PRODUCTION OF BIODIESEL FROM MARINE AND FRESHWATER MICROALGAE: A CRITICAL REVIEW

ABSTRACT

The increase in the annual global energy consumption over the past century has relayed heavily on fossil fuels. Fossil fuel burning have accelerated CO_2 emissions on a global scale. CO_2 makes up 63 % of the greenhouse gasses present in the atmosphere. The environmental concerns associated with greenhouse gas emissions emphasise the need for alternate energy sources that are more environmentally friendly. The aim of this paper was to review the availability of various types of algae for the production of biodiesel and other value added products and to investigate the factors that affect cell growth and lipid production in the cells, the various oil extraction methods, and the methods of conversion of the extracted lipids into biodiesel. Microalgae are abundant in nature and can be used as an alternate source of energy. They are photosynthetic microorganisms that are capable of growing in marine and fresh water environments and converting organic substances to oil. Their high growth rate, ability to produce large amounts of lipids which can be used for biodiesel production and to utilize CO_2 present in the atmosphere for growth, makes them a good alternative to fossil fuel. Microalgae generate oil in the form of triacylglycerols which can be converted into biodiesel, via chemical or enzymatic a transesterificaition process. Biodiesel is a renewable fuel that generates the same amount of energy as that generated from petroleum diesel without the release of harsh compounds into the atmosphere, it is biodegradable and nontoxic and can be utilized in existing diesel engines without modification. Currently, the use of microalgae for biodiesel production is not economically feasible because of the high harvesting and pre-treatment costs associated with the production process. This can be overcome by extractingproteins, vitamins, carotenoids, nucleic acid, carbohydrates and lipids from the algae and processing the algae biomass into various value added products such as ethanol, methane, animal feed and fertilizer. Additionally, theglycerol produced as a by-product duringlipid conversion into biodiesel can be further fermented to produce products such as methanol, lactic acid, ethanol and hydrogen. By producing these value-added products in addition to the biodiesel, the economics of the harvesting, pre-treatment and processing of microalgae into biodiesel can be improved significantly.

Keyword: Microalgae, biodiesel, transesetraficaiton, oil extraction, lipids, carotenes, vitamins, carbohydrates, proteins, nucleic acid, ethanol, methane, animal feed, nutraceuticals

1. INTRODUCTION

The increase in the annual global energy consumption over the past century has relayed heavily on fossil fuels (oil, coal, and natural gas) for powering up cars, farms and factories and for the production of electricity (Areva, 2011). The world consumption of crude oil, coal and natural gas in 2009 was 84.2 million barrels/day, 7.99 million short tons/day and 2 831 billion m^3, respectively (Ratner, 2010). In 2011, the total sales of gasoline and diesel in Canada amounted to 40.4 billion litres and 17.8 billion litres, respectively(Statistics Canada, 2012).

Fossil fuel burning has accelerated CO_2 emissions on a global scale from 1.1 % per year in 1990 to more than 3 % per year in 2004 (Raupach et al. 2007). Carbon dioxide contributes about 63 % of the greenhouse gasses emitted into the atmosphere (Mendelsohn et al. 1994; Hoffmann et al. 2006). A change in global warming would increase the atmospheric temperature and negatively impacts all living organisms (Root et al. 2003). An increase in the earth's temperature results in a decline in the Adelilie penguins spices, melting of glaciers, increased precipitationcausing floods(National Geographic, 2011; Hanna et al. 2008) and increased sea level causing loss of property and productive agricultural lands (Meehl et al. 2005). In Canada, global warming effects are already felt across the nation. Forest fires, floods, insect infestations and drought arethe results of global warming (Dai, 2010; Westerling et al. 2006; Gazette, 2005).

The environmental concerns of greenhouse gas emissions emphasise the need for alternate energy sources that are more environmentally friendly. Various biomass materials can be used as renewable sources of energy that offer immediate prospects for producing renewable liquid biofuels such as biodiesel and bioethanol which can be used as substitutes for petroleum products (Singh and Gu, 2010). Using biofuels over the traditionally used fuels offers the benefits of greater energy security, foreign exchange savings and reduced environmental problems (Balat, 2009; Yenikaya et al., 2009; Kan, 2009). Biomass feedstocks that can be used for energy production include food waste, municipal waste, agricultural waste, fish processing waste, animal rendering waste, edible and nonedible oilseeds and aquatic plants. Oilseeds are currently for biofuel production, but are considered a food source for millions of people around the world (Singh and Gu, 2010; Demirbas, 2005).

Algae, which are abundant in nature, can be used as an alternate fuel source (Chisti, 2007; Hu et al. 2008). Using microalgae as an energy source is ideal because of their high growth rate and their ability to produce lipids that can be used for the production of biodiesel (Song et al. 2008). The

majority of lipids produced by microalgae have a low degree of unsaturation, making them a good replacement for the current fossil fuels (Singh and Gu, 2010). Microalgae are photosynthetic microorganisms capable of surviving in marine and freshwater environments. Their advantages include: (a) they tend to have a much higher oil yield than terrestrial plants and require much less land spaceas shown in Table 1,(b) they can produce and store large amounts of oil without the production and release of harmful wastes into the environment, (c) they are extremely resilient and often unaffected by fluctuations in the environment, (d) they utilize carbon dioxide for their growth, thus reducing greenhouse gas emissions and (e) they are not considered a traditional food, hence they do not compete with traditional agriculture (Aresta et al. 2005; Wahlen et al., 2011; Singh et al. 2010; Demirbas, 2010; Pokoo-Akins et al. 2010; Demirbas, 2011; Chen et al. 2009).

Biodiesel, as a liquid fuel, can be produced by transestraficaiton of oil extracted from algae (Leung et al. 2010; Demirbas and Demirbas, 2011; Wahlen et al. 2011). Algae generate oil in the form of triacylglycerols which can be converted into biodiesel by the addition of methanol and the use of a catalyst (acid, base or enzyme) (Demirbas, 2005; Chen et al. 2009). The waste generated from the algal biomass can be further processedto produce other biofuels such as methane via anaerobic digestion or bioethanol via fermentation. Additionally, the algal biomass waste can be converted to other value added products such as protein supplement in animal feed, organic fertilizers, cosmetics and pharmaceutics (Demirbas, 2010; Demirbas, 2011; Chen et al. 2009; Banerjee et al. 2009). It is important to note that different types of microalgae contain different cellular compositions (as percent of dry weight). Algae with higher lipid composition are better suited for biodiesel production while those possessing higher sugar content are more appropriate for production of bioethanol (Demirbas, 2010).

In terms of efficiency, biodiesel provides 2% less energy than petroleum diesel. Thus, in 2011 Canada would have required 44.9 billion litres of biodiesel to meet its transportation needs (Chen et al. 2009; Statistics Canada, 2012). The biggest advantage of biodiesel, compared with other alternative transport fuels, is that it can be utilized in existing diesel engines without much modification (Singh and Gu, 2010). Other advantages of using biodiesel generated from microalgae include: no release of harsh compounds (carbon monoxide and carbon dioxide) into the atmosphere (Maceiras et al. 2011), it is biodegradable and nontoxic, a much cleaner energy source and far more environmentally friendly than the current energy sources being used (Ulusoy et al. 2004; Kalam and Masjuki, 2005; Demirbas, 2005; Maceiras et al. 2011). In addition, microalgae can generate 44.9 billion L of biodiesel in 345-764 thousand hectares of land per year (depends on algal composition)

95 **Table 1.** Biomass feedstock oil yield comparison (DOE, 2009; Chisti, 2007; Lele, 2009).

Crop	Oil Yield (L/ha/year)	Land required to produce 44.9 L* of biodiesel (million ha)
Corn	172	261
Soybean	446	100
Peanut	1 059	42.4
Sunflower	952	47.2
Canola	1 190	37.7
Rapeseed	1 190	37.7
Jatropha	1 892	23.7
Oil Palm	5 950	7.55
Microalgae	58 700-130 000	0.764-0.345

96 *Amount of biodiesel required to meet the transportation demand of Canada in 2011.

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which is far less than the land required for the same amount of biodiesel to be generated using other terrestrial crops (Table 1).

2. TYPES OF ALGAE

Algae, is a term that refers to thallophytes or photosynthetic oxygen producing organisms that lack roots, stems, leaves and vascular tissues, making them different from plants (Barsanti and Gualtieri, 2006). Their size can range from 0.2 μm to 60 m in length. They are either prokaryotic or eukaryotic and can be divided into two groups:macroalgae and microalgae (Barsanti and Gualtieri, 2006; Demirbas 2010; Carlsson et al. 2007; Chen et al. 2009). Both macroalgae and microalgae are found in marine and freshwater environments (Barsanti and Gualtieri, 2006). They formthe base of the food chain by providing nutrition and an accommodating environment for other organisms (Brodie and Lewis, 2007; Banerjee et al. 2009). Algae can either be subaerial or aquatic, but the main bulk of algae are microscopic aquatic organisms that are visible to the eye when colonies are formed (Diaz-Pulido and McCook, 2008).

2.1. Macroalgae

Macroalgae are multicellular organisms that can also be referred to as “seaweeds”. They are fast growing plants that can reach up to 60 m in length, growing in salty or freshwater environments. They lack roots, stems and leaves and are composed of thallus with a stem and foot (Carlsson et al. 2007). Based on pigmentation,macroalgae are broadly classified to three main groups (Figure 1): brown seaweed (Phaeophyceae), red seaweed (Rhodophyceae) and green seaweed (Chlorophyceae). In order to provide buoyancy, some of these species have gas filled structures (Carlsson et al. 2007; Barsanti and Gualtieri, 2006). Microalgae are mainly used for food production and hydrocolloid extraction (Demirbas 2010; Carlsson et al. 2007). They are found to produce both carbohydrates and lipids. The carbohydrates are used as their main energy storage compound and the small amount of lipids produced (Table 2) are used to make up the cell membrane structure (Sheehan et al. 1998). Some species such as *P. fermandeziana* can store high level of lipids as shown in Table 2.

