J Ind Microbiol Biotechnol (2010) 37:1271–1287
DOI 10.1007/s10295-010-0884-5

REVIEW

Microbial lipids from renewable resources: production and characterization

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Received: 24 June 2010 / Accepted: 18 September 2010

Abstract A number of microorganisms belonging to the genera of algae, yeast, bacteria, and fungi have ability to accumulate neutral lipids under specific cultivation conditions. The microbial lipids contain high fractions of polyunsaturated fatty acids and have the potential to serve as a source of significant quantities of transportation fuels. This paper reviews the current state of the art of this field. It summarizes the various microorganism used, feed stocks available, environmental factors that influence growth of cells and accumulation of lipids, major fatty acid composition of lipids, and the technology.

Keywords Algae · Yeast · Fatty acid composition · Lipids · Economics

Introduction

Energy outlook and need for alternative energy

Petroleum is the largest source of energy consumed by the world's population, exceeding coal, natural gas, nuclear, hydro, and renewables [21, 95]. Consumer products derived from petroleum are present everywhere in our society. But this consumption of petro-products is rapidly exhausting global crude oil, increasing the concentration of greenhouse gases in our environment, and creating expensive and challenging waste recycling issues. However, since there is a strong correlation between energy consumption and standard of living and world population growth, even higher demand for crude oil and petroleum-based products is expected in future. Global demand for petroleum is predicted to increase up to 40% by 2025 [77]. Concerns about energy security, climate change, and soaring oil prices are driving policymakers and scientists towards energy alternatives that would allow us to break our dependence on imported fossilized oil. Fuels derived from renewable resources are arguably one of the best options to lead the transition away from petroleum fuels in the near-term.

Biodiesel produced from vegetable oils, plant oils, or animal fats by transesterification with low molecular weight alcohols is an important renewable fuel [115, 202]. Vegetable oils have been used in motor vehicles since the beginnings of the automobile industry. The German engineer Rudolph Diesel demonstrated his first compression ignition engine at the 1898 World Exhibition in Paris with peanut oil as fuel. Diesel engines ran on vegetable oils as fuel until 1920s when the engines were modified to use petroleum hydrocarbons as fuel [196].

Biodiesel can be used in diesel engines alone or as a mixture with petroleum-based diesel fuel. The most common blend currently used is "B20", a 1:4 mixture of biodiesel and petroleum diesel. "B100" implies pure biodiesel. The advantages of biodiesel include that it is a renewable resource, is easy to manufacture, and has a positive fossil energy imprint (requiring only 0.31–0.39 units of fossil energy to make a 1 unit of fuel [164, 185], superior emissions characteristics, compatibility with existing engines, distribution infrastructure, and supports domestic agriculture. Vehicles using biodiesel limit the impact of carbon dioxide on global warming when compared to petrodiesel,

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although they get slightly fewer miles per gallon. Another favorable environmental property of biodiesel is its very low sulfur content [11, 202]; use of biodiesel as fuel would reduce sulfur and carbon monoxide emissions from our vehicles by 30 and 10%, respectively. It can decrease air toxicity by 90% and cancers by 95% compared to petrodiesel [184]. Finally, biodiesel is better than petrodiesel in terms of flash point and biodegradability [136].

Global biodiesel production

According to Global Renewable Fuels Alliance [66], Europe and the USA are the two largest markets for biodiesel. Europe leads the world in biodiesel production and consumption; production started there in the early 1990s and grew to over 500 million gallons by 2004 [197]. The 2009 production of biodiesel in Europe stood at 2.6 billion gallons (S&T^2 [37]). Biodiesel production in the USA started in earnest in 2000 and grew from 25 million gallons in 2004 to 678 million gallons in 2008 [96]. In 2009, market conditions dictated a reduction of biodiesel production in the USA to 440 million gallons, but it is expected to rise again [148]. Biodiesel production of late has been driven by mandatory alternative fuel-use legislations [197]. Other producers are not yet large, but they are increasing their production capacity rapidly (S&T^2 [37]). The total global production of biodiesel in 2009 was 4.3 billion gallons. China at 50 million gallons was a small producer of biodiesel because it is a big net importer of all the major edible vegetable oils and lacks the land for crop production for biodiesel. Currently, most of China's biodiesel production is based on animal fat or waste vegetable oil from oil crushing plants or restaurants [199]. The production in India in 2009 was 6 million gallons, again from waste oil, but the Government of India (GoI) has launched a National Mission on Biofuels with the aim of achieving a target of 20% blending of biodiesel by 2012 [177]. In Brazil, the targeted biodiesel production in 2010 is 500 million gallons per year [26].

Biodiesel feed stocks

Biodiesels are esters of fatty acids and can be produced from any vegetable oil, plant oil, or animal fat. At present, biodiesel is produced mainly from soybean and vegetable oils [24], palm oil [6], sunflower oil [11], rapeseed oil [163], Jatropha oil [76], and restaurant waste oil [19, 45]. Oils and fats are primarily composed of triacylglycerols (TAGs), three fatty acid molecules attached to a glycerol backbone by ester bonds. These may contain lesser amounts of diacylglycerols (DAGs) and also monoacylglycerol (MAGs). The fatty acids in biodiesel can be of varying chain lengths. Longer chain length of fatty acids results in biodiesel with a higher cetane number and reduced NO_x emissions in engine exhausts [11, 115, 202]. Wastes such as used cooking oils and fats can also be used as raw materials, but these may contain large amounts of free fatty acids and require additional processing [44, 72].

Limitations of agricultural feedstocks for biofuels

The rapid expansion in biofuel production has stretched the potential resources that can be used as raw materials. There have been growing concerns about the impact of rising commodity prices on the global food system. World food prices rose 10% in 2006 because of increases in corn, wheat, and soybean prices, primarily from demand-side factors, including rising biofuel demand [40]. The Chinese government identified many potential non-grain feedstocks such as cassava and sweet potatoes for biofuel production to avoid high food prices. Mexico capped tortilla prices in early 2007 to contain food price inflation from higher priced corn imports. Real sugar prices hit a 10-year high in 2006, stressing budgets of low-income people in Brazil and elsewhere. The Indonesian Government increased the export duty on crude palm oil, also used in biodiesel production, in mid-2007 to slow the rising cost of domestic cooking oil [207]. Prices have since declined, but these issues brought forth the debates of food vs. fuel.

