Abstract

Petroleum prices are increasing day by day and for fueling we are completely dependent on the fossil fuels. Sources of fossil fuels are depleting continuously due to the increasing demand of petroleum products. Renewable carbon neutral transport fuels are necessary for environmental and economic sustainability. There are many options in this area, but unlike solar, nuclear, and fossil fuels, biofuels such as bioethanol, biodiesel, and green diesel have the capability of providing a fuel source ideally suitable for existing infrastructure within the transportation industry. Biodiesel, when blended with petroleum diesel, can be used in unmodified diesel engines. It has higher lubricity than petroleum diesel, so it helps provide for greater longevity within diesel engines. There is currently great interest in using microalgae for the production of biofuels, mainly due to the fact that microalgae can produce biofuels at a much higher productivity than conventional plants and that they can be cultivated using water, in particular seawater, and land not competing for resources with conventional agriculture. The main focus of this review article is to illustrate the role of microalgae in the production of biofuels, their biosynthesis, commercial production, extraction and purification.

Keywords- Microalgae, Biofuels, Methane, Hydrogen, alternative energy.

Introduction

Microalgae are classified as the most primitive form of plants. The mechanism of photosynthesis in microalgae is similar to that in higher plants, but they are usually more efficient converters of solar energy because of their simple cellular structure. They normally grow in suspension within a body of water (Chang, 2007). They can double every few hours during their exponential growth period (Metting, 1996). They commonly double every 24 hrs. During the peak growth phase, some microalgae can double every 3.5 hrs (Chisti, 2007). They contain large amounts of lipids within their cell structure, and so they are increasingly becoming an interest as a biofuel feedstock. The Oil contents of microalgae are usually between 20-50% (dry weight) while some strains can reach as high as 80% (Metting 1996; Spolaore, Joannis-Cassan et al., 2006). Due to the fact that they grow in aqueous suspension, they have more efficient access to water, CO₂, and other nutrients. These factors account for the ability of microalgae to produce larger quantities of oil per unit area of land as compared to terrestrial oilseed crops.

Macroalgae and microalgae are two different groups and they are completely different from each other in morphology. Macroalgae are the large (measured in inches), multi-cellular algae often seen growing in ponds. The largest multi cellular algae are called seaweed; an example of which is the giant kelp plant which can be more than 100 feet long. Microalgae, on the other hand, are tiny (measured in micrometers), unicellular algae that normally grow in suspension within a body of water. Microalgae have many different species with widely varying compositions and live as single cells or colonies without any specialization. Although this makes their cultivation easier and more controllable, their small size makes subsequent harvesting more complicated. Macroalgae are less versatile, there are far fewer options of species to cultivate and there is only one main viable technology for producing renewable energy: anaerobic digestion to produce biogas.

Present scenario-

According to Exposy news 2007, biodiesel production is constantly increasing with an average annual growth rate of over 40% during the period 2002-2006. In 2006 the amount of biodiesel production in the world ranged 5-6 million tones, with 4.9 million tons produced in Europe (of which 2.7 million tones in Germany), and great part of the remaining quantity produced in the USA (Martinot, Eric, Renewable 2007 Global status Report, 2008). Moreover the global production of vegetable oil for all
purposes in 2005-2006 touched 110 million tones, of which about 34 million tones is of palm oil and soybean oil (Biopower London, Biodiesel to drive up the price of the cooking oil, 2006).

According to Nastari (2008), world ethanol production has grown, on average, 12 per cent per year between 2000 and 2007. In 2007, world ethanol production for energy reached 49.5 billion liters (13 billion gallons). This amount represents 4.4 per cent of global gasoline consumption (1.117 trillion liters or 295 billion gallons).

The estimated production of ethanol by 2012 in the United States is between 45.2 and 51.4 billion liters (12–13.5 billion gallons), about two and a half times current production. Brazil is expected to produce between 35.4 and 40.5 billion liters by the same year (9.3–10.7 billion gallons), double the amount of its 2007 production (ICONE, 2007).

Sources of Biofuels-

First generation biofuels can be produced from vegetable oil extracted from many plants like soybeans, rape seeds, sunflower seeds, and palm oil. These biofuels have a number of problems. First, there is not enough available farmland to provide more than about 10 percent of the developing countries’ liquid fuel needs. The use of first generation biofuels also raises the price of animal feed and ultimately increases the cost of food (Huber et al., 2009).