Industrial applications of macroalgae vary widely. Some of the applications for the polysaccharides formed by the macroalgae species include cosmetics, food, paint, textiles, rubber, paper and building industries (Banerjee et al. 2009). Also, their anticoagulant, antiviral, antitumor and brinolytic properties make them a great asset for use in medicine and pharmacology (Boopathy

(a) Phaeophyceae

(b) Rhodophyceae

(c) Chlorophyceae

Figure 1. Main groups of macroalgae (Peters, 2006; Baker et al. 2012).

138 **Table 2.** Chemical composition of macroalgae species (Agrimer, 2013; Goecke et al. 2012).

Class	Species	Protein (%)	Carbohydrate (%)	Lipid (%)
Brown	*P. fernandeziana*	6-8	1.8-2	30-44
	Ascophyllum nodosum	5-13	45-60	2-4
Red	*Chondrus crispus*	5-25	50-60	0.6-6
	Palmaria palmate	8-30	43-66	1-4
Green	*Enteromorpha sp.*	7-20	30-45	1-3
	Ulva lactuca	7-30	41-62	1-3

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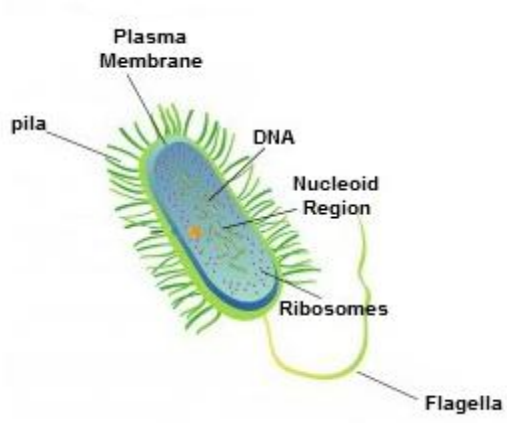
and Kathiresam, 2010). Boopathy and Kathiresam (2010) noted that macroalgae are promising agents against cancer because of their metabolites, protein, vitamins, iodine and minerals. The protein content of these organisms varies with type. The brown seaweeds class tend to possess low proteinsin the range of 5-15% (dry weight), while the green and red seaweeds have a higher protein range of 10-30% (dry weight), Table 2. Proteins present in these species are especially important in the food industry, particularly in developed countries (Banerjee et al. 2009). The lipids present in these species represent only 1-5% of the algal dry mass and are mainly made up of omega 3 and omega 6 polyunsaturated fatty acids which play an important role in prevention of osteoarthritis, diabetes and cardio vascular diseases (van Ginneken et al. 2011). Van Ginneken et al. (2011) reported a total lipid content of seven macroalgae species screened to range from 7 to 45 mg/g (dry matter) and that omega-3 and omega-6 polyunsaturated fatty acids ranged from 2 to 14 mg/g (dry matter). Maceiras et al. (2011) screened fourteen different species of macroalgae collected from Galician coast and noted that they all contained a low oil content that ranged from 0.25 to 3.25% (dry weight). The low lipid composition of macroalgae makes them unsuitable for biodiesel production (Bruton et al. 2009; Maceiras et al. 2011). However, they can be used for the production of biogas and bioethanol and also as a source of medicine (Bruton et al., 2009).

2.2. Microalgae

Photosynthetic microscopic organisms are known as microalgae (Demirbas 2010; Chen et al. 2009). They are single celled organisms and are categorized into several groups on the basis of pigmentation, basic cellular structure and life cycle (Sheehan et al. 1998: Demirbas 2010). Microalgae can be further broken down into prokaryotic cyanobacteria (Figure 2) and eukaryotic algae(Figure 3). Table 3 lists a few of the divisions found in prokaryotic and eukaryotic algae. Eukaryotic algae are classified into 12 divisions (Chen et al. 2009). In terms of abundance, the four most important groups of microalgae are diatoms (Bacillariophyceae), green algae (chlorophyceae), golden algae (chrysophyceae), and the blue-green algae (cyanophyceae), Figure 4 (Sheehan et al. 1998; Chen et al. 2009). They are capable of tolerating extreme temperatures and pH conditions as well as being able to live in various environments such as freshwater, marine water and wastewater (Chen et al. 2009; Maceiras et al. 2011).

Microalgae are made up of proteins, carbohydrates, lipids and nucleic acid. The proportions vary widely among species. The protein, carbohydrate, lipid and nucleic acid contents are in the ranges of 6-71, 4-64, 1.9-40 and 1-6 % (dry basis), respectively (Pimentel et al. 2008). There are microalgae species capable of achieving up to 40% lipids which makes them suitable for biodiesel production. In addition, microalgae in comparison with macroalgae have a much more



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175 **Figure 2.** Cell structure of a prokaryotic organism (TutorVista, 2013).

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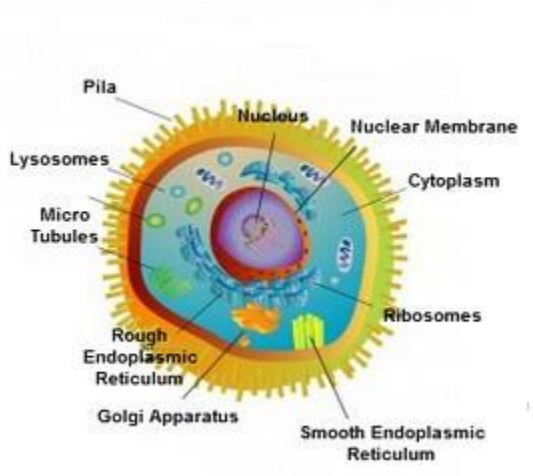

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178 **Figure 3.** Cell structure of a eukaryotic organism (TutorVista, 2013).

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180 **Table 3.** Microalgae group classification (Barsanti and Gualtieri, 2006).

Kingdom	Division
Prokaryota eubacteria	Cyanophyta
	Prochlorophtya
Eukaryota	Glucophyta
	Rhodophyta
	Heterokontophtya
	Haptophyta
	Cryptophyta
	Dinophyta
	Euglenophyta
	Chlorarachniophyta
	Chlorophyta
	Bacillariophyta
	Xanthophyta
	Phaeophyta

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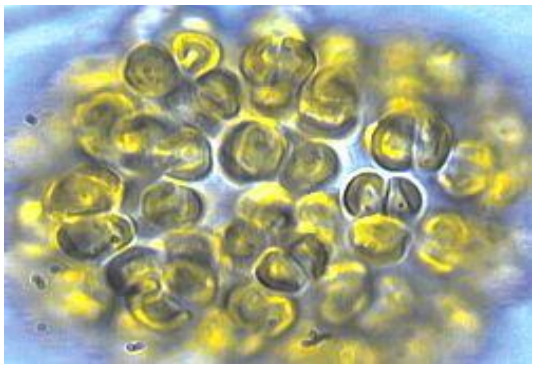
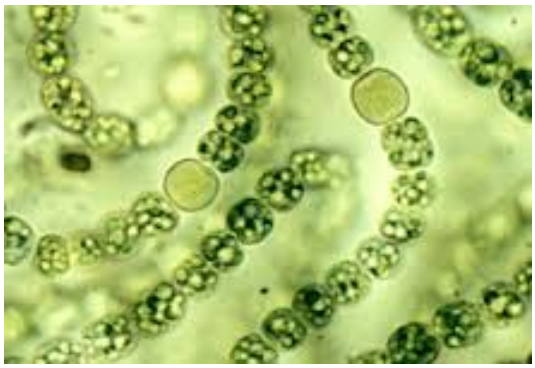
(a) Bacillariophceae

(b) Chlorophyceae



(b) Chrysophyceae



(d) Cyanophyceae

Figure 4. The four most important groups of microalgae (Darling, 2013; Garcia et al., 2003; Zeenews, 2013).

192 rapid growth rate under optimal conditions and under unfavourable conditions they alter their
193 metabolic products to form natural oils (triaclyglycerols) (Chen et al. 2009). These characteristics
194 add to their increased appeal for utilization in biodiesel production.

195 Li et al. (2011) reported 95% biodiesel yield from oil extraction from *Chlorella pyrenoidosa*.
196 Johnson and Wen (2009) achieved a biodiesel conversion efficiency of 95.9 % from the oil extracted
197 (57 %) of *Schizochytrium limacinum*. Miao and Wu (2006) reported a biodiesel yield of 70% from
198 oil extracted from *Chlorella protothecoides*. Stephenson et al. (2010) reported that *Chlorella*
199 *vulgaris*can produce a total biodiesel of 8200 L/ha/year. Rodolifi et al. (2009) noted that
200 *Nannochloropsis* species can produce 23000-34000 L/ha/year of biodiesel.

201 After using microalgae for the production of biodiesel, the remaining biomass residues can be
202 further used to produce numerous by-products (Figure 5).These include: bioethanol via fermentation
203 and hydrolysis (Demirbas, 2011), food ingredients, animal feed, nutraceuticals and pharmaceutical
204 (Pulz and Gross, 2004). The glycerol can also be converted to several chemical products by
205 fermentation including biofuels such as hydrogen and ethanol. Processing of algal biomass after the
206 extraction of lipids would improve the economics of biodiesel (Williams and Laurens, 2010).

207 Harun et al. (2010a) reported a bioethanol production of 3.83 g/L, using 10 g/L of lipid-extracted
208 microalgae (*Chlorococum sp*.) debris via yeast (*Saccharomyces bayanus*) fermentation. Kim et al.
209 (2012) used hydrothermal treatment to fractionate *Schizochytrium* sp. for the production of bioethanol
210 and achieved 11.8 g/L of ethanol form 25.7 g/L of glucose. Chisti et al. (2007) illustrated that in
211 order for an economical balanceto be reached, the biomass that remains after lipid extraction needs to
212 be transformed into methane via anaerobic digestion which can also recycle back nitrogen and
213 phosphorus.Becker (2007) noted that nutritional and toxicological analyses of algae biomass protein
214 illustrated that it is highly suitable as a supplement feed similar to soybean meal, fish meal and rice
215 Bran. Currently 30% of the world's algae production is used as feed supplements. Soletto et al.
216 (2005) noted that the microalgae *Arthrospira* possess a high protein content, and it is for this reason
217 that they are used in human nutrition today.

218 **3. MICROALGAE ENVIRONMENT**

219 Microalgae have the ability to withstand various environments. Their main requirements for
220 biomass production include water, light and carbon dioxide. Microalgae can grow in salt, fresh and
221 wastewater environments (Demirbas, 2011).