Biodiesel derived from oilseeds or animal fat can deliver only a small fraction of the existing demand for transport fuels without committing excessively large acreages of quality agricultural land for cultivation of oilseed crops targeted away from food production [32]. Therefore, it is necessary to explore new raw materials that (1) deliver superior environmental benefits over the fossilized materials they displace, (2) are economically competitive, (3) can be produced in quantities sufficient to meet the energy demands, (4) provide a net energy gain over the energy sources used to produce it, and (5) also do not compete with food production [143]. Microbial oils fit these bills if these can be produced economically and, therefore, have received much interest in order to resolve the worldwide crude oil and greenhouse-gas crisis [32]. Microorganisms have many advantages over plants for production of lipids, such as short life cycles, less labor required, less demand on space, venue, season and climate, and ease of scale up. Photosynthetic microorganisms have 100-fold higher yield of lipids per hectare than plants [179]. Lipids produced by oleaginous microorganisms are considered as promising candidates for biodiesel production because fatty acid composition is similar to that of vegetable oils [89]. Microbial lipids are rich in specific polyunsaturated fatty acids also and are often used in dietary supplements and for infant nutrition [171, 189].

Microorganisms for lipid production

Microorganisms belonging to several different families, such as microalgae, bacillus, and fungi (molds and yeasts), possess the ability to produce and accumulate a large fraction of their dry mass as lipids [111, 146]. Those with lipid content in excess of 20% are classified as 'oleaginous' [174]. Several microorganisms with potential for microbial oil production are listed in Table 1.

Micro algae

Microalgae, also called 'miniature sunlight-driven biochemical factories' [201], are capable of producing large amounts of lipids and hydrocarbons in the presence of sunlight and carbon dioxide from flue gases. Microalgae can be a promising alternative feedstock for the next generation of biofuels, as they have a relatively high lipid content, grow fast, and can be harvested daily [69, 219]. Doubling times of algae are on the order of 4–24 h [33] and as short as 3.5 h [32] during exponential growth. Algae have a number of advantages over terrestrial energy crops, such as higher photosynthetic efficiency, surface area productivity, absence of need for arable land, and low nutritional needs [32]. Algae can be sources of several different types of renewable biofuels [39], including biodiesel from neutral lipids [63], bio hydrogen [104], hydrocarbons [14, 112], ethanol [25], and methane [97]. The average lipid content of algal cells varies between 1 and 70% [69, 139, 141, 217], but can reach as high as 90% of dry weight under specific conditions [144].

Table 1 Oil content of some oleaginous microorganisms [32, 143]

Microorganisms	Oil content (% dry weight)
Microalgae	
Botryococcus braunii	25–75
Cylindrotheca sp.	16–37
Chlorella sp.	28–32
Crypthecodinium cohnii	20
Dunaliella primolecta	23
Isochrysis sp.	25–33
Monallanthus salina	>20
Nannochloris sp.	20–35
Nannochloropsis sp.	31–68
Neochloris oleoabundans	35–54
Nitzschia sp.	45–47
Phaeodactylum tricornutum	20–30
Schizochytrium sp.	50–77
Tetraselmis sueica	15–23
Bacteria	
Arthrobacter sp.	>40
Acinetobacter calcoaceticus	27–38
Rhodococcus opacus	24–25
Bacillus alcalophilus	18–24
Yeast	
Candida curvata	58
Cryptococcus albidus	65
Lipomyces starkeyi	64
Rhodotorula glutinis	72
Molds	
Aspergillus oryzae	57
Mortierella isabellina	86
Humicola lanuginose	75
Mortierella vinacea	66

The growth of cells and lipid accumulation by algae under phototrophic conditions is influenced by the intensity of light, pH, dissolved oxygen concentration, fraction of carbon dioxide in sparging gas, concentration of nutrients such as nitrogen, phosphorous, silicon, and iron, and presence of organic carbon sources.

To enhance the economic feasibility of algal oil production, biomass productivity (production per unit volume per unit time), cellular lipid content, and overall lipid productivity are the three key parameters that need to be improved. These requirements are not always compatible, and in general, conditions favoring a high growth rate of cells result in a low lipid fraction in the cells and vice versa [135].

High cellular lipid content in algae is usually achieved under environmental stress [121]. The stress may be caused by limitations of nitrogen [50, 94, 121, 123, 125, 180, 182, 186, 204], phosphorous [175, 180, 210], silicon [70], salinity [167], and iron [131]. Lipid accumulation in the cells also depends on the growth phase of cells [34, 181, 193].

Effect of nutrient limitations

Nitrogen and/or phosphorous limitations in the medium result in increased production of lipids in algal cells as well as in increased lipid productivity [43, 180]. Illman et al. [94] studied batch growth of five strains of the green alga *Chlorella* in Watanabe medium and in a low-nitrogen medium in a 2-l stirred bioreactor. Higher lipid content was seen for all the five strains in the low-nitrogen medium when compared to that in Watanabe medium. The greatest increases were seen in *Chlorella vulgaris*, for which the lipid content (percent dry weight) increased from 18% in Watanabe medium to 40% in low-nitrogen medium; the lipid fraction in *Chlorella emersonii* increased from 29 to 63% of dry weight [94].

Scragg et al. [182] performed further studies with the *C. vulgaris* and *C. emersonii* strains in tubular reactors using the same Watanabe and low-nitrogen media. The specific growth rate of *C. vulgaris* in Watanabe medium was 0.4 day^{-1}. In the low-nitrogen medium, growth was biphasic with an initial specific growth rate of 0.69 day^{-1}, and it decreased to 0.12 day^{-1} after day 4. The specific growth rate for *C. emersonii* was 0.38 day^{-1} for both the Watanabe and the low-nitrogen medium. Lipid content increased for both strains in low-nitrogen medium, increasing from 28 to 58% dry weight for *C. vulgaris* and from 25 to 34% dry weight for *C. emersonii* [182].

Lipid accumulation in algal cells is not growth associated. Chiu et al. [34] reported that the lipid contents of *N. oculata* cells in nitrogen-limited medium during logarithmic, early stationary, and late stationary phases were 30.8, 39.7, and 50.4%, respectively, congruently with a decrease in nitrogen content in the broth. Exhaustion of nitrogen in medium may cause cessation of cell division, but carbon metabolism continues, resulting in diversion of carbon to lipid production [17]. Total biomass concentration may still increase mainly due to increase in lipid content of cells. A similar effect, hampered cell division due to nutrient limitation, was observed for diatoms in silica-depleted media. However, the results for lipid accumulation were found to be different for different strains of diatoms. Some strains showed an increase in lipid content, particularly neutral lipids. However, lipid content of other strains remained unchanged while the growth rate decreased, resulting in much lower total biomass [186]. Even for heterotrophic algae *Chlorella protothecoides*, the lipid production increased under nitrogen limitation [187]. However, a higher lipid fraction in cells does not necessarily translate into higher productivity of lipids. Weldy and Huesemann [204] studied the combined effect of light intensity, N deficient and N sufficient conditions on growth of *Dunaliella salina*. They recorded higher lipids productivity (66 mg l^{-1} day^{-1}) under N-sufficient conditions and high light intensity than under N-deficient conditions (12 mg l^{-1} day^{-1}).