Second generation biofuels are made from cellulosic biomass. Sources include wood residues like sawdust and other cellulosic sources like construction debris, agricultural residues like corn stalks and wheat straw, fast growing grasses and woody materials that are grown for the sole purpose of making biofuel. The advantage of second generation biofuels is that they are abundant and do not interfere with the production of food. Most of these energy crops can be grown on marginal lands that would not otherwise be used as farmland (Huber et al., 2009).

Third generation biofuels includes fuel produced from algae and cyanobacteria. Algae grown in ponds can be far more efficient than higher plants in capturing solar energy especially when grown in bioreactors. If algal production could be scaled up to industrial capacity, less than 6 million hectares would be needed worldwide to meet the current fuel demand. This consists of less than 0.4% of arable land which would be an achievable goal from global agriculture. For example, in Texas which has a land mass of 67,835,300 hectares, only 271,300 hectares would be required for the growth of algae. In addition, many of the most efficient algal species are marine which means that no freshwater would be necessary in the culture phase (Gressel, 2008).

<table>
<thead>
<tr>
<th>Crop</th>
<th>Oil yield(L/ha)</th>
<th>Land area needed (M ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>172</td>
<td>1540</td>
</tr>
<tr>
<td>Soybean</td>
<td>446</td>
<td>594</td>
</tr>
<tr>
<td>Canola</td>
<td>1190</td>
<td>223</td>
</tr>
<tr>
<td>Jatropha</td>
<td>1892</td>
<td>140</td>
</tr>
<tr>
<td>Coconut</td>
<td>2689</td>
<td>99</td>
</tr>
<tr>
<td>Oil palm</td>
<td>5950</td>
<td>45</td>
</tr>
<tr>
<td>&quot;Microalgae&quot;</td>
<td>136,900</td>
<td>2</td>
</tr>
<tr>
<td>&quot;Microalgae&quot;</td>
<td>58,700</td>
<td>4.5</td>
</tr>
</tbody>
</table>

"70% oil (by wt) in biomass; ‘30% oil (by wt) in biomass.

Biofuels from Microalgae

Today, biodiesel production by algae is of major interest. Many species of algae accumulate large amounts of oils. The algal oil is converted into biodiesel through a trans-esterification process.

Microalgae have two major advantages over higher plants with respect to biofuel production: First, biomass productivities are expected to be significantly greater for microalgae. Second the cultivation of microalgae does not require the arable land or fresh water; it can be carried out in shallow ponds on hardpan soils, using saline or brackish water. Many species of microalgae, such as Dunaliella, grow in sea water, allowing its utilization for CO₂ enriched air. The combination of high biomass productivities and the lack of need for arable land and freshwater allows the large scale production of microalgal biofuels. Without affecting agricultural commodities prices, thereby avoiding the ethical conflict that arise when diverting crops that are...
desperately needed to feed a growing population for biofuels production.

Algal oils have been found to be very high in unsaturated fatty acids. Some of these unsaturated fatty acids that are found in different algal species include: arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, gamma-linolenic acid, and linoleic acid. When comparing the lipid yield of algae to vegetable sources, algae can produce between 20,000 and 100,000 liters per hectare.

Parent oil used in making biodiesel consists of triglycerides in which three fatty acid molecules esterifies with a molecule of glycerol (Qiang et al., 2008). In making biodiesel, triglycerides are reacted with methanol in a reaction known as transesterification or alcoholysis (Bradshaw et al., 1942). Transestrification produces methyl esters of fatty acids that are biodiesel and glycerol (Figure 1).