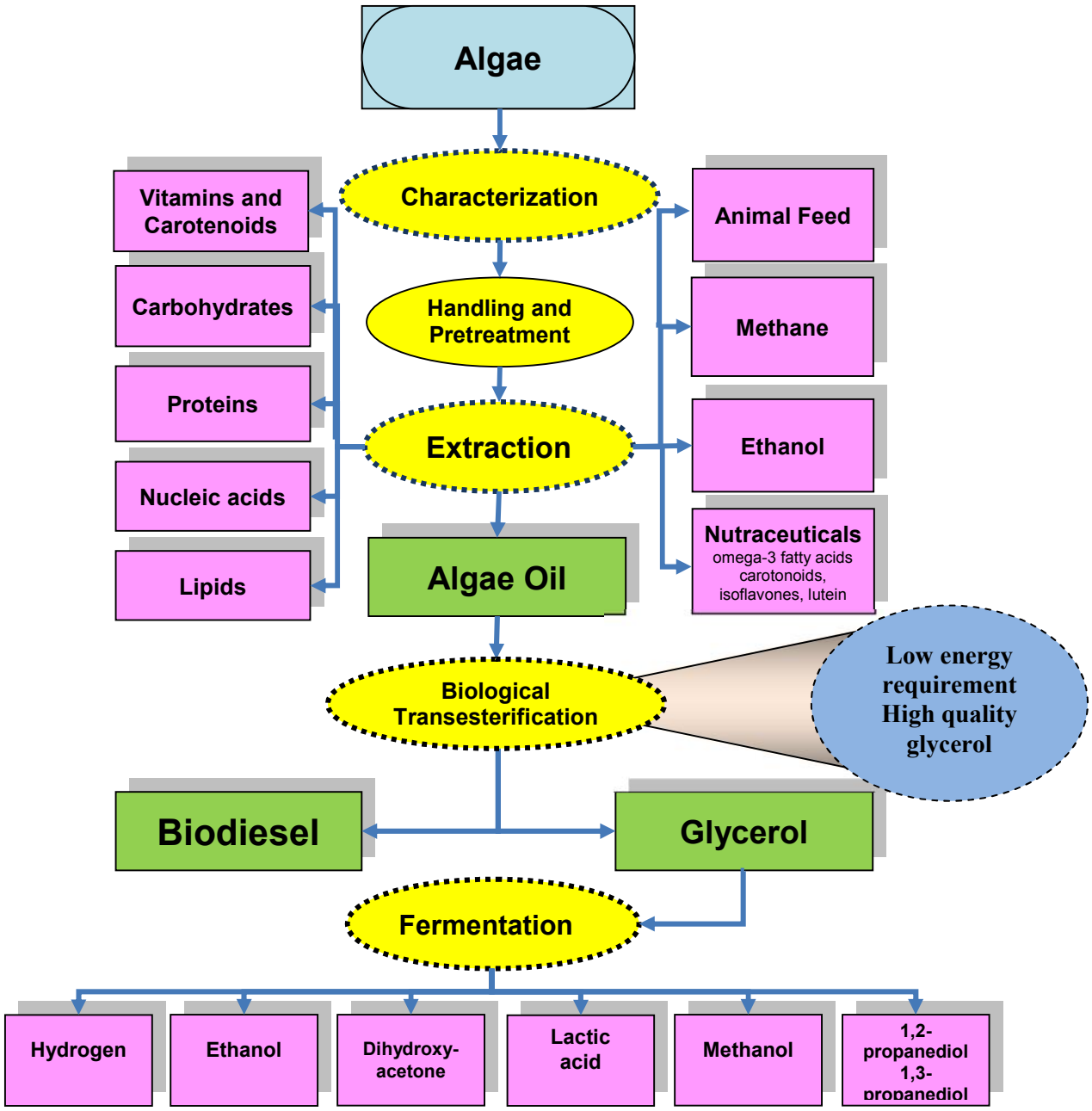


Figure 5. Schematic of value added products that can be produced from algae processing.

3.1. Freshwater

Freshwater (Table 4) contains a few or no saltscompared to marine water. It has a freezing point and a boiling point of 0°C and 100°C, respectively. Therefore, nutrients must be supplied into the media for algae to grow(Aquarius, 2011). Since it has been observed that triacylglycerols (TAG) content in lipid increases with N-deprivation, growing algae in freshwater allows for the manipulation of nitrogen content in order to achieve high algal biomass and high oil content (Chen et al. 2009).

3.2. Marine

Marine water environments are simply seawaters with varying salt contents. Variation in the salt content is a result of varying water evaporation and precipitation in different parts of the ocean. Numerous elements (>70) make up seawater, however only a small number (6) make up more than 99 % of the dissolved salts (Table 5). All of these dissolved salts are in the form of electrically charged atoms or groups of atoms (Badea et al. 2007). Utilizing marine water for microalgae growth would minimize the need for additional nutrients into the production system.

3.3. Wastewater

Wastewater is mainly comprised of water (98.0-99.9%) with small amounts of suspended and dissolved organic and inorganic solids. Table 6 shows the major composition of strong, medium and weak domestic wastewaters. Organic compounds present include: fats, soap, proteins, detergents, natural and synthetic organic chemicals, lignin and carbohydrates. Inorganic substances contain: nitrogen, sodium, calcium, magnesium, phosphorous, potassium and chlorine (FAO, 1992). Growing algae on municipal wastewaters has the advantage of removing certain elements such as phosphorous and nitrogen in addition to the removal of CO_2 from the atmosphere, which if left undealt with are harmful to the environment (Chen et al. 2009).

4. BIOLOGICAL COMPOSITION OF MICROALGAE

Microalgae use light energy in a process called photosynthesis as well as water and carbon dioxide and convert them into lipids, carbohydrates, proteins and nucleic acids in different proportions, depending on the algal type as shown in Table 7 (Demirbas and Demirbas 2011; Singh and Gu, 2010; Lewin, 1962).

Table 4. Composition of freshwater (Swenson, 2013).

Ion	Percentage of Total Salinity
Chloride (Cl^-)	8.64
Sodium (Na^+)	6.98
Sulphate (SO_4^{2-})	12.41
Magnesium (Mg^{2+})	4.54
Calcium (Ca^{2+})	16.62
Potassium (K^+)	2.55
Bicarbonate (HCO_3^-)	31.90
Silica (SiO_2)	14.51
Iron (Fe)	0.74
Nitrate (NO_3^-)	1.11

Table 5. Composition of marine water (Badea et al. 2007).

Element	Percentage
Chloride (Cl^-)	55.05
Sodium (Na^+)	30.61
Sulphate (SO_4^{2-})	7.68
Magnesium (Mg^{2+})	3.69
Calcium (Ca^{2+})	1.16
Potassium (K^+)	1.1
Bromide (Br^-)	0.2
Bicarbonate (HCO_3^-)	0.41
Boric acid (H_3BO_3)	0.07
Strontium (Sr^{2+})	0.03

Table 6. Composition of major constituents in wastewaters (FAO, 1992).

Constituents	Concentration (mg/L)		
	Strong	Medium	Weak
Total Solids	1200	700	350
Dissolved Solids	850	500	250
Suspended Solids	350	200	100
Nitrogen (as N)	85	40	20
Phosphorus (as P)	20	10	6
Chloride	100	50	30
Alkalinity (as $CaCO_3$)	200	100	50
Grease	150	100	50
BOD_5	300	200	100

257 **Table 7.** Microalgae chemical composition (Demirbas, 2011).

Species of sample	Proteins	Carbohydrates	Lipids	Nucleic acid
Scenedesmus obliquus	50–56	10–17	12–14	3–6
Scenedesmus quadricauda	47	-	1.9	-
Scenedesmus dimorphus	8–18	21–52	16-40	-
Chlamydomonas rheinhardii	48	17	21	-
Chlorella vulgaris	51–58	12–17	14–22	4–5
Chlorella pyrenoidosa	57	26	2	–
Spirogyra sp.	6–20	33–64	11–21	-
Dunaliella bioculata	49	4	8	-
Dunaliella salina	57	32	6	-
Euglena gracilis	39-61	14-18	14-20	-
Prymnesium parvum	28-45	25-33	22-38	1-2
Tetraselmis maculata	52	15	3	-
Porphyridium cruentum	28-39	40-57	9-14	-
Spirulina platensis	46–63	8–14	4–9	2–5
Spirulina maxima	60–71	13–16	6–7	3–4.5
Synechoccus sp.	63	15	11	5
Anabaena cylindrica	43–56	25–30	4–7	-

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4.1. Proteins

Proteins in microalgae make up to 8-71 % of the cells (Table 7) and function as the photoreceptive center of the cells. These molecules are present in microalgae because their function is light detection. There are two main types of photoreceptors in microalgae that are responsible of algal vision: rhodopsin-like proteins and flavoproteins. Rhodospin-like proteins function in the visible light region, while flavoproteins function in the near UV-light region (Barsanti and Gualtieri, 2006). It is important to note that these receptive molecules do not play a role in the color of the organism, because the color plays an important role in the classification of the organism (Ahmed and Hellebust, 1993).

Proteins extracted from algae biomass can be used in industrial, therapeutic and diagnostic applications (Hempel et al., 2011). The incorporation of algae protein in food processing is still in its trail phase (Beker, 2007). Algae proteins have been noted to possess antioxidant peptides that play a vital role in the human health (Sheih et al., 2009; Samarakoon and Jeon, 2012). Toxicological and nutritional evaluation of algae protein deem it suitable as a feed supplement or a substitute for current protein sources such as fish meal, rice bran and soybean meal (Beker, 2007; Hempel et al., 2011).

4.2. Carbohydrates

Carbohydrates can make up to 4-64 % of the cell(Table 7). They are a form of storage of the end products of photosynthesis inmicroalgae. They are formedbyCO_2 fixation and can bein theform of sucrose, paramylonand starch. In eukaryotic algae, the fixation process of CO_2 takes place in the storma, while that of prokaryotic algae takes place in the cytoplasm (Barsanti and Gualtieri, 2006). Somealgae produce carbohydrates as their primary energy storage compounds.