The nature of nitrogen source can also impact algal cell growth and lipid productivity [94, 121, 180]. According to Li et al. [123], the order of specific growth rate of freshwater algae *Scenedesmus* sp. on different nitrogen sources is NH_4-N > urea-N > NO_3-N. However, H^+ released from consumption of NH_4^+ ions has the potential of reducing medium pH to values inhibitory to cell growth. Green algae *Neochloris oleoabundans* cultivated in the presence of 5 mM $NaNO_3$ resulted in cells with lipid fraction of 33% of dry matter, whereas the cells had only 19 and 17.5% lipids, respectively, in the presence of 5 mM ammonium bicarbonate and urea [121]. The lipid productivity values were 133, 33 and 57 mg l^{-1} day^{-1}, respectively, with the three nitrogen sources. On the other hand, Fidalgo et al. [61] reported that total fatty acid content in algal cells is influenced by the nitrogen source, but the gross biochemical composition is affected more by the growth phase than by the nitrogen source.

Effect of carbon dioxide

The fraction of carbon dioxide in sparging gas has a profound effect on cell growth and lipid accumulation [29, 34, 83, 84] both due to its effect on medium pH as well as on the availability of bicarbonates used by the cells as carbon source. Chiu et al. [34] reported that *Nannochloropsis oculata* cells grew faster (0.571 day^{-1} vs. 0.194 day^{-1}) and to a higher maximum cell concentration (1.277 g l^{-1} vs. 0.268 g l^{-1}) as the carbon dioxide fraction in sparging air was increased from 0.03 to 2%. Further increase in the CO_2 fraction decreased cell production with complete inhibition of cell growth at 5% CO_2 in inlet air. The toxicity of carbon dioxide to microalgal cells above 5% (v/v) was reported by several other researchers as well [27, 42, 190]. On the other hand, pre-adapting the cells to higher carbon dioxide fractions in gas phase and use of high inoculums levels helped the *N. oculata* cells [34] overcome the CO_2 toxicity in a semicontinuous reactor, suggesting that the observed carbon dioxide toxicity in the batch system may have been due to an associated phenomenon. In experiments conducted in our laboratory with cultivation of *Scenedesmus* cells (unpublished data), sparging medium with pure carbon dioxide resulted in unfavorable pH conditions in broth and growth inhibition. This condition could be reversed by sparging the medium with air and adjustment of pH. Several researchers have been able to cultivate *Botryococcus braunii* at high carbon dioxide concentrations [64, 168, 217]. Ge et al. [64] cultivated their cells in a 3-l photobioreactor illuminated with cool white fluorescent lights (150 μmol m^{-2} s^{-1}) at 25°C and sparged continuously with air containing up to 20% CO_2 without any pH adjustment. A maximum cell density of 2.3 g l^{-1} along with a hydrocarbon content of 24.5% and lipid content of 12.7% was obtained with 20% CO_2 on the 25th day.

Effect of PAR photon flux (light intensity)

Light (photon intensity) is critical to cell growth and lipid accumulation under phototrophic conditions. The specific growth rate of cells increases with increasing photon irradiation flux (characterized in μmol photon m^{-2} s^{-1}) in the photosynthetically active range (PAR 400–700 nm), but shows a characteristic saturation/inhibition behavior with irradiation flux beyond an optimum value [16, 71, 117]. The photon flux saturation behavior is manifested as the photon flux reaches the culture's capability to process the energy captured by the cellular photosynthetic machinery

[67]. Higher values of photon flux result in dissipation of photonic energy as heat and may cause photo inhibition of cellular functions [134]. In most algal photobioreactors, the photonic flux optimum for growth of cells occurs between 345 and 1,125 μmol photon m^{-2} s^{-1}. However, there is evidence that proper design of bioreactors can help push this limit to several-fold higher values [67, 71]. It can also be achieved through use of light-emitting diodes [107, 194], light pulsations [178], mixing in dense cultures [86], or genetic engineering [147, 166, 195]. The genetic approaches focus either on decreasing the photosynthetic antenna size [67] or on enhancing the rate of carbon fixation (dark) reactions that are characterized by the largest time constants in photosynthesis [194]. A recent study by Theodoridou et al. [198] suggests that the same effect may be obtained by chemical means also. These authors investigated the enhancement of phototrophic growth of *Scenedesmus obliquus* by low concentrations of methanol and observed a decrease in size of light-harvesting complex concomitant with increased photosynthesis and respiration rates. Algae grown at low irradiance had a relatively higher content of the total lipids compared to those exposed to high light intensity [108].

Effect of environmental parameters

Cultivation time, initial pH, and medium composition are the key parameters for enhancement of biomass growth, lipid content, and fatty acid profile [192, 209]. Concentrations of NaCl and nitrogen in the medium significantly affect the total lipids, unsaponifiables contents, and fatty acid composition for the algal strain *D. salina* [135]. A higher yield of total lipids and higher proportion of polyunsaturated fatty acids (C18 and C16) were obtained when the NaCl concentration was increased from 8 to 16% [50]. Since nutrients represent a major cost factor in the production of microbial oils, Kim et al. [109] explored and found that supplementation of medium with fermented and treated swine urine could improve the cost efficiency of microalgal biodiesel production. The organic acids, enzymes, and hormones generated by bacteria during the fermentation of swine urine serve to accelerate the physiological and biochemical activities of the growing cells, and also cause a delay in the onset of stationary phase of the cell divisions, despite the shortage of inorganic nutrients within the medium. In *Chlorella* sp., the production of fatty acids increased with an increase in sodium thiosulphate concentration, but addition of glucose was counterproductive [59].

Optimum temperatures for cultivation of algae range from 25 to 35°C. These were reported to be 25–27°C for *Rhodomonas* sp., 27–30°C for *Prymnesiophyte*, *Cryptomonas* sp., *Chaetoceros* sp., and *Isochrysis* sp., and 33–35°C for *Chaetoceros* sp. by Renaud et al. [176]. More biomass was obtained at 30°C than at 35°C with *S. platensis* [36]. Increasing temperature from 20 to 25°C increased the lipid content of *N. oculata* from 7.90 to 14.92%; but the lipid content of *C. vulgaris* decreased from 14.71 to 5.90% when the temperature was increased from 25 to 30°C [38]. The optimum temperature range for *Cyclotella cryptica* was found to be 22.5 to 25°C [156].