Fig. 1. The transesterification process (Sheehan et al., 1998)

Table 2. Chemical composition of algae on a dry matter basis (%)

<table>
<thead>
<tr>
<th>Species of sample</th>
<th>Nucleic acid</th>
<th>Proteins</th>
<th>Carbohydrates</th>
<th>Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scenedesmus obliquus</td>
<td>50–56</td>
<td>10–17</td>
<td>12–14</td>
<td>3–6</td>
</tr>
<tr>
<td>Scenedesmus quadricauda</td>
<td>47</td>
<td>—</td>
<td>1.9</td>
<td>—</td>
</tr>
<tr>
<td>Scenedesmus dimorphus</td>
<td>8–18</td>
<td>21–52</td>
<td>16–40</td>
<td>—</td>
</tr>
<tr>
<td>Chlamydomonas rheinhardii</td>
<td>18</td>
<td>17</td>
<td>21</td>
<td>—</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>51–58</td>
<td>12–17</td>
<td>14–22</td>
<td>4–5</td>
</tr>
<tr>
<td>Chlorella pyrenoidosa</td>
<td>57</td>
<td>26</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Spirogyra sp.</td>
<td>6–20</td>
<td>33–64</td>
<td>11–21</td>
<td>—</td>
</tr>
<tr>
<td>Dunaliella bioculata</td>
<td>49</td>
<td>4</td>
<td>8</td>
<td>—</td>
</tr>
<tr>
<td>Dunaliella salina</td>
<td>57</td>
<td>32</td>
<td>6</td>
<td>—</td>
</tr>
<tr>
<td>Euglena gracilis</td>
<td>39–61</td>
<td>14–18</td>
<td>14–20</td>
<td>—</td>
</tr>
<tr>
<td>Prymnesium parvum</td>
<td>28–45</td>
<td>25–33</td>
<td>22–38</td>
<td>1–2</td>
</tr>
<tr>
<td>Tetraselmis maculata</td>
<td>52</td>
<td>15</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Porphyridium cruentum</td>
<td>28–39</td>
<td>40–57</td>
<td>9–14</td>
<td>—</td>
</tr>
<tr>
<td>Spirulina platensis</td>
<td>46–63</td>
<td>8–14</td>
<td>4–9</td>
<td>2–5</td>
</tr>
<tr>
<td>Spirulina maxima</td>
<td>60–71</td>
<td>13–16</td>
<td>6–7</td>
<td>3–4.5</td>
</tr>
<tr>
<td>Synechoccus sp.</td>
<td>63</td>
<td>15</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Anabaena cylindrica</td>
<td>43–56</td>
<td>25–30</td>
<td>4–7</td>
<td>—</td>
</tr>
</tbody>
</table>

(Source: Becker, 1994.)

**Production of lipid by microalgae**

Some microalgae accumulates neutral lipids particularly triglycerol (TAGs) under certain conditions like nutrient deficiency (nitrogen and phosphate) and other environmental stresses such as extreme pH values, salinity, or heavy metal toxicity. These lipids and triglycerides can be directly converted into biodiesel.

Von Witsch (1948, 1953) first reported that some algae contains upto 80% TAGs, under certain environmental conditions. Piorreck, found that nitrogen limitation increases the percentage of lipid in two microalgae *Chlorella vulgaris* and *Scenedesmus obliquus* upto 70% (Piorreck et al., 1984). Suen et al (1987) reported that under nitrogen deficient conditions, *Nannochloropsis sp.* accumulates 55% lipids, consisting of 79% TAGs.
The earliest work compared digestion of wastewater sludge and green microalgal biomass, *Scenedesmus* and *Chlorella*, harvested from wastewater ponds (Golueke et al., 1957). They found that these algae could yield as much as 0.25-0.50 L CH₄/g VS input at an 11-day retention time when incubated at 35-50°C. The lower value was 32% less than the yield from the wastewater sludge. In addition, the maximum VS destruction was about 45% for the algae, compared to 60% for the wastewater sludge. They suggested that the relatively low digestability and thus yield of microalgal biomass was the result of cell walls resisting bacterial degradation, but being more readily digested by bacteria at the higher temperature.

Sheehan et al., (1998) reported that nitrogen deficiency led to increased lipid content in many microalgae like *Ankistrodesmus* (from 24% to 40%), *Isochrysis* (from 7% to 26%) and *Nannochloris* (from 21% to 35%).

It is found that nutrient limitation like nitrogen deficiency (limitation) showed the adverse effect on the lipid production. Shifrin and Chrisholm (1981) found that the lipid content in *D. tertiolecta* decreased slightly under nitrogen starved condition. In another study it was found that total lipids per cell decreased in a nitrogen limited culture of *D. viridis* grown in (0.035% CO₂), but not at 1% CO₂.