Carbohydrates can be extracted and converted into value added products such as ethanol and methane gas. Conversion of carbohydrates into ethanol is achieved by fermentation process (Lee et al., 2011; Nahak et al. 2011; Harun et al. 2010b). This process involves the conversion of carbohydrates by yeast (*Saccharomyces cerevisiae*) under anaerobic conditions into ethanol and carbon dioxide (Gupta et al. 2012). Methane production can be achieved through anaerobic digestion of carbohydrates (Chisti, 2007; Sialve et al. 2009). This process takes place under anaerobic conditions. The anaerobic microorganisms degrade and stabilize organic materials into methane and carbon dioxide (Kelleher et al. 2000).

290 **4.3. Lipids**

291 Another form of energy storage for microalgae is triacylglycerol (TAG) lipids (Table 7). The
292 main building blocks of TAGs and all other cellular lipids are fatty acids. The enzyme acetyl CoA
293 carboxylase is the enzyme responsible for regulating the rate of fatty acid synthesis, which is
294 synthesized in the chloroplast (Hu et al. 2008). Small amounts of spherical lipid droplets are
295 contained in the chloroplasts between the thylakoids, which play a role in the growth and synthesis of
296 lipoprotein membranes within the chloroplast (Lee, 2008). Additionally, these lipids function as a
297 cell structural support and as metabolic organelles in photosynthesis (Chen et al. 2009). Surfactant
298 lipids work to stabilize the structure of the mitochondria and in photosynthesis metabolism (Lewin,
299 1962). Algae typically have 5-20 % DCW lipid oil when grown under optimal conditions, while
300 growth in unfavourable conditions can increase the lipid content to 20-50 % DCW (Hu et al. 2008;
301 Pokoo-Aikins et al. 2010).

302 Lipids extracted from microalgae can be used for biodiesel production. Lipids are converted into
303 biodiesel through transesterification technology which works by using an alcohol and an acid or
304 base. The alcohol plays the role of both the solvent (extracting the lipids from the biomass) and the
305 reactant (converting the lipids into fatty acid methylesters) (Demirbas, 2010; Chen et al. 2009). The
306 alcohol most commonly used is methanol and the reaction catalyst can be an acid or base (Demirbas
307 and Demirbas, 2011; Wahlen et al. 2011).

308 **4.4. Nucleic Acid**

309 Nucleic acid makes up to 1-6 % of the cell and plays a vital role in cell growth and cell repair.
310 During the growth of algae, the accumulation of nucleic acid and other cellular constituents take place
311 and the RNA and protein synthesis are formed in a fixed ratio(Lavens and Surgeloos, 1996).Nucleic
312 acid contains high amounts of phosphorous and nitrogen which can be recycled back by the anaerobic
313 digestionprocess. Also, the biomass containing nucleic acid can be used as fertilizer (Demirbas,
314 2011; Chen et al. 2009).

315 **4.5. Vitamins**

316 Microalgae have been noted to contain numerous essential vitamins such as vitamin A, B_1, B_2, B_6,
317 B_{12}, C, E, nicotinate, biotin, folic acid and pantothenic acid. The amount of vitamins varies with
318 species and cultivation techniques. The range of vitamins present in a few species ranges from 0.4 to
319 554 mg Kg^{-1}as shown in Table 8(Beker, 2004). These vitamins haveimportant antioxidant properties
320 and play vital roles in immune function, vision, reproduction, preventing against kidney disease, birth

defects, depression and asthma (Letcher and Scott, 2012; ODS, 2013; UMMC, 2011). Extraction of these vitamins from microalgae would provide an additional renewable source for vitamin extraction.

4.6. Carotenoids

Microalgae contain numerous carotenoids(β-carotene, zeaxanthin, lutein, violaxanthin, diadinoxanthin, pyrrhoxanthin, peridinin, neoxanthin, fucoxanthin, echinenone, canthaxanthin and astaxanthin) which play important roles in photosynthesis. Carotenoidstypically make up 25 % of the microalgae cells (Fernandez-Sevilla et al. 2010). Theyare synthesized and accumulated in the plastids and can be extracted and used as value added products. Accumulation of these compounds is affected by environmental conditions. Cells exposed to environmental stress accumulate higher amounts of carotenoids (Aburai et al. 2013). Caroteniodshave important anti-inflammatory, antioxidant and anti-tumor activity (Soontornchaiboon et al. 2012;Yoshida et al. 2007), which make microalgae a great source of providing essential compounds for maintaining good human health. These compounds can be used in pharmaceutics, cosmetics, nutraceuticals and medical industries. Carotenoids also have industrial uses acting as coloring agents in natural foods such as egg yolk, fish and chicken (Del Campo et al. 2007; Aburai et al. 2013).

5. FACTORS AFFECTING ACCUMULATION OF LIPIDS IN MICROALGAE

On average, oleaginous green algae consist of 25.5 % lipid of their dry cell weight (DCW). However, when they are grown under stressed environments, the lipid content can double or even triple (Chen et al. 2009; Rodolfi et al. 2009; Scott et al. 2010; Li et al. 2011; Demirbas and Demirbas 2011). Unfavourable conditions alter the synthesis of membrane lipids to the storage of neutral lipids (TAG). The average lipid content of oleaginous green algae grown under unfavourable conditions has been reported to reach 45.7 % DCW (Hu et al. 2008). Oleaginous diatoms showed similar results indicating that under stress conditions the algal lipid content increases significantly.

Certain species such as *Chlorella* (Illman et al., 2000; Hsieh and Wu, 2009), *Dunaliella* (Takagi et al., 2006), *Nannochloropsis* (Rodolfi et al. 2009) and *Neochloris* (Li et al. 2008) have been noted to alter their metabolic pathways from producing proteins to the production of lipids (TAGs) by diverting the fixed carbon under stress conditions. However, lipid content and biomass productivity are inversely related as depicted in Table 9 (Rodolfi et al. 2009).

Table 8. Vitamin content of various microalgae, values are mg Kg^{-1} (Beker, 2004).

	A	B_1	B_2	B_6	B_{12}	C	E	N	B	FA	PA
Spirulina Platensis	840	44	37	3	7	80	120	-	0.3	0.4	13
Chlorella pyrenoidoisa	480	10	36	23	-	-	-	240	0.15	-	20
Scenedesmus quadricauda	554	11.5	27	-	1.1	396	-	108	-	-	46

N= Nicotinate
B=Biotin
FA= Folic Acid
PA= Panthotenic Acid

Rodolfi et al. (2009) noted that of the marine species screened, *Chaetoceros* was found to produce the highest lipid content of 40 %, but resulted in the lowest biomass productivity. Alonso et al. (2000) noted that an increase in the total lipid content in the *P. Tricornutum* cells due to unfavorable conditions resulted in a biomass reduction. Hu et al. (2008) reported an increase in lipid content of oleaginous diatoms from 22.7 % DCW under normal conditions to 44.6 % under unfavourable conditions.

Lipid synthesis (i.e. non-polar TAGs) is the best substrates for biodiesel production which can be formed by alternating some of the algal growth conditions (Rodolfi et al. 2009). These conditions can be either chemical or physical environmental stimuli (Guschina and Harwood, 2006; Hu, 2004; Hu et al., 2008). Physical stimuli include temperature and light intensity and chemical stimuli include pH and depravation of nutrients (Hu et al. 2008).

5.1. Temperature

Temperature plays a major role in the lipid yield and composition of fatty acids in algae (Hu et al. 2008). The optimal temperature for algalgrowth is in the rangeof 18-20°C. Temperatures that do not fall within this range are found to affect the rate of growth or result in the death of the organisms (Chen et al. 2009; Demirbas and Demirbas, 2011). However, increases in lipid content have been noted by several research's in microalgae grown at temperatures both above and below the optimal range (Chen et al. 2009; Converti et al. 2009; De Castro and Garcia 2005; Jiang and Gao, 2004; Renaud et al. 2002; Macedo and Alegre, 2001).

Renaud et al. (2002) tested the temperature effect on the growth rate and lipid content of three algal species *Isochrysis* sp., *Nitzschiaclosterium* and *Nitzschia paleacea*. They noted that over the temperature range of 10-35°C, the *Isochrysis* sp. had the highest growth rate and a lipid content at 20°C. Significantly lower growth rates were observed at temperatures of 10, 15,25 and 30°C. *Nitzchia closterium* did not grow at temperatures above 30°C or lower than 20°C with no significant difference in growth rate at temperatures of 30, 25 or 20°C. The maximum lipid production of20.1 % was noted at 20°C. Finally, *Nitzschia paleacea*was toleranttolow temperatures, although the growth rate at 10°C was very low. The maximum growth rate for this species was noted at 15°C while the maximum lipid content of 21.2 % was noted at 10°C.

Converti et al. (2009) noted that the species *N. Oculata* produced double the lipid content (from 7.9 to 14.9 %) as the temperature increased from 20 to 25°C. De Castro and Garcia (2005) reported

387 **Table 9.** Microalgae species screened for biomass productivity, lipid content and lipid productivity
388 (Rodolfi et al. 2009).
389

Microalgae	Biomass Productivity (g/L/day)	Lipid Content (% biomass)	Lipid Productivity (mg/L/day)
Marine strains			
Prophyridium Cruentum	0.37	9.5	34.8
Tetraselmis Suecica	0.32	8.5	27.0
Nannochloropsis	0.21	29.6	61.0
Isochrysis	0.17	22.4	37.7
Chaetoceros calcitrans	0.04	39.8	17.6
Freshwater strains			
Chlorococcum	0.28	19.3	53.7
Scenedesmus	0.26	21.1	53.9
Chlorella	0.23	18.7	42.1
Scenedesmus quadricauda	0.19	18.4	35.1
Chlorella Vulgaris	0.17	19.2	32.6

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that the lipid content of *Chaetoceros cf. wighamii* increased with lower temperatures over the range of 20-30°C. Jiang and Gao (2004) showed that the lipid content of the marine specie *Phaeodactylum tricornutum* increased as the temperature was lowered from 25 to 10°C. Renaud et al. (2002) noted that the *Chaetoceros* sp. grew well at temperatures of 33-35°C. However, the highest lipid content of 16.8 % was achieved at a temperature of 25°C. Macedo and Alegre (2001) reported a 3 fold increase in lipid content of *Spirulina* species when exposed to temperatures below the optimal range.