Medium pH is an important parameter in photoautotrophic as well as heterotrophic cultivation of algae. The optimal pH range for C*yclotella crypticaI* is 7.2–9.0 [98, 156]. The favorable initial pH of the medium for the growth of *Trichosporon fermentans* is 6.5 [220] and that for C*haetoceros* sp., *Rhodomonas* sp., and *Cryptomonas* sp. is 8.3 [176].

Some algae strains can grow both autotrophically and heterotrophically. Heterotrophic cultivation on organic carbon sources has been used to overcome the issues of photonic energy delivery to cells in photoautotrophic growth. Li and coworkers [118, 212] reported cultivation of *C. protothecoides* heterotrophically in fed-batch bioreactors. Up to 52 g/l cell density with 50% lipid content in dry cells was obtained in these systems. High biomass and high lipid yield have been found when heterotrophic algae are placed in low light and supplied with organic carbon rather than carbon dioxide as carbon source [145, 213]. Miao and Wu [145] reported that autotrophic *C. protothecoides* possessed a lipid content of 14.57%, whereas the heterotrophically grown cells had 55.2% lipids. Autotrophic and heterotrophic growth of algal cells has been reported by Oh et al. [153] and Shen et al. [187]. Oh et al. [153] observed that cells of marine alga *Porphyridium cruentum* grew at a faster pace under 18:6 h light:dark cycle (0.042 h^{-1}) than under 12:12 h cycle (0.031 h^{-1}). However, the lipid fraction in cells was higher (19.3% w/w) in the case of a 12:12 h light:dark cycle. Mixotrophic growth conditions resulted in the highest and fastest lipid production by the cells, due perhaps to less loss of cell mass during the dark cycle [35]. The macro algae strain *Oedogonium* had higher algal oil production than *Spirogyra*, but *Spirogyra* had a higher biomass content than *Oedogonium* after oil extraction [81]. Both Chojnacka and Noworyta [35] and Andrade and Costa [8] found that mixotrophic growth can result in a several fold increase in specific growth rate and maximum cell density of cells.

Algae can be cultivated heterotrophically on organic carbon in photobioreactors to produce lipids while treating organic wastes. A summary of these possibilities has been presented by Brennan and Owende [21]. In the heterotrophic and mixotrophic cultivation of algae, the cost of carbohydrates represents a major cost factor towards the cost of lipids. Use of carbohydrates in wastes for growth of cells not only reduces this cost, it may even result in credit for

mitigating wastes and thus improve the economics of lipid production [60].

Molds

Some oleaginous molds (filamentous fungi) can store up to 80% of their biomass as lipids [150]. The lipids in fungi are mainly influenced by the nature of the nitrogen source, carbon source, C/N ratio, temperature, agitation, and pH in broth. Molds produce a high concentration of γ-linolenic acid (GLA) and arachidonic acid (AA) and are, therefore, often cultivated to produce these higher value products more than other lipids for biofuels. As in most other microbes, lipid production in cells increases with increasing C/N ratio [161]. But Rasheva et al. [169] did not find any influence of C/N ratio and nitrogen source on the neutral, phospholipid, and glycolipid composition.

The oleaginous fungus, *Mucor rouxii*, is known to accumulate a high level of intracellular lipids and GLA [138]. Eroshin et al. [52] reported production of as much as 4.5 g l^{-1} AA by *Mortierella alpina* with a productivity of 19.2 mg l^{-1} h^{-1} with potassium nitrate as nitrogen source. AA in the cells was more than 18% of dry cell mass and over 60% of the total lipids in the cells. Aki et al. [2] succeeded in producing 7.1 g.l^{-1} arachidonic acid (AA) using the fungus *Mortierella alliacea* in a 50-l jar with a 25-l working volume; a medium containing 12% glucose and 3% yeast extract produced 46.1 g l^{-1} cells with 42.3% lipids in 7 days. When starch was used as carbon source, the volumetric concentration of AA obtained was 5 g l^{-1}.

The production of these polyunsaturated fatty acids in the cells is related to the age of the mycelia. Fakas et al. (57, 58) found that their fraction was highest in young mycelia, and it decreased as the cells grew older. Enhanced biomass of 28.1 g l^{-1} and a lipid content of 62.4% were achieved for *T. fermentans* by Zhu et al. [220] with peptone as nitrogen source, glucose as carbon source, and a C/N ratio of 163. Similarly remarkable lipid productions have been reported also by Andre et al. [9] and Papanikolaou et al. [158] for *Aspergillus niger* and *Mortierella isabellina*.

Oleaginous molds can also be used for the production of cocoa butter substitutes. Cocoa butter has a high saturated fatty acid content of up to 60%; of this 35% is stearic acid and 25% is palmitic acid [47].

Effect of carbon source and environmental conditions on lipid production by molds

Carbon sources can strongly influence the production and composition of fatty acids in lipids of the fungi due to differences in their metabolism. Glucose, lactose, starches, oils, corn steep liquor, and agricultural produce have been used as carbon sources for production of lipids from fungi [1, 28, 49, 129, 152, 188]. Somashekar et al. [188] reported that glucose was a better carbon source than lactose for fungi *Mucor rouxii* and *Mucor* sp.1b. The cells grown on glucose had high lipid (30% by weight) as well as high GLA content (3–17% of lipids). Although cells cultivated on sesame oil had a higher lipid content (44%), there was no GLA production with plant oil as carbon source. With *Cunninghamella echinulata*, a maximum GLA production of 1.35 mg l^{-1} was obtained when using soluble starch as carbon source [28]. Lipid yield of 0.11 g g^{-1} of dry weight of sweet sorghum was achieved with *M. isabellina* using solid-state fermentation [49]. A lipid content of 4.41% was obtained when orange peel extracts were used as carbon source for *Geotricum candida*; the lipids contained mainly the TGA (24.31%), FFA (16.74%), sterols (12.0%), and polar lipids [221].

A high GLA content of 18.3% was obtained by Hiruta et al. [78] with *Mortierella ramanniana* under the optimized condition of pH (4), inoculum spore concentration (5×10^3 ml^{-1}), and agitation (800 rpm). The effect of medium composition, temperature, pH, culture time, and substrate concentration was studied by Xian et al. [211] for the production of γ-linolenic acid (GLA) by *M. isabellina* on octadecanol, and the maximum production was obtained using 2% octadecanol, 1% yeast extract, and 25 mmol l^{-1} of Mg^{2+} at 23°C for 5 days. Under the optimized conditions of the ratio of steam-exploded wheat straw (SEWS) to wheat bran (WB) of the dry substrate, initial moisture content, and incubation temperature, the maximum single-cell oil production of 8% of dry cells was obtained in *Microsphaeropsis* sp. by Peng and Chen [162].