**Production of Methane by microalgae**

Anaerobic digestion involves the microbial conversion of biodegradable material in the absence of oxygen in to biogas, a mixture of methane (CH₄) and carbon dioxide (CO₂) and may have small amounts of hydrogen sulphide (H₂S), and moisture. A wide variety of biomass feedstock and waste have been used to generate methane by anaerobic digestion, with lignocellulosic biomass (wood, crop residues, etc.) resulting in little or no gas production while more putrecible substrates (food wastes, waste water slugs, animal wastes, etc.) generate considerable biogas, typically expressed in terms of methane per gram of volatile solids (VS) destroyed (Gunaseelan 1997). The natural anaerobic digestion of microalgae has been demonstrated to attain a 40–80% conversion at 20°C in 200 days (Foree and McCarty, 1970). Application of a concentrated algal biomass mixture as a feeding for anaerobic digesters resulted in a lower performance than that with raw sewage sludge as substrate, yet values of 0.5 m³ biogas kg⁻¹ algal organic dry matter (VS) supplied could be obtained (62.5% CH₄) (Golueke et al., 1957).

Relatively few studies have been published on the anaerobic digestion of microalgae (reviewed recently by Sialve et al., 2009). The earliest work compared digestion of domestic wastewater sludge and green microalgal biomass, *Scenedesmus* and *Chlorella*, harvested from wastewater ponds (Golueke et al., 1957). They found that these algae could yield as much as 0.25-0.50 L CH₄/g VS input at an 11-day retention time when incubated at 35-50°C. The lower value was 32% less than the yield from the wastewater sludge. In addition, the maximum VS destruction was about 45% for the algae, compared to 60% for the wastewater sludge. They suggested that the relatively low digestability and thus yield of microalgal biomass was the result of cell walls resisting bacterial degradation, but being more readily digested by bacteria at the higher temperature.

The anaerobic digestion of *Spirulina maxima* resulted in a biogas yield of 0.3-0.37 m³ biogas kg⁻¹ VS, with 70% methane and conversion efficiencies up to 48% (Samson and Leduy, 1982, 1983a,b, 1986). Maximum yields were obtained with a retention time of 30 days and an algal concentration of 20 kg VSm⁻³. In contrast to the study of Golueke et al., (1957), a mesophilic temperature (35°C) was found most preferable for the degradation of the algal biomass (Samson and Leduy, 1986). Biogas productivity could be increased by mixing the proteinaceous algal biomass with carbon-rich wastes such as sewage sludge (Samson and Leduy, 1983b) or waste paper (Yen and Brune, 2007), thereby increasing the C/N ratio of the digester feeding. Mechanical and thermochemical pretreatments have been applied on algal biomass to increase the biodegradability of the algae. The resulting higher solubility of the biomass entailed a positive effect for the acid forming bacteria. The methanogenic bacteria, however, appeared to be only influenced by the chemical composition of the culture medium (Samson and Leduy, 1983a). Good results were obtained with a thermochemical pretreatment at 100°C for 8 h without NaOH, which could increase the efficiency of methane fermentation with 33%, up to 0.32 m³ kg⁻¹ VS (Chen and Oswald, 1998). Algae biomass typically has a high protein content (40-50%; C:N ratio 6:1), which contributes to high total ammonia concentrations in the sludge. Co-digestion with high-carbon, low-nitrogen substrates has potential for diminishing any ammonia toxicity and also increasing the biogas production per unit volume of digester tank. Methane yield and productivity were doubled when equal masses of wastewater sludge and *Spirulina* biomass were co-digested (Samson and Leduy, 1983). Similarly, Yen (2004) and Yen and Brune (2007) added waste paper (50% w/w) to aquacultural microalgal sludge to adjust the C:N ratio to around 20-25:1 which, in turn, doubled the methane production rate from 0.6 L/L day to 1.2 L/L day at 35°C and with a hydraulic retention time of 10 days.

The economics of anaerobic digestion depend on the process used, with a wide range of engineering options available, such as fixed tank, mechanically mixed, heated digesters, used for digestion of waste water sludge.