5.2. Light

Light is a major factor that regulates and stimulates algal growth. Light is an electromagnetic radiator characterized by its quality, possession of different wavelengths and intensities. In order for algae to be able to detect light and react to it, they must possess a photocycling proteinsthat have a high sensitivity to light (Barsanti and Gualtieri 2006).

Through the process of photosynthesis microalgae are capable of taking photons from a light source and converting them into algal biomass (Round, 1973). The absorption of light photons depends on the algal cell pigmentation, culture density and the cells specific position (Chen et al. 2009; Barsanti and Gualtieri, 2006). Therefore, in an open pound system the algae cultures must be adjusted in order to be able to sense the light photons for the different seasons and to avoid shading by other cells (Chen et al. 2009).

Phototrophic algae obtain their energy and nutrients via photosynthesis. Photosynthesis in algae occurs in the chloroplast and is driven by solar energy which is converted to chemical energy. This chemical energy is stored in organic matter via cycling of carbon from atmospheric CO_2 to organic carbon (Chen et al. 2009). Scott et al. (2010) stated that algal biomass requires light for photochemical reactions to take place (producing energy for the cell). Algae are capable of storing more of the absorbed light when it is in low amounts, but high levels of light have been noted to cause photoinhibition and biochemical damage to photosynthetic machinery. However, the highest efficiency for photoynthetic reactions has been noted at low light intensities.

The quality and intensity of natural light varies from place to place and also varies during the day. Algae cultures growing on water surface (or close to it) may obtain their photons for photosynthesis, but algae growing blow a certain depth may not sense the photons from the natural light. If the photons do not reach the algae then a decrease in biomass production will result. Therefore, fluctuations in the light intensity may not yield the expected biomass (Chen et al. 2009).

The pigments (chlorophylls) in charge of light absorption have the best absorption at wavelengths of 440 and 680 nm (Chen et al. 2009; Barsanti and Gualtieri, 2006). Thus, the white light, which encompasses the whole visible spectrum, is not fully absorbed. The unabsorbed light is reflected off or transmitted as wasted energy. Theoretically speaking, artificial light that corresponds to the wavelengths of 440 and 680 nm will be most efficient for algal growth (Chen et al. 2009).

Rodolfi et al. (2009) grew the marine species *Nannochloropsis*with one sided illumination and noticed that an increase in light intensity from 115 to 230 μmol photons/m^2/s increased the biomass productivity from 0.61 to 0.85 g/L/d. They also noted that the fatty acid content increased from 14.7 to 19.6 %with the increase in light intensity. However, when the same specie was tested under two-sided illumination and the light intensity was increased from 115 to 230 μmol photons/m^2/s, both the biomass production and fatty acid content increased from 0.97 to 1.45 g/L/day and 24.3 % to 32.5 %, respectively. The increase in fatty acid content was the result of the increase in saturated and monounsaturated fatty acids. More light illumination resulted in more biomass production as appose to one sided light illumination with a higher light intensity. This suggests that light penetration into the reactor had a great effect on algae growth.

Yoshimoto et al. (2005) noted that flashing light enhanced the photosynthetic process in *Chaetoceros calcitrans*. Nedbal et al. (1996) reported higher rates of growth using flashing light as opposed to continuous light. Rai and Gaur (2001) noted that the photosynthesis in culture environments undergoing nutrient depravation is utilized for the formation of reduced storage products (fats). Pal et al. (2011) noted that by varying the light intensity and salinity, the total fatty acid content increased to 47% DCW. Jacob-Lopes et al. (2009) experimented with the species *Aphanothece microscopia* and noted a reduction in biomass (5000 to 100 mg/L) as the light duration period was decreased from 24 to 2 hours. Cheirsilp and Torpee (2012) noted that the highest lipid content of the four microalgae species tested (Marine *Chlorella*) resulted in a lipid production of 117 mg/L in phototrophic conditions as appose to heterotrophic.

5.3. pH

In geothermal and mining areas, the natural water may be acidic whereas the water in saline deserts is mostly alkaline. Only a few algal organisms are capable of growing in extreme pH environments because the extent of ionization of metabolites is affected by the pH, which in turn affects the organism's reactivity and its ability to uptake nutrients. High levels of photosynthesis may cause fluctuations in pHdue to carbon dioxide removal from the medium (Rai and Gaur, 2001).

The pH of a medium affects the toxicity of the surrounding metals that are present. The ability of algae to uptake nutrients from the environment is affected by the toxicity of the metals, which is dependent on the pH (Franklin et al. 2000). Increasing the pH decreases the competition between the metal ion and the H^+ at the cell surface (Franklin et al. 2000; De Schamphelaere et al. 2003). Therefore, the pH must be maintained at the appropriate levelfor a sufficient nutrient uptake.

Skrupski et al. (2013) reported an increase in *Chlorella* lipid content from 15to 45% (DCW) with incremental pH increases. Wang et al. (2010) grew *Chlorella vulgaris* at various pH levels (6-8.5) and achieved the best growth rate at a pH of 6.5-7.0. However, the best lipid accumulation was achieved within the pH range of 7-8.5. Wilde et al. (2006) noted that inhabitation of growth rate of *Chlorella* sp. was affected by the toxicity of copper and zinc which increased over the pH range of 5.5-8, but the sensitivity to copper was greater than that of zinc. De Schamphelaere et al. (2003) noted that Cu^{2+} toxicity increased at higher pH values over the pH range of 5.9-8.5 and inhibited cell growth in *Chlorella* sp. and *Pseudokirchneriella subcapitata* species. Rodolfi et al. (2009) maintained the pH of the microalgae culture in the range of 7.5-8.1 by introducing air/CO_2 into the system at a ratio of 97/3 (v/v).

5.4. Nutrients

Various nutrients in the surrounding media of the microalgae play a role in lipid production. Altering the amount of these nutrients affects the rate of growth and the organisms ability to synthesize lipids. Such nutrients include: carbon, nitrogen, and phosphorous.

5.4.1. *Carbon*

A carbon source is required for algal cell production (Table 10). Half of the biomass dry weight is made up of carbon. Carbon can be obtained from carbon dioxide, sugars, and/or lignocellulose based substances. Algae convert carbon dioxide (CO_2) into biomass. During daylight, algae require a continuous carbon dioxide supply (Demirbas and Demirbas, 2011) which can naturally be obtained from the atmosphere(Demirbas, 2011). Microalgae are capable of tolerating high levels of CO_2 as shown in Table 11 (Demirbas, 2011). One ton of algalbiomass can fix 1 ton of CO_2byeither autotrophic or heterotrophic metabolism (Chen et al. 2009). Under heterotrophic conditions, the organism is grown in the dark and exposed to glucose as a form of energy whereas in photoautotrophic conditions the organism is exposed to light for a certain period of time. Liu et al. (2011) reported that in *Chlorella zofingiensis* 51.1 % lipid was observed under heterotrophic

484 **Table 10.**Oil content of algae species and optimal conditions (Chen et al. 2011; Lavens and
485 Sorgeloos, 1996; Rushing, 2009; Demirbas, 2011).
486

Species	Oil Content (% dry basis)	Carbon Sources	pH	Temperature (°C)
Nannochloropsis oculata	22-30	CO_2/$NaHCO_3$/glucose	8.4	20-30
Tetraselmis suecica	15-23	CO_2/$NaHCO_3$/glucose	7.6-8.4	20-30
Chaetoceros muelleri	33.6	CO_2/$NaHCO_3$	8	20-30
Freshwater				
Chlorella protothecoides	15-55	CO_2/$NaHCO_3$/glucose	6.0-6.5	20-30
Chlorella saccharophila	36-47	CO_2/$NaHCO_3$/glucose	7.5-8.1	20-24
Scenedesmus obliquus	11-55	CO_2/$NaHCO_3$/glucose	7.5-8.1	20-24

487

488 **Table 11.** Consumption rate of CO_2 by microalgae.

Species	pH	Rate of CO_2 uptake (mg/g/day)	Reference
B. braunii	7.2	160.7	Sydney et al. (2010)
S. platensis	9	146.3	Sydney et al. (2010)
D. tertiolecta	7.2	126.5	Sydney et al. (2010)
C. vulgaris	7.2	128.6	Sydney et al. (2010)
S. obliquus	6.5	156.5	Tang et al. (2011)

489

490 conditions and only 25.8 % lipid was observed under photoautotrophic conditions. The use of
491 heterotrophic conditions would eliminate the need for light, bringing forward the possibility of
492 increased productivity and cell density (Perez-Garcia et al. 2011). However, growing microalgae in
493 heterotrophic conditions is difficult in large scale systems because bacteria thrive on sugars (unless
494 this is overcome) and may result in biomass contaminations (Future Science, 2010).

495 Sugars such as glucose can provide the carbon source required by algal cells for growth (Table
496 12). However, they are not as productive as the lignocellulose based materials such as rice straw,
497 corn powder and sweet sorghum in their hydrolysate forms. Table 13 compares biomass and lipid
498 productivity using various carbon sources. Amongst these various hydrolysate materials, rice straw
499 has been noted to result in the highest cell lipid content of 56.3 % while running at the shortest culture
500 time of 48 hours (Li et al. 2011).

501 Liu et al. (2011) noted that *Chlorella zofingiensis* grown under heterotrophic conditions and fed
502 glucose produced a higher lipid content as opposed to phototrophic conditions, which resulted in a
503 lipid yield of 79.5 % of which 88.7 % are made up of TAGs. Qiao and Wang (2009) noted that
504 *Chlorella sorokiniana* exhibited an increase in lipid content (from 0.053 g/L to 0.272 g/L) when
505 glucose was used as the carbon source under heterotrophic conditions.

506 Liang et al. (2009) reported that *Chlorella vulgaris* produced the highest amount of lipid when
507 grown with glucose and light, and the fastest growth rate was achieved using 1 % glucose
508 concentration. However, the highest lipid content and lipid productivity were noted at 2 % glycerol
509 concentrations. Johnson and Wen (2009) noted that the microalgae specie *Schizochytrium limacinum*
510 grown in heterotrophic conditions resulted in the production of lipids suitable for biodiesel
511 production.