The highest value of GLA of 36% w/w of lipids and 2.7% w/w of cells was produced by *M. inaquisporus* when growing exponentially in batch cultures [51]. Sergeeva et al. [183] studied the synthesis of lipids with the fungal species *P. moreaui, P. caucasica*, and *P. anomala*, and estimated the fatty acid profiles of each species. The maximum stearic acid content was 11.8–15.8% in *P. moreaui*, and 4.1–9.6% in *P. anomala* and *P. caucasica*. In all the species, 0.4–1.4% eicosanoic acid and 0.6–2.7% of unsaturated fatty acids (palmitoleic and eicosenoic acids) were found. The cellulolytic fungus, *Aspergillus oryzae* A-4, yielded a lipid content of 36.6 mg g^{-1} dry substrate by direct microbial conversion of wheat straw in suspended cultures and 62.87 mg g^{-1} dry substrate in solid substrate fermentation under optimized conditions [91]. With peptone and glucose as C and N sources, *M. rouxii* and *Mucor* sp. resulted in production of 30% lipids with up to 17% GLA in lipids; the major fatty acids produced were palmitic, stearic, and oleic acids [188]. A C/N molar ratio of

163, initial pH of 6.5, and 25°C were the optimum conditions for lipid production with *T. fermentans* [220].

Some fungal cells (*A. niger, A. oryzae*) are net producers of lipids, whereas others (*Pleurotus ostreatus*) are net consumers of lipids; the total lipid content of sweet potato fermented with *A. niger*, *A. oryzae* cells in solid-state fermentation increased from 1.93 to 3.17% and 8.71%, respectively, but decreased from 1.93 to 0.54% when fermented with *P. ostreatus* [1].

Yeast

Oleaginous yeasts are single-celled fungi having at least 20% of their dry weight made up of lipids [174]. Oleaginous yeasts have a fast growth rate and high oil content, and their triacylglycerol (TAG) fraction is similar to that of plant oils. These organisms can grow on a multitude of carbon sources (glucose, xylose, arabinose, mannose, glycerol, and other agricultural and industrial residues). Most oleaginous yeasts can accumulate lipids at levels of more than 40% of their dry weight and as much as 70% under nutrient-limiting conditions [17]. However, the lipid content and fatty acid profile differ between species [17, 122, 143]. Some of the yeasts with high oil content are *Rhodotorula glutinis, Cryptococcus albidus, Lipomyces starkeyi*, and *Candida curvata* [143]. The main requirement for high lipid production is a medium with an excess of carbon source and other limiting nutrients, mostly nitrogen. Hence, production of lipids is strongly influenced by the C/N ratio, aeration, inorganic salts, pH, and temperature [159].

Effect of carbon sources

Yeasts are able to utilize several different carbon sources for the production of cell mass and lipids. These sources can be glucose, xylose, glycerol, starch, cellulose hydrolysates, and industrial and municipal organic wastes. In all cases, accumulation of lipids takes place under conditions of limitations caused by a nutrient other than carbon. Easterling et al. [48] explored production of lipids by the yeast *R. glutinis* on different carbon sources (dextrose, xylose, glycerol, mixtures of dextrose and xylose, xylose and glycerol, and dextrose and glycerol). The highest lipid production of 34% TAG on a dry weight basis was measured with a mixture of dextrose and glycerol as carbon source [48]. The fraction of unsaturated fatty acids in the TAGs was dependent on carbon source, with the highest value of 53% on glycerol and lowest value of 25% on xylose. With whey permeate for production of lipids by different yeast strains, *L. starkeyi* ATCC 12659 was found to have the highest potential of accumulating lipids among *Apiotrichwn curvatum* ATCC 10567, *Cryptococcus albidus* ATCC 56297, *L. starkeyi* ATCC 12659, and *Rhodosporidium toruloides* ATCC. The yeast *L. starkeyi* is unique in that it is known not to reutilize the lipids produced by it [80] and it produces extracellular carbohydrolases [103]. Angerbauer et al. [10] explored production of lipids from sewage sludge using yeast *L. starkeyi*. While there was no growth on untreated sludge, pretreatment of sludge with alkali or acid or heat, or even ultrasound resulted in cell growth and lipid accumulation. Sludge itself had no inhibitory effect on cell growth.

Production of microbial lipids from glucose and sweet potato starch has been studied [161, 206]. These authors confirmed the earlier reports [10] of the effect of C/N ratio on production of lipids by *L. starkeyi* and that the conditions favoring accumulation of lipids result in reduced growth of cells. The cells could consume liquefied starch in batch culture and produced cells containing 40% lipids at a cell yield of 0.41 g dry weight per g starch [206]. The yield on starch was higher than when glucose was used as carbon source.

C/N ratio, pH, temperature and other environmental parameters

Culture temperature and pH influence the total cell number and lipid content in yeast cells [151, 17]. In minimal medium with glucose as carbon source, the yeast *L. starkeyi* accumulates large fractions of dry weight as lipids with a high yield in the pH range of 5.0–6.5 [10]. But Patil [161] found that cell yield on glucose was higher at pH of 5.5 than at 6.

The lipid fraction and fatty acid composition in yeast *C. curvata* varied with temperature, pH, and medium composition, but octadecenoic, stearic, and linoleic acids remained the principle fatty acids in the yeast cells [116]. In *Saccharomyces cerevisiae* cells, the degree of unsaturation increased and the chain length in fatty acids decreased when the cells were cultivated at lower temperatures [15]. At higher temperatures, the cellular lipid content, the glucose conversion efficiency, and the specific lipid production rates in *L. starkeyi* were high, but the degree of fatty acid unsaturation was low [191]. Fastest growth of *L. starkeyi* cells occurred at 28°C (specific growth rate 0.158 h^{-1}), and the lipid fraction in cells under these conditions was 55%. However, the fraction of oleic acid in the lipids increased from 52 to 60% of lipids when the accumulation phase temperature was reduced from growth temperature of 28–15°C.