**Production of Ethanol and Other solvents by microalgae**

According to Nastari (2008), world ethanol production has grown, on average, 12% per year between 2000 and 2007. In 2007, world ethanol production for energy reached 49.5 billion liters (13 billion gallons). This amount represents 4.4% of global gasoline consumption (1.117 trillion liters or 295 billion gallons).
There are two different processes by which ethanol can be generated by microalgae. First, by yeast fermentation of carbohydrate storage products such as starch in green algae, glycogen in cyanobacteria, or even glycerol that is accumulated at high salinities. Second by an endogenous self-fermentation of carbohydrate storage products by algal enzymes induced in the absence of oxygen.

Matsumoto et al., (2003) tested the saccharification of starch produced by marine microalgae using a salt tolerant amylase from a marine bacterium since terrestrial amylases were found to be inactive in saline cell suspensions.


Production of Hydrogen by Microalgae

Hydrogen production plays a very important role in the development of hydrogen economy. Producing H₂ using conventional methods defeats the purpose of using H₂ as a clean alternative fuel. The production of H₂ from non-fossil fuel sources has become central for better transition to H₂ economy. Certain microorganisms can produce enzymes that can produce H₂ provides an attractive option to produce hydrogen through microbial process. A large number of microbial species, including significantly different taxonomic and physiological types, can produce H₂.

It is reported that some bacteria and green algae are capable of biologically evolving H₂ under certain conditions [Miyake et al., 1999]. One possible source is the green alga, Chlamydomonas reinhardtii which is found all around the world as green pond scum. This alga has the potential to produce large amounts of hydrogen because it can directly split water into hydrogen and oxygen using the enzyme, hydrogenase. The first report on H₂ production by green algae was dated back to 1942 (Gaffronet et al., 1942). Gaffron and Rubin (Gaffronet H., 1942) found that green alga Scenedesmus obliquus under anaerobic conditions could evolve H₂ in both dark and light. H₂ evolution in green algae requires a certain period of anaerobic incubation in the dark to induce the reversible hydrogenase. The hydrogenase then functions to combine protons and electrons to form H2 (Greenbaum et al., 1977, Greenbaum et al.,1982, Happe et al.,1994, Yildiz et al.,1994). The high quantum yield of photosynthesis makes it feasible to produce H₂ by green algae using the two most abundant resources of light and water in our planet. Moreover, it is even more preferable to photobiologically generate H₂ using seawater.

The processes of biological H₂ production can be broadly classified into following distinct approaches: 1) Direct biophotolysis 2) Indirect biophotolysis 3) Photofermentation 4) Dark fermentation (Benemann 1996, Nath and Das 2004)

Dark fermentation involves the anaerobic conversion of microbial reduced substrates (e.g. starch, glycogen, glycerol, etc.) into hydrogen, solvents, and mixed acids. This process is either carried out by externally supplied anaerobic heterotrophs (e.g., clostridia, enteric bacteria, etc.) or, in some case, by microbial cell itself.

H₂ can also be produced by microalgae via direct or indirect biophotolysis in which the fundamental concept is to use microalgae to catalyze the conversion of solar energy and water into H₂ fuel, with O₂ as a byproduct.

Markov et al., 1997 investigated the indirect biophotolysis with Cyanobacterium Anabaena variabilis exposed to light intensities of 45–55 Amol/m² and 170–180 Amol/m² in the first stage and second stage, respectively. Photoproduction of hydrogen at a rate of about 12.5 ml H₂/gdw h (cdw:cell dry weight) was found. In the study on indirect biophotolysis with Cyanobacterium Gloeocapsa alpicola by Troshina et al. (Troshina et al., 2002), it was found that maintaining the medium at pH value between 6.8 and 8.3 yielded optimal hydrogen production. Increasing the temperature from 30°C to 40°C can increase the hydrogen production twice as much. The hydrogen production rate through indirect biophotolysis is comparable to hydrogenase-based hydrogen production by green algae.

Commercial Production systems

For Macroalgae (seaweed) and microalgae different culture systems are required. Because of their small (μm) size, microalgae have to be cultivated in a system designed for that purpose (placed on land or floating on water).