512 Liu et al. (2011) reported that the cell biomass rate of *Chlorella zofingiensis* under
513 photoautotrophic conditions was 1.9 g/L while that under heterotrophic was 9.7 g/L. They also noted
514 that the cells grown under heterotrophic conditions consumed nitrate much more rapidly. In addition,
515 the algae cells grown under heterotrophic conditions produced natural lipids, oleic acid and TAGs
516 which are better for biodiesel production as appose to the oils produced by the cells grown under
517 photoautotrophic conditions.

518 During phototrophic conditions, algae photosynthesize and use CO_2 as the carbon source (Mata
519 et al. 2010). During this process the CO_2 is converted into algal biomass, which releases oxygen into
520 the atmosphere (Packer, 2009). In seawater, low CO_2concentrations is a result of: (a) different

521 **Table 12.** Consumption rate of $NaHCO_3$ by microalgae.

Species	pH	Rate of uptake (mg/L/day)	Reference
C. vulgaris	7	125	Blake et al. (2006)
	9	70	Blake et al. (2006)
S. obliquus	7	126	Blake et al. (2006)
	9	50	Blake et al. (2006)
N. oculata	8	45.8	Merrett et al. (1996)

522

523

524 **Table 13.** Impact of various carbon sources have on the biomass growth and lipid content of algal
525 cells (Li et al. 2011).
526

Hydrolysate materials	Maximum biomass concentration (g/L)	Biomass productivity (g/L/day)	Lipid content (%, w/w)
Glucose	0.92	0.37	50.3
Rice straw	2.83	1.10	56.3
Corn powder	3.92	0.65	55.3
Cassava starch	7.20	0.72	28.9
Cassava	4.26	0.82	50.2
Sweet sorghum	5.10	1.02	53.3

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habitats, (b) seawater equilibrated air contains 180 times more inorganic carbon in the forms of bicarbonate and carbonate than CO_2, (c) the algae are capable of changing the way in which the carbon forms are utilized based on the surroundings and (d) the degree of the presence of inorganic carbon (Rai and Gaur, 2001). Low levels of CO_2 results in: (a) lower levels of growth, (b) lower photosynthesis and the over excitation of photosynthesis apparatus which in turn results in the decrease of photosynthetic activity (Rai and Gaur, 2001) and (d) damage to the photosynthetic apparatus which is irreversible (Demmig-Adams and Adams, 2000). Some photosynthetic organisms have developed various pathways forcontrolling the amount of light allowed to be trapped in order to avoid damage to the photosynthetic apparatus (Demmig-Adams and Adams 1992, 2000). These stress the importance of avoiding low CO_2 concentrations (Rai and Gaur, 2001).

Huntley and Redalje (2007) reported that*Haematococcus pluvialis* capable of taking up 16-34 % carbon dioxide. De Morris and Costa (2007) noted that *Chlorella kessleri* was capable of taking up 18 % CO_2. Sobczuk et al. (2000) stated that the species *Phaeodactylum tricornutum* was 63 % efficient in the uptake of CO_2. Wahlen et al. (2011) reported that CO_2 is introduced into the culture by aeration with air at 1 % (v/v) of CO_2. Rodolfi et al. (2009) introduced CO_2 into the system by flushing it with air and CO_2 at 95/5 (v/v).

The various sources of carbon (bicarbonate, carbonate and carbon dioxide) exist at various pH levels. At acidic pHs (below 5) carbon is in the form of CO_2, bicarbonate (HCO_3^-) is the carbon form that exists in the pH range of 7-9, and at high pHs (above 9.5) carbonate (CO_3^{2-}) is the carbon form present (Round, 1973). Algae are also capable of using bicarbonate as a carbon source (Table 9).

Blake et al. (2006) reported of sodium bicarbonate uptake rates of 125 and 126 mg/L/day at a pH of 7 by*C. vulgaris* and *S. obliquus,*respectively. Huertas et al. (2000) noted that bicarbonate uptake by *Nannochloropsis gaditana*was activated by light. Merrett et al. (1996) reported a sodium bicarbonate uptake rate of 45.8 mg/L/day by *N. oculata*at a pH of 8.

5.4.2. *Nitrogen*

Nitrogen is the most critical nutrient which plays a large role in algal lipid (mainly TAGs) accumulation (Hu et al. 2008; Huesemann and Benemann, 2009). Large scale production of microalgae for oil requires 8-16 tons/ha/year of nitrogen fertilizer (Demirbas 2011). The green microalgae species*C. pyrenoidosa* showed a multiple fold increase in lipid content upon nitrogen depravation while almost no change/slight reduction in lipid content in the species *Dunaliella* and *Tetraselmis suecica* (Borowitzka, 1988). However, within the same genus *Chlorella* was found to produce starch under nitrogen stress (Hu, 2004).

In nitrogen deficient environments, the photosynthesis products shift from protein to carbohydrate and then to lipid production (Rodolfi et al. 2009). However, the accumulation of lipid results at the expense of biomass production (Rodolfi et al. 2009; Scott et al. 2010). Cell division in culture under nitrogen deficiency are halted in the stationary phase, as a result of accumulation of inhibitory products that are formed in nitrogen starved surroundings (Rai and Gaur, 2001). Young cells grown in nitrogen deficient environments are able to grow and divide, generating a second generation of cells with an alteration in their metabolic pattern. Instead, these cells favour the production of carbohydrates (Fogg and Thake, 1987).

Rodolfi et al. (2009) noted that the marine species *Nannochloropsis* accumulated 60 % DCW lipid content under nitrogen starvation. Rai and Gaur (2001) noted that *Synechococcus* showed signs of protein destruction in nitrogen limited environments. Li et al. (2008) tested the effects of sodium nitrite concentrations (3 mM and 5mM) on the biomass yield and lipid productivity of *Neochloris oleoabundans* and noted biomass yield and lipid productivity of 40.91 g and 0.125 g/L/day at a sodium nitrite concentration of 3 mM and biomass yield and lipid productivity of 34.13 g and 0.133 g/L/day at a sodium nitrate concentration of 5mM, respectively.

The nitrogen source nitrate maybe reduced by the process illumination in green algae, through which the hydrogen donors are, generated photochemically (Lewin, 1962). Round (1973) stated that nitrogen source affects the pH, if ammonium salt is present and absorbed by the algal cells the pH decreases whereas if the nitrate salt is present and absorbed by the algal cells the pH increases. In cultures where nitrogen starvation is in effect, the algal cells assimilate ammonium much more rapidly than normal cells do (Montesinos et al. 1998; Tapia et al. 1996).

Lewin (1962) stated that the amount of extracellular products does not vary with the different nitrogen sources (nitrate, nitrite and or ammonium)used. In the dark, the nitrogen starved cells continue to assimilate ammonium until they have exhausted their carbohydrate reserves. Solovchenko et al. (2008) indicated that the cells recovering from nitrogen deficiency (with addition of nitrogen) experience an increase in chlorophyll content in order to restore the original rate of photosynthesis.

Future Science (2011) noted that under nitrogen sufficient conditions,*C. vulgaris* consisted of 13.7 % dry cell weight (DCW) lipids, of which only 3 % were triacylglycerol's(TAGs) that can be used for biodiesel production. However, when nitrogen deficiency was increased, the lipid content increased to 20 % (DCW) with TAGs making up to 50 %. Under nitrogen sufficient environments, the culture reached its maximum cell density of 4.2 g/L after 9 days, with a biomass, lipid and

biodiesel productivities of 480, 66 and 2 mg/l/day, respectively. Under nitrogen deficient conditions, it was noted that the cultures with low initial densities resulted in the highest cell TAG content. However, the highest TAG content was noted in cells coming from very dense cultures. Therefore, an optimal initial cell density must be established for maximum cell production of TAG, when taking the initial cells from a nitrogen sufficient media into a nitrogendeprived environment. The effect of various initial nitrogen concentrations were tested, and results indicated that over the nitrate concentrations of 10-550 mg/L, the highest TAG content of 39-46 % was achieved at initial nitrate exposure of 100-200 mg/L.

It was noted that high TAG content within the cells, as well as high productivities were achieved in environments that use up all of the nitrogen present as appose to exposing the algal cells to an environment with no nitrogen present. In this environment, as the TAG concentration was increased, a decrease in protein and chlorophyll was noted. Generally, cultures that are nitrogen deprived show a higher lipid content, but lower biomass production than those for which nitrogen was sufficient (Rodolfi et al. 2009).

5.4.3.*Phosphorous*

Phosphorous as a nutrient plays an important role in the building blocks of nucleic acids, phospholipids, complex carbohydrates (Rai and Gaur, 2001) and normal algal growth (Xin et al. 2010), it also plays a central role in catabolic and anabolic pathways and in the conversion of energy through the energy rich phosphoanhydride bonds (Rai and Gaur, 2001). It has been reported by several researchers that phosphorous limitation enhances the lipid accumulation in microalgae cells (Xin et al. 2010; Rodolfi et al. 2009; Hu et al. 2008; Khozin-Goldberg and Cohen, 2006).

Xin et al. (2010) reported on enhancing lipid accumulation to 53 % of algal biomass of *Scenedesmus sp*. by limiting phosphorous concentrations to 0.1 mg/L. Rodolfi et al. (2009) reported that during phosphorous starvation, the lipid content of *Nannochloropsis* species increased from 13.2 % to 50.1 % (total biomass), of which 67 % consisted of TAGs. Khozin-Goldberg and Cohen (2006) noted that *Monodus subterraneus*lipid content increased from 6.5 to 39.3 %, mainly TAG, due to phosphorus limitation over the range of 0-175µM.

6. MICROALGAE PRODUCTION SYSTEMS

Microalgae cultivation can be done in either open or closed systems. These microorganisms can grow virtually anywhere as long as the right nutrients and environmental requirements for growth are

present. However, it is much harder to control the amount of nutrients entering the open system, while closed systems are much easier for algal cultivation but cost much more than open systems. Sunlight energy for algal cultivation can be utilized in open systems and closed systems (Demirbas, 2011). Current microalgae production systems include: open pond (circular and raceway), enclosed photobioreactores (tubular and plate), and hybrid systems.