High lipid accumulation in cells of oleaginous yeast is obtained under limiting nitrogen concentration conditions [17, 54, 161]. The oleaginous yeast *L. starkeyi* delivered lipid content of 68% at a C/N ratio of 150 compared to 40% in the presence of a C/N ratio of 60 while growing on

digested sewage sludge [10]. Similar results were obtained by Patil [161] also while using a semi-synthetic medium for cultivation of *L. starkeyi*. Patil [161] reported a maximum specific growth rate of cells at 0.1 h^{-1} and lipid content of 27% fed-batch fermentation, which is slightly higher than the specific growth rate of 0.08 h^{-1} and lipid content of 23% in batch fermentation of *L. starkeyi* on glucose in a semi-synthetic medium with C/N molar ratio of 56.7. The key fatty acids produced were C16:0, C16:1, C18:0, and C18:1. Accumulation of lipids by *Cryptococcus curvatus* cells also required a high C/N ratio of 50 in batch and fed-batch cultures [75]; the fatty acids produced were mainly oleic (C18:1), palmitic (C16:0), and stearic (C18:0). The highest fraction of stearic acid (18:0) in batch cultures was 14 and 19% in fed-batch culture.

Under optimal fermentation conditions in a batch reactor (100 g l^{-1} glucose as carbon source, 8 g l^{-1} yeast extract, and 3 g l^{-1} peptone as nitrogen sources, initial pH of 5.0, inoculation volume of 5%, 28°C temperature, and 180 rpm agitation in a 5-l bioreactor), *Rhodotorula glutinis* can accumulate lipids up to 49% of cell dry weight and 14.7 g l^{-1} lipid [41]. In continuous culture, the cell biomass, lipid content, and lipid yield increase with decreasing growth rate [4]. Dai et al. [41] obtained 60.7% lipids in cells and 23.4 g l^{-1} lipid production in a continuous mode of operation. In *R. toruloides* cultivated in fed-batch mode, oleic, palmitic, stearic, and linoleic acids were the main fatty acids [119]. Also in *R. mucilaginosa* TJY15a, 85.8% long-chain fatty acids were composed of palmitic, palmitoleic, stearic, oleic, and linolenic acids [124]. Under continuous culture conditions, Ratledge and Hall [173] recommend nitrogen-limited medium and a dilution rate of about one-third of the maximum to achieve the maximum content of lipids in a microorganism.

Bacteria

Bacteria demonstrate high cell growth rates under simple cultivation methods [143]. Bacterial species such as *Mycobacterium*, *Streptomyces, Rhodococcus*, and *Nocardia* can accumulate triacylglycerols (TAG) at high concentrations. The compositions and structures of bacterial TAG vary considerably depending on the microorganism and on the carbon source [3]. The Actinomycete group of bacteria are capable of accumulating remarkably high amounts of intracellular fatty acids as TAGs (up to 70% of the cell dry weight) from simple carbon sources like glucose under growth-limited conditions [102]. The bacterial species *Rhodococcus* and *Nocardia corallina* accumulate primarily TAGs with minor amounts of diacylglycerols (DAGs) and wax esters under nitrogen-limiting conditions [5]. The accumulation takes place mostly during the stationary phase of growth, i.e., after the cessation of net protein synthesis [154].

Not all bacteria, however, accumulate large quantities of fatty acids. Bacterial strains *Dietzia maris* sp. S1, *Stappia* sp. AG2, *Nocardioides* sp. S3, *Sphingomonas* sp. AG6, *Oceanicaulis alexandrii* sp. AG4, *O. alexandrii* sp. AG7, and *Micrococcus* sp. AG10 isolated from marine living cells, contain a total fatty acid (TFA) content from 0.3 to 4% dry weight [218]. Bacterial growth in batch operations is affected by two variables: micro- and macro-nutrient limitations. Excess micronutrients support very high biomass concentrations. However, macronutrients are consumed progressively, and consequently they cause a slowing down of and finally halt in growth [113].

Bio-resources available for lipid production

Commercial production of lipids is hampered by the high cost of the substrates. Hence, various low-cost and effective alternative feedstocks have been explored. These include carpet mill effluents [30, 31], sweet sorghum juice [49, 62, 126], sweet potato waste [1, 205, 206], tomato waste [56], dairy farm and municipal wastewater [74, 99, 106, 114, 140, 155, 203, 208], biodiesel-derived glycerol [9, 12, 53, 127, 128, 137, 149, 160], fertilizer effluent [7], sewage sludge [10], urea [82], lignocellulosic materials [88], waste molasses [105, 220], beet molasses [55, 65, 116], soluble starch [28, 161], sugar cane molasses [4, 68], orange peel extracts [221, 68], industrial glycerol [157], power plant flue gas [100, 217], prickly pear juice [74], agro industrial byproducts [68, 152], corn steep liquor [129], cassava starch [124], wheat straw mixed with wheat bran [162], waste rice straw [89], and starch wastewater [215]. Most of these substrates are locally available and thus are expected to support mainly small production facilities. The exceptions are the sewage sludge and power plant flue gas, both of which have potential for very widespread usage. Given carbon dioxide production of 452–920 kg per MWh [93] and algae yield of 0.55 kg per kg CO_2, each power plant has a potential for producing 1,900–4,000 tons of algae (or 1–2 kton algae assuming a 50% CO_2 capture efficiency) per MW capacity per year. With 30–35% extractable lipids in algae, this potential translates into 0.1–0.2 million gallons of lipids along with 0.7–1.4 kton algal cake per year for each MW power plant capacity while reducing carbon dioxide emissions in half.

Modes of cultivation for microbial lipid production

Batch, repeated batch, fed-batch, and continuous cultivations all have been used to produce microbial oils in the

laboratory. Agitation systems are critical in the bioreactors as was shown by Hiruta et al. [79]. These authors compared the Maxblend impeller to turbine impeller in agitated fermenters and showed that the mixing capacity of the Maxblend impeller was higher. Consequently, the mixing time was less than 50% of the turbine impeller, and a higher GLA content in the lipid was achieved.

Batch operation

Most studies on microbial lipid accumulation have been conducted using batch cultivation. The cell growth and the production of neutral lipids, carbohydrates, and proteins by the alga *Botryococcus sudeticus* was studied by Duhalt and Greppin [46] in batch culture for a period of 18 weeks, and a maximum production of 4.5% protein, 7.5% carbohydrate, and 22.0% neutral lipid on a dry weight basis was obtained during the stationary phase. During the exponential growth phase of the batch operation, most of the metabolic energy is used for synthesis of cell constituents; in the stationary phase, the energy is predominantly used for the synthesis of extracellular compounds [46]. The fungal strain *M. inaquisporus* has been used for production of lipids in batch reactors. The degree of unsaturation and *GLA* content of the lipid increased with increasing lipid content in *M. inaquisporus* during the logarithmic growth phase and decreased during the stationary growth phase while the lipid content in the biomass remained constant [51]. At bench scale, the maximum GLA content obtained was 2% (w/w) dry biomass. Some commercial strains can accumulate up to 4% GLA during batch processing [170].