(i) Photobioreactors

Photobioreactors are different types of tanks or closed systems in which algae are cultivated (Richmond, 2004). Algal cultures consist of a single or several specific strains optimized for producing the desired product. Water, necessary nutrients and CO₂ are provided in a controlled way, while oxygen has to be removed. Algae receive sunlight either directly through the transparent container walls or via light fibres or tubes that channel it from sunlight collectors. A great amount of developmental work to optimise different photobioreactor systems for algae cultivation has been carried out and is reviewed in Janssen et al., (2003), Choi et al., (2003), Carvalho et al., (2006), and Hankamer et al., (2007). It has also been suggested to grow heterotrophic algae in conventional fermentors instead of photobioreactors for production of high-value...
products (Jiang and Chen 1999; Wen and Chen 2003). Instead of light and photosynthesis, heterotrophic algae are relying on utilizable carbon sources in the medium for their carbon and energy generation (Ward and Singh, 2005).

(ii) Open pond systems
Open pond systems are shallow ponds in which algae are cultivated. Nutrients can be provided through runoff water from nearby land areas or by channelling the water from sewage/water treatment plants. The water is typically kept in motion by paddle wheels or rotating structures. (Borowitzka, 1999 and Chaumont, 1993).

Harvesting of micro-algae
The micro-algae are typically small with a diameter of 3 – 30 μm, and the culture broths may be quite dilute at less than 0.5 g l-1. Thus, large volumes must be handled. The harvesting method depends on the species, on the cell density, and often also on the culture conditions. Harvesting costs may contribute 20 – 30% to the total cost of algal biomass (Molina Grima et al., 2003).

Conventional processes used to harvest micro-algae include concentration through centrifugation (Heasman et al., 2000), foam fractionation (Cordas and Wang, 2004), flocculation (Poelmann et al., 1997; Knucckey et al., 2006) membrane filtration (Rossignol et al., 2000) and ultrasonic separation (Bosma et al., 2003).

The oil from the dry algae biomass can be extracted through various methods. One of the least expensive extractions is simply through cold pressing. Up to 70% of the oil contained within the algae can be extracted this way (Danielo, 2005). The use of organic solvents can increase this extraction level to 99%, but there is an increased cost in processing to achieve this (Metzger and Largeau, 2005). Using direct transesterification allows for a single step process that extracts and the algal oils and reacts them with methanol to result in biodiesel.

Most extraction methods are based on a method developed by Bligh and Dyer in 1959 (Lewis et al., 2000). There are a number of modifications to this method (White, 1979; Dunstan 1993; Smedes 1999). However, algal tissue is much different from animal tissue, for which the Bligh and Dyer method was developed. Research has reported that the lipid in algae is more difficult to extract with these methods (Ahlgren, 1991). Some direct transesterification reactions involve a mix of solvent, alcohol, and catalyst. The solvent works to extract the lipid as the alcohol and catalyst convert it into methyl esters. Others use heat combined with methanol and catalyst to remove and transform the fatty acids (Bo Liu, 2007). These processes use less solvent than the extraction process followed by transesterification process (Lewis et al. 2000). This is an important factor to consider since most organic solvents are toxic and must be recovered. Lewis (2000) found that direct transesterification greatly increased the total amount of fatty acids extracted.

Whichever way the oil is extracted (or directly reacted), it undergoes a transesterification reaction to produce the fatty acid methyl esters. After transesterification, the biodiesel is separated from the rest of the reactants. Glycerol must be removed with multiple washings with water (Wen, 2006). If direct transesterification was used, there will be particulate matter from the algal biomass in the mix, and it has to be removed via filtration. The biodiesel can be used as fuel after washing.

The main concerns with algal biodiesel production are some of the limitations that make algal biodiesel too expensive to commercialize (Sheehan et al., 1998). These include contamination with unwanted species, low oil yields, and the overly expensive harvesting step to recover the algal biomass from the growth medium.

Conclusion
Microalgae appear to represent the only current renewable way to generate biofuels. Microalgae biofuels are also likely to have a much lower impact on the environment and on the world’s food supply than conventional biofuel-producing crops. When compared with plants biofuel, microalgal biomass has a high caloric value, low viscosity and low density, properties that make microalgae more suitable for biofuel than lignocellulosic materials, as well as due to their inherently high-lipid content, semi-steady-state production, and suitability in a variety of climates.

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Lewis, T., Nichols, P. D. et al. 2000. Evaluation of extraction methods for recovery of fatty acids from lipid-