6.1. Open ponds

Open ponds are the simplest and oldest methods known for mass cultivation of microalgae. Open pond systems for algal cultivation are shallow with nutrients entering the system via runoff water. Nutrients may also be obtained from sewage water (Demirbas, 2011; Demirbas, 2010). Although open ponds require low operation and construction costs. However, they have many limitations which include low productivity, temperature fluctuation, water loss via evaporation, high harvesting costs and lower carbon dioxide transfer, and are prone to contamination by predators (Chen et al. 2009). Open pond productivity is assessed based on the biomass production per day per unit surface area available (Demirbas, 2010). There are various types of open pond systems which include circular and raceway ponds.

6.1.1. *Circular Pond*

Agitation in circular ponds is provided by the rotation arm as shown in Figure 6 (Chen et al. 2009; Demirbas, 2010). The pivoted agitator arm can extend to 45 m in diameter. The average size of circular ponds is limited to 1000 m^2 with a depth of 0.3 m because agitation through rotation arm is no longer possible in larger ponds (Borowitzka, 2005; Lundquist et al. 2010). The productivity of microalgae grown in circular ponds can range from 1.5-16.5 g dry weigh/ m^2 /d (Chen et al. 2009). Circular ponds are less popular then raceway ponds because of the high expenses associated with construction. They are made up of concrete and consume a lot of energy for stirring. They are also inefficient in land use and face high complexity when it comes to supplying CO_2 (Chen et al. 2009).

Henrikson (2011) reported on mass cultivation of *Spirulina* and *Chlorella* in Taiwan using circular ponds producing hundreds of tons of algae per year. Borowitzka (1999) reported on a large scale production facility in Taiwan achieving *Chlorella* spp. volumes of 15 000 L using circular ponds with a rotating arm. Ranga Rao et al. (2012) noted a biomass productivity in *Botryococcus braunii* of only 1.25 g/L in a circular pond as appose to 1.75 g/L in a raceway pond. Sheehan et al. (1998) cultivated*Oscillatoria* in an open circular pond and achieved a biomass productivity of 15 g/m^2/d. Kanazawa et al. (1958) achieved a biomass productivity of 2.43-13.52 g/m^2/d from Scenedesmus sp. grown in a circular pond.

655

656 **Figure 6.** Circular algae ponds in Yaeyama, Japan (Chen et al. 2009).

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660 **Figure 7.** Raceway pond, for algal cultivation (Algae Energy, 2013).

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663 **6.1.2. *Raceway Pond***

664 The raceway pond (Figure 7) consists of a paddlewheel, propeller or air lift pumps which function
665 to circulate and mix the algae and nutrients around the pond. Agitation and circulation are produced
666 by the paddlewheel which operates continuously in order to bring the algae to the surface of the water
667 and to prevent sedimentation. Shallow ponds are necessary for algal exposure to sunlight due to
668 limited light penetration in the water, which is typically 15-25 cm deep (Demirbas, 2011; Chen et al.
669 2009; Demirbas, 2010). Raceway ponds can be made of concrete or more simply earth dug and lined
670 with a plastic liner. The plastic liner prevents the water from penetrating into the ground. In this
671 system the water and nutrients are fed into the pond continuously, while the water containing the
672 algae is removed from the other end (Demirbas, 2011).

673 Blanco et al. (2007) reported of lutein rich cells from *Muriellopis* sp. cultivated in a raceway pond
674 operated with a wheel peddle. Moheimani and Borowitzka (2006) achieved a 33 % lipid content and
675 a productivity of 0.19 g/L/day using*Pleurochrysis carterae* in a raceway pond. Jimenez et al. (2003)
676 cultivated*Spirulina* in raceway pond and obtained a productivity of 10.3 g/m^2 per day over the
677 duration of 9 months. Olguin et al. (2003) noted a productivity of 9-13 $g/m^2/d$ of*Spirulina platensi*s
678 grown in a raceway pond. Moreno et al. (2003) achieved a productivity of 9.4-23.5 $g/m^2/d$
679 for*Anabaena sp*. grown in a raceway pond. Garciaet al. (2003) achieved a biomass productivity of
680 1.6-3.5 $g/m^2/d$ of *Dunaliella salina*in raceway pond.

681 **6.2. Enclosed Photobioreacters**

682 Although the cost of enclosed production systems is much higher than open ponds, they require
683 less light and land area for cultivation. Cultivation of algae in photobioreacters (PBR) does not only
684 function to grow the algae but also to remove nutrients from wastewaters as well as reducing the
685 amount of gases released into the atmosphere by power plants and transportation industry (Demirbas,
686 2011). PBR are closed bioreactors consisting of a light source. An open pond may also be
687 considered as a PBR if it is enclosed within a greenhouse, in which all the required nutrients are
688 introduced into the system for cultivation purposes (Demirbas, 2011). Enclosed PBR mediums can
689 achieve high cell densities and are easily maintained because of their enclosed structure (Chen et al.
690 2009).

691 Tubular and plate PBR are the major types. Their advantages over open pond systems include:
692 their narrow light path (1.2-1.3 cm) which allows for more cell concentration, large illuminating area
693 and less contamination. The enclosed PBR disadvantages include: wall growth, fouling, formation of
694 dissolved oxygen and CO_2 along the tube, pH gradients, hydrodynamic stress and the high cost (Chen

et al. 2009). Singh and Gu (2010) stated that although enclosed photobioreacters produce higher fuel per hectare as appose to open ponds, the start-up cost is much greater.

6.2.1. *Tubular Photoreactors*

Tubular PBR are made up of several horizontal tubes running parallel to one another (Figure 8) and made of transparent glass or plastic (Demirbas, 2011; Chen et al. 2009). Due to limited sunlight penetration ability into the tubes, the diameter is 0.1 m or less in order to achieve high cell productivity and high biomass yield (Demirbas, 2011). The shape of tubular PBR can be horizontal, vertical, conical, or inclined. Mixing of the biomass can be achieved in the system by use of an airlift or pump system (Chen et al. 2009). The advantages of using a tubular photobioreactor include: its large illumination surface area, biomass productivity and its suitability for outdoor operation. However, scale up of this system is poor because the mass transfer problems (Ugwu et al. 2008).

Kong et al. (2009) achieved a productivity of 2.0 g/L/day of *Chlamydomonas reinhardtii* grown in a vertical coil reactor. Ugwu et al. (2008) reported on*Porphyridum cruentum* grown in an airlift tubular photobioreactor achieving a productivity of 1.50 g/L/day. Barbosa et al. (2004) achieved a biomass productivity of 0.5 g/L/d from the species *P. cruententum* grown in a vertical tubular reactor. Alias et al. (2004) achieved a productivity of 0.25 g/L/d from *S. Platensis* grown in a horizontal tubular reactor. Lee (2001) reported on *Chlorella pyrenoidosa* grown in an inclined tubular PBR that achieved a biomass density of 2.90 g/L/day.

6.2.2. *Plate Photobioreactor*

Plated PBRs (Figure 9) are made up of a transparent plastic material. The large surface area allows for more illumination which increases photosynthetic activity (Demirbas, 2011; Demirbas, 2010). Plate PBRs also possess low concentrations of accumulated dissolved oxygen (Demirbas, 2011). The plate PBR can be horizontal, vertical or inclined (Chen et al. 2009).

Ugwu et al. (2008) reported on*Nannochloropsis sp*. achieving a productivity of 0.27 g/L/day when grown in a flat plate reactor. Lee (2001) noted that *Spirulina platensis* grown in an inclined plate achieved a productivity of 4.30 g/L/day. Zhang et al. (2001) noted a biomass productivity of 1 g/L/day using the species *Synechocystis aguatillis* in an outdoor flat plate photobioreactor. Cuaresma et al. (2009) cultivated *Chlorella sorokiniana* in a flat panel photobioreactor and achieved a productivity of 12.2 g/L/d. Meiser et al. (2004) noted a productivity of 1.37 g/L/d for *Phaeodactylum tricornutum* grown in a flat panel airlift reactor.

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728

729 **Figure 8.** Tubular photobioreactor (ATSE, 2013).

730

731

732 **Figure 9.** Plated microalgae photobioreactor (IGV Biotech, 2003).

6.3. Hybrid Systems

A hybrid system is a combination of both an open pond and a closed bioreactor as shown in Figure 10 (Demirbas, 2011; Chen et al. 2009). Since open ponds are very proficient but are easily contaminated, a mix of both is most likely the most cost effective algae cultivation method (Demirbas, 2011).

Putt et al. (2011) noted a CO_2 mass transfer efficiency of 83 % using a hybrid system for microalgae growth as opposed to a 37 % conventional system. Rodolfi et al. (2009) described a two stage hybrid production plant that was dedicated to producing 22 % biomass and 78 % oil production. Huntley and Redalje (2007) reported on*Haematococcus pluvialis,* cultivated in a two stage system for the production of oil and astaxanthin (salmon feed), achieving an oil production rate of 10 tons/ha annually. Pulz (2001) noted that *Haematococcus* grown in a combination of closed and open systems for use in astaxanthin production resulted in a productivity of 50 tons/acre. Kizilisoley and Helvacioglu (2008) reported a productivity of 0.35 g/L using *Spirulina* cultivated in a closed pond system.

7. ALGAE HARVESTING AND OIL EXTRACTION

The algal biomass must be harvested and treated to release TAGs which can then be utilized to produce biodiesel. It is important to find a method for releasing the TAGs from the cells in the most economical and energy efficient way, while utilizing minimal amounts of solvent. It is best to release the oil from the algal biomass while avoiding contamination of DNA and chlorophyll which are also present in the cells and can be extracted and used in the medical industry (Scott et al. 2010).

7.1 Algae Harvesting

The algal biomass must be harvested to separate the algal cells from their surrounding liquid media. Numerous techniques can be deployed including: membrane filtration, chemical flocculation, air flotation, centrifugation and ultrasound waves.

7.1.1. *Membrane filtration*

Membrane filtration is a simple method used for water removal and collection of algal cells. This method can be used with the aid of suction or a vacuum pump. The membranes used are made of modified fibers or cellulose. The problem associated with this method is membrane clogging or fouling due to the ability of algal cells to penetrate into the membrane. Drum filters and disc filters have been developed to overcome this problem. In order to avoid cell penetration into the filter, a

763 

764 **Figure 10.** Hybrid microalgae production system (enclosed pond) (National Geographic, 2013).