Fed-batch and repeated batch operation

Fed-batch culture has proved effective in increasing both the cell density and lipid contents of oleaginous yeasts. Microbial lipid production by the oleaginous yeast *Rhorosporidium toruloides* was studied in batch, flask fed-batch, and pilot-scale fed-batch (15-l stirred-tank fermenter) mode using glucose as carbon source [118–120]. Flask fed-batch mode resulted in a cell concentration of 151.5 g l^{-1} with a cellular lipid content of 48.0% w/w in 25 days. Lipid yield in this case was 0.26 g per g glucose, and volumetric lipid productivity was 2.91 g l^{-1} day^{-1}. Flask fed-batch was conducted in shake flask with intermittent feeding of sugar to keep the glucose concentration above 20 g l^{-1}. When the same cells were cultivated in a 15-l stirred bioreactor with controlled agitation, pH, and aeration with the same sugar feeding scheme, the cells grew to a concentration of 106.5 g l^{-1} within 134 h. The lipid content in the cells in the bioreactor was 67.5%, resulting in lipid productivity of 0.54 g l^{-1} h^{-1} [118–120]. Xue et al. [214] obtained a similar trend for batch and fed-batch mode with *Rhodotorula glutinis* and monosodium glutamate (MSG) wastewater. Both authors found that fed-batch mode is a better option than batch mode for higher productivity. Meesters et al. [142] and Yamauchi et al. [216] also reported higher lipid productivity with *Zygomycets*, *C. curvatus*, and *L. starkeyi* in fed-batch cultivation, respectively. Hsieh and Wu [82] compared biomass production and lipid productivity for a *Chlorella* sp. in intermittent fed-batch and in a repeated batch mode of operation. These authors achieved higher productivity with the repeated batch mode of operation. Repeated batch operation was investigated also by Chiu et al. [34] with *Nannochloropsis oculata*, Veloso et al. [200] with *Phaeodactylum tricornuturn*, and Feng et al. [60] with *C. vulgaris*. Feng et al. [60] reported that lipid productivity increases with increased volumes of daily withdrawals even though cell density goes down.

Continuous operation

In general, high cell yields occur when the cells are cultivated under steady-state conditions and when carbon is used with the same efficiency at each stage of the growth cycle [172]. Brown et al. [22] studied growth and lipid accumulation with the yeast *C. curvata D* in both batch and continuous mode and indicated that the specific lipid accumulation rate increases during the course of batch fermentation and as dilution rate is increased in continuous cultivation. But the rate of non-nitrogenous non-lipid biomass production undergoes a decrease under the same conditions. Papanikolaou and Aggelis [157] reported that the microorganism *Y. lipolytica* is capable of producing huge quantities of lipid during growth on raw glycerol in nitrogen-limited continuous cultures. In highly aerated continuous cultures, lipid production was favored at low dilution rates, and the highest lipid productivity achieved was 0.12 g l^{-1} h^{-1} at the lowest studied dilution rate of 0.03 h^{-1}. Increasing dilution rates resulted in increased cell mass yield but with a decreasing lipid fraction. Fatty acid composition of the lipids was not affected by dilution rate. *R. glutlnis* was investigated for its ability to accumulate lipids in continuous culture with molasses under nitrogen-limiting conditions [4]. The maximum lipid content of 39% (w/w) of dry cell biomass was obtained at a dilution rate of 0.04 h^{-1}. Banerjee et al. [13] reported that only continuous systems are realistically feasible systems for cultivation of the fungus *B. Braunii* for microalgal biomass.

Composition of fatty acids in microbial lipids

Oils and fats are primarily composed of triacylglycerols (TAGs). TAGs serve as a primary storage form of carbon

and energy in microorganisms [87]; their fatty acid composition is also superior to that of other cellular lipids (phospholipids and glycolipids) for biodiesel production [165]. Fatty acid composition impacts on the saponification number, iodine value of the particular lipids [101], and influences the quality of biodiesel, such as cetane number, heat of combustion, oxidative stability, cloud point, and lubricity [110]. Although fatty acids in microbial lipids range from lauric acid (C12:0) to docosahexaenoic acid (C22:6), palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acids constitute the largest fraction. Of these, palmitic and oleic acids are most abundant. Considering the saturated and unsaturated acid components, approximately 25–45% are saturated fatty acids, and 50–55% are unsaturated. Thus, the ratio of unsaturated to saturated fatty acids in microbial oils ranges between 1 and 2, which is somewhat similar to that in plant oils (such as palm). As a result, the quality of biodiesel produced from microbial oils can be expected to be similar to that produced from palm oil.

When cultivated under appropriately optimized conditions, microorganisms are capable of producing significant quantities of γ-linoleic (C18:2) and arachidonic (C20:4) acids. These fatty acids have high nutraceutical value, and microbial oils are generally marketed as extracted oils as health food. Technologically, the production of these high-value compounds is accompanied by production of significant quantities of other neutral lipids. Hence, separation of non-neutraceutical fatty acids from the PUFA needs to be explored.

The fatty acid composition of algal lipids is mainly influenced by the medium composition [20, 50, 1, 59, 61, 85, 132, 133]. Liu et al. [132, [133] cultivated the microalgae *Chlorella zofingiensis* with glucose, and they found that C16:0, C16:2, C18:1, C18:2, and C18:3 (n-3) were the major fatty acids produced, out of which oleic acid contributes 35.7% of the total fatty acids. Polyunsaturated fatty acids C18:3ω3, C16:4ω3 were produced with the algae *D. salina* by manipulating the NaCl concentration of the medium [50]. The nitrogen content present in the medium significantly altered the saturated and unsaturated fatty acid compositions [20]. Hu and Gao [85] also found that the fatty acid composition was influenced by the concentrations of nitrate and phosphate in the medium and reported that the key fatty acids produced with *Nannochloropsis* sp. were C16:0, C16:1, and C18:1. Cultivation temperature can also alter the fatty acid composition significantly [73]. They found that at temperatures below 20°C, a higher production of eicosapentaenoic acid (EPA), GLA, and dihomo-GLA was obtained at the expense of AA and α-linoleic acid. Renaud et al. [176] also found that temperature affects fatty acid composition. C16:0, C18:0, and C18:1 fatty acids constituted 95% of all the fatty acids produced with the oleaginous microalgae *Pseudochlorococcum* sp. growing on starch [123–125], out of which 42% was oleic acid (C18:1) alone. C16:0, C16:2, C18:1, C18:2, and C18:3 (n-3) were the major fatty acids in *C. zofingiensis* cultured with glucose as the carbon source, and here too C18:1 accounted for 35.7% of the total fatty acids produced.

