outdoors in a greenhouse (Nakajima and

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**TABLE 26.4**

Comparison of the Properties of Open Pond Raceway Culture Systems and Closed Photobioreactors

<table>
<thead>
<tr>
<th>Species range</th>
<th>Open Ponds</th>
<th>Closed Photobioreactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most species, but control of contamination by other algae and/or predators is a potential problem, but this can be controlled for a number of species (e.g., in all of the ones listed below)</td>
<td>Can only grow species that are shear-tolerant because the need for circulation (air-lifts, pumps) damages many species. “Sticking” of some algae to the walls of the reactor is also a problem</td>
<td></td>
</tr>
<tr>
<td><em>Chlorella</em>, <em>Spirulina</em>, <em>Scenedesmus</em>, <em>Dunaliella</em>, <em>Phaeodactylum</em>, <em>Pleurochrysis</em>, <em>Monodus</em>, <em>Nitzschia</em>, <em>Nannochloropsis</em></td>
<td><em>Spirulina</em>, <em>Chlorella</em>, <em>Tetraselmis</em>, <em>Isochrysis</em>, <em>Phaeodactylum</em>, <em>Nannochloropsis</em></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Key Factors limiting productivity</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>Some capacity to optimize light environment for cells by controlling pond depth and/or cell density</td>
<td>Can control light environment by controlling diameter of tubing or width of plate reactor as well as cell density. Sticking of the algae to the bioreactor surface also limits light availability and growth in many species. For maximal productivities, the light path (reactor thickness) should not exceed ~400 mm</td>
</tr>
<tr>
<td>Temperature</td>
<td>Limited capacity to control temperature in large ponds; however, maximum pond temperature does not exceed ~30–35°C because of evaporation, so no cooling is required.</td>
<td>In high light the system heats up rapidly and there is a need to cool system. Requires energy (and large amounts of freshwater if evaporative cooling system is used)</td>
</tr>
<tr>
<td>CO₂, O₂ (high O₂ inhibits photosynthesis)</td>
<td>Can be added</td>
<td>Can be added</td>
</tr>
<tr>
<td>O₂ built up during the day by photosynthesis is lost from system by exchange on pond surface</td>
<td>Requires efficient degassing system. In tubular photobioreactors O₂ limits length of tubes that can be used.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cost factors</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Capital cost</td>
<td>Relatively high because of the need for pond liners</td>
<td>Very high</td>
</tr>
<tr>
<td>Power requirement</td>
<td>Mixing using paddlewheels is very energy-efficient.</td>
<td>Power requirements for circulating the culture are high. Power may also be required for cooling during the day.</td>
</tr>
</tbody>
</table>

Ueda 1997, 1999; Polle et al. 2003). Recently, Mussgnug et al. (2007) used RNA interference (RNAi) to downregulate the expression of light harvesting antenna complex proteins of *C. reinhardtii* with the recombinant strain showing higher resistance to photodamage and increased light penetration in the culture.

Alternatively, genetic engineering may be used to enhance key parts of the pathway of lipid synthesis. For example, the acetyl-CoA carboxylase (ACC) gene from *Cyclotella cryptica* has been transformed into the diatoms *C. cryptica* and *Navicula saprophila*, resulting in overexpression of the ACC gene, *acc1*, enhancing enzyme activity 2- to 3-fold. However, there was no significant increase in lipid accumulation in the transgenic diatoms (Roessler et al. 1994; Dunahay et al. 1995, 1996), suggesting that there is a secondary limiting step in the TAG pathway. As an alternative approach, Courchesne et al. (2009) proposed to enhance lipid overproduction by overexpressing transcription factors regulating the metabolic pathways involved in the production of lipids.

These potential improvements in the efficiency of light use and metabolic redirection to lipid synthesis, coupled with essential improvements in culture and harvesting systems, will contribute to the development of commercially viable and environmentally sustainable production of biofuels from algae.

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