Fakas et al. [57, 58] reported that the fugal strains *Cunninghamella echinulata* and *M. isabellina* have the same fatty acid composition, and the composition is independent of the carbon source. Linoleic acid (18:2) and GLA are the key fatty acids produced during the initial stages of growth. But when the lipid accumulation exceeded 20%, oleic and palmitic acids were predominant [57, 57, 58]. Fatty acid composition of lipids in *M. isabellina* was 24–35% palmitic acid, 49–54% oleic acid, 2–11% linoleic acid, 0.4–2% GLA, 1–2% palmitoleic acid, and 3.5–8.0% stearic acid [49]. Palmitic acid (27.3%), palmitolic acid (3.5%), stearic acid (3.0%), oleic acid (46.1), and linoleic acid (20.0) were present in *Microsphaeropsis* sp. Huang et al. [89] and Zhu et al. [220] have reported lipid production by fungi *T. fermentans* CICC1368 with glucose and/or xylose as carbon source. With peptone as nitrogen source, glucose as carbon source, C:N ratio of 163, pH 6.5, and 25°C temperature, Zhu et al. [220] found that these cells could produce as much as 62.4% of their dry weight as lipids after 7 days of fermentation. With a cell density of 28 g l^{-1}, this amounted to 17.5 g lipid per liter of broth.

Myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid are the main fatty acids in lipids from yeasts [118–120, 120, 130]. Patil [161] investigated the effect of the C/N ratio on lipid production and on fatty acid composition of lipids in *L. starkeyi* cultivated under different operating conditions. In batch shaken flasks, the percent lipids in cells increased from 19 to 30% as the C/N ratio was changed from 20 to 61. But the cell mass yield decreased from 0.44 g DW per g glucose to 0.29 g DW per g glucose. C16:0 (38.7–44.8%) and C18:1 (40.7–50.2%) were the main fatty acids in the lipids, although small amounts (5.9–14.5%) of C16:1 were also produced. As the C/N ratio was increased, there was a perceptible shift in fatty acids from C16 to C18 fatty acids. Lipid profiles in *L. starkeyi* and other yeast cells have been reported by a number of researchers [10, 216]. Angerbauer et al. [10] reported lipid composition in *L. starkeyi* cells grown on sewage sludge. Here, too, the main fatty acids were C16:0 (56%), C16:1 (2%), C18:0 (14%), and C18:1 (26%). Small amounts of C14:0 (myristic acid), C18:3 (linolenic acid), C20:0 (arachidic acid), C20:1 (gadoleic acid), and C22:0 (behenic acid) were also present; these were all under 1% by weight and mostly under 0.5% by weight. Yamauchi et al. [216] reported the fatty acid profile of lipids produced by *L. starkeyi* cells grown on ethanol in

a fed-batch culture. These authors found that fatty acid composition in the cells undergoes change with time during fed-batch cultivation; the fractions of C16:0 and C18:1 fatty acids in the intracellular lipids increase as time increases up to 90 h. The fractions of C18:0 and C18:2 went down with time, while that of C16:1 remained unchanged. C16:0 accounted for 28–32% of all the fatty acids and C18:1 for 50–54%.

The nature of substrate affects the composition of fatty acids in yeast lipids. Patil [161] noticed that cultivation of *L. starkeyi* on starch as substrate resulted not only in a higher fraction of lipids in cells, but also in more oleic acid in cellular lipids than when the cells were cultivated on glucose as carbon source.

Economics of microbial lipid production

Economically viable biofuels should be cost competitive with petroleum fuels. The single-cell oil production cost depends mainly upon the species chosen for cultivation [18], lipid concentration within cells, and the concentration of cells produced [21]. The cost of feed stock or carbon source required for the production of microbial lipids accounts for 60 to 75% of the total costs of the biodiesel [90]. Hence, the economics of single-cell oil production can be improved by using carbon in wastes such as wastewater, municipal, and other carbonaceous industrial wastes and CO_2 in flue gases from boilers and power plants [57, 58]. Patil [161] conducted economic analysis of biodiesel production with *L.starkeyi* and starchy waste from the sweet potato processing industry as carbon source and determined that microbial lipids could be produced at a factory gate price of $2.30 per gallon and that it would support a biodiesel price of $3.00 per gallon with a continued subsidy of $1.00 per gallon of biodiesel. The cost of lipid production was influenced strongly by the cost of medium nutrients (50%) needed for cultivation of cells and the cost of solvent (25%) for the extraction of lipids from biomass.

Utilization of algae for commercial production of lipids depends on use of efficient cultivation systems. Huntley and Redalje [92] suggested that microalgal biodiesel production is economically feasible using a two-stage open pond system and estimated the price of oil derived from microalgae was at US$2.00 per gallon. Kadam [100] reported that the unextracted algal lipid can be produced at a cost of US$ 1.4 per gallon using power plant flue gases. The production cost of algal biodiesel can be reduced by using economical photobioreactors that have advantages like high productivity, low contamination, efficient CO_2 capture, continuous operation, and controlled growth conditions. Unfortunately, the capital and operating costs of photobioreactors are very high [23]. Hence, Feng et al. [60] evaluated utilization of heterotrophic cultivation and reported that the algal biofuels can be competitive with crude oil at US$ 63.97 per barrel with *C. vulgaris* from artificial wastewater after accounting for the wastewater treatment cost of US$ 0.4 m^{-3} and energy costs of $0.22/KWh. Similarly, the biodiesel production cost with algae *C. zofingienesis* growing heterotrophically on glucose as sole carbon source was estimated by Liu et al. [132] at US$ 0.9 l^{-1} or $3.40 per US gallon. These authors considered a bulk glucose price of $100/ton and lipid yield of 0.21 g g^{-1} glucose and suggested that the production price of algae can be reduced considerably if a lower cost carbon source can be used for cultivation of cells [132].

Conclusion

Microbial lipids offer potential for sufficient production of renewable fuels to impact consumption of fossil fuels. In order to succeed in this endeavor, a suit of autotrophic, heterotrophic, and mixotrophic microbial systems utilizing diverse substrates is available. These have been described in this review. In order to be cost effective, it will be necessary to use innovative combinations of cultivation systems involving all the carbonaceous materials including wastes. Several recent efforts in this area use domestic and industrial wastes. Economic analyses have indicated the need to minimize costs of medium components and for further research dealing with microbial systems capable of producing lipids at relatively high productivities in minimal media.

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