765

766 reverse-flow vacuum filter method is utilized to move the liquid upward across the membrane (Chen
767 et al. 2009).

768 Tsukalhara and Sawayama (2005) used membrane filtration on *B. braunii* to achieve high cell
769 density. Sawayama et al. (1992) filtered *Botryococcus braunii* through filter paper of 0.2 μm pore
770 size to recover the algal cells grown in treated sewage from domestic wastewaters. Grima et al.
771 (2003) noted that microfiltration is cost effective for small volumes of algae (less than 2 m^3/d), than
772 centrifugation.

773 **7.1.2. *Chemical Flocculation***

774 Algal cells possess a negatively charged surface which allows the separation from one another
775 upon suspension. The surface charge of the cells may be disrupted by the addition of iron, alum,
776 lime, cellulose, salts, polyacrylamide polymers, surfactants and chitosan. These chemicals result in
777 cell flocculation and settling. Filtration through this method has been reported to achieve 95 %
778 recovery of the algal cells from the culture media (Chen et al. 2009).

779 Bilanovic and Shelef (1988) noted that microalgae flocculation was effective at salinity levels
780 less than 5 g/L. Sukenik et al. (1988) reported on the marine microalgae *Isochrysis galbana* and
781 *Chlorella stigmatophora* requiring 5-10 times more flocculent dosages than those required by
782 freshwater microalgae. Morales et al. (1985) noted a 100 % flocculation efficiency using chitosan
783 concentration of 40 mg/L for 20 L batch cultures of *Chlorella* sp. and *Thalassiosira nordenskoldii*.

784 **7.1.3. *Air Flotation***

785 Air flotation method for filtration of algal cells is performed by the generation of fine air
786 bubbles. Air is then adhered to the cells, causing them to float to the surface of the column in a form
787 of foam. The foam of cells formed on the surface may be removed or the water may be drained from
788 below the cells. Different types of flotation methods may be performed including air floatation,
789 dissolved air flotationand suspended air flotation. These methods work by generating small air
790 bubbles that adhere to the algal cells and float to the surface (Chen et al. 2009; Wiley et al. 2009).
791 Suspended air flotation differs from dissolved air flotation in that the bubbles generated are coated
792 with a surfactant (Wiley et al. 2009). This method is expensive and impractical because air
793 compression requires a tremendous amount of energy (Chen et al. 2009).

794 McMahon (2013) achieved a microalgae harvesting efficiency of 95 % using dissolved air
795 floatation method. Elder (2011) optimized dissolved air floatation method for algae harvest and noted
796 a capture efficiency of 68-70 %. Wiley et al. (2009) reported suspended air flotation and dissolved air

flotation capture efficiencies of microalgae of 76.6 % and 84.9 %, respectively. Boussiba et al. (1988) reported of a 100 % biomass removal efficiency by means of flocculation with 180 mg/L of $FeCl_3$ and dissolved air flotation using *Isochrysis galbana*. Chen et al. (1998) noted that removal of microalgae is more efficient by means of flotation as appose to sedimentation.

7.1.4. *Centrifugation*

This method is more commonly used either on its own or as a second step for further water removal. Cream separator centrifuges are used to separate large volumes of algal cultures. In this method, the algal cells form a paste on the walls of the centrifuge tubes (Chen et al. 2009).

Heasman et al. (2000) reported a harvesting efficiency of 95-100 % and a cell viability of 88-100 % using centrifugation at 13000xg. Chen et al. (2011) reported a 80-90 % microalgae recovery efficiency from the liquid media using centrifugation at 500-1000x g. Grima et al. (2003) noted that the preferred method for microalgae biomass recovery is centrifugation, especially producing extended concentrates for shelf-life. Sim et al. (1988) compared air flotation, drum filtration, and centrifugation and noted that the most efficient method for biomass recovery was by means of centrifugation. Golueke and Oswald (1965) compared flotation, filtration and centrifugation for algal removal efficiencies and concluded that centrifugation is the only one of the three that is economically feasible.

7.1.5. *Ultrasound Wave*

In this method, the algal cells agglomerate to the low pressure nodes of ultrasound waves generated by low energy ultrasound waves. The particle-particle interaction and acoustic interaction forces aid the mass collection of the cells. When the ultrasonic field is turned off the cells settle due to gravity. This technique for dewatering of algal biomass is non-fouling, free of mechanical failures (no movement of parts involved) and offers continuous operation. However, this method requires high consumption of power and is only capable of being used on low concentrations of biomass (Chen et al. 2009).

Joen et al. (2013) noted that increasing sonication time (10- 60 min) of algal biomass decreased the algal surface area from 75 to 28 %. Bosma et al. (2003) reported efficiencies higher than 90 % using ultrasound separation of *Mondus subterraneus*. Zhang et al. (2006) noted that ultrasonic irradiation improved algae settleability, but also change the structure of the algal cell. Yin et al. (2004) reported that ultrasonication significantly hydrophilizes algal cells.

7.2. Oil Extraction

There are five common methods for oil extraction: oil press, solvent extraction, supercritical fluids, ultrasound and liquefaction (Demirbas and Demirbas, 2011). Table 14 summarizes some of the advantages and limitations of the various methods for oil extraction.

7.2.1. *Oil Press*

The oil press (or expeller) method is one of the simplest ways known for oil extractions that is capable of extracting 70-75 % of the algal oil (Demirbas and Demirbas, 2011; Singh and Gu, 2010). The expeller press is a mechanical process that squeezes the oil out of the raw materials under high pressure. The raw materials are supplied to the press in a continuous feed and pressure is applied to break the cells in order to compress the oil out of the cells. (Singh and Bargale, 2000). For maximum efficiency when utilizing this method, the algae should first be dried (Singh and Gu, 2010). Despite itshigh extraction efficiency and simplicity, this method has been noted to be less desirable than other methods because of the long extraction time required (Popoola and Yangomodou, 2006).

Shah et al. (2012) achieved an oil extraction of 115 ml from 500 g of *Scenedesmes dimorphus* using the expeller method. Topare et al. (2011) experimented with filamentous algae obtained from an open pond and obtained an oil extraction efficacy of 75 % using the expeller method. Demirbas (2009) achieved a microalgae oil extraction efficiency of 70-75% using the oil press method. Popoola and Yangomodou (2006) reported an oil extraction efficiency of 75% using the oil press method. Govindarajan et al. (2009) achieved a 95 % oil extraction using the combined methods of expeller and solvent.

7.2.2. *Solvent Extraction*

Oil extraction from microalgae can also be performed by the solvent extraction method. In this method, the oil in the wet algae paste is extracted utilizing organic solvents (benzene, cyclo-hexane, hexane, acetone, or chloroform) that break down the algal cell walls and extract the oil from the aqueous medium, due to its solubility in the organic solvents as oppose to water. The oil can then be separated via distillation from the solvent extract (Singh and Gu, 2010).

For maximum lipid extraction efficiency, the solvent used should possess several features: (a) has a lipid polarity that matches those of the cells, (b) economical, (c) easy to recover, (d) non-toxic, (e) water insoluble and (f) recyclable (Chen et al. 2009). Despite the simplicity of this method, it is

857 **Table 14.** Advantages and limitations associated with various microalgae oil extraction methods.
858

Extraction Method	Advantages	Limitations	Reference
Oil Press	No solvent required Easy to use	Time consuming Large amount of sample required	Mata et al. (2010)
Solvent Extraction	Inexpensive solvents Reproducible	Organic solvents are highly flammable or toxic Energy intensive solvent recovery Large volume of solvent required	Herrero et al. (2004) Galloway et al. (2004)
Supercritical fluid extraction	Non-toxic Non Flammable Simple operation	Often fails in large extractions of polar analyte Insufficient interaction between supercritical CO2 and the sample	Macias-Sanchez et al. (2005) Pawliszyn, (1993)
Ultrasound	Reduced extraction time Reduced solvent use Higher solvent penetration Improves release of cell content into the medium	High power consumption Scale up difficulty	Luque-Garcia and Luque De Castro, (2003) Martin, (1993)

859

860

impractical for use on a large commercial scale because these solvents are environmentally destructive and costly (Singh and Gu, 2010; Demirbas, 2011).

Demirbas (2009) and Serrato (1981) stated that hexane is the most efficient solvent for algal oil extraction because it is cost effective and has the highest extraction capability. Demirbas and Demirbas (2011) stated that hexane is the most inexpensive chemical known for algal oil extraction. Xiou and Xu (2005) reported that butanol is effective in extracting lysophospholipids but high boiling point of this solvent makes it difficult to evaporate and its high polarity tends to extract more impurities.

Fajardo et al. (2007) reported an 80 % lipid recovery via two stage extraction, the first stage by ethanol extraction and the second stage by hexane in order to purify the lipids. Long and Abdelkader (2011) reported that the mixture of chloroform-methanol provided the highest extraction efficiency of microalgal lipids (25-27 %) from *Nannochloropsis*.

Li et al. (2011) noted that methanol is a poor solvent for oil extraction and since the solvent plays a major role in transesterification for biodiesel conversion, n-hexane or chloroform should be used. The authors tested solvent volumes in the range of 2-8 mL of hexane and chloroformand reporteda 10 % higher biodiesel yield with hexane as appose to chloroform. Although a higher biodiesel yield was achieved with the chloroform solvent, it contained solid residues whereas the hexane was found to be light yellow with no residues. The solid residues found using the chloroform may be a result of its high polarity which resulted in a better solubilityof proteins. Therefore, for the production of biodiesel one must consider using nonpolar solvents in order to avoid the solid residues that result in polar solvents.

7.2.3. *Supercritical Fluid*

Fluids above their critical point are known as supercritical fluids. Their diffusivity is enhanced and the viscosity of this fluid is decreased upon its critical point. Such properties allow fluids to diffuse easily through solid materials (Chen et al. 2009). The supercritical fluid extraction (SFE) method for oil extraction is the most efficient of all extraction methods because of its high selectivity, time efficiency and non-toxicity (Demirbas and Demirbas, 2011; Chen et al. 2009; Singh and Gu, 2010). This, results in a product of high purity (Demirbas and Demirbas, 2011; Chen et al. 2009). In this method, if carbon dioxide is the chemical used for extraction, it would have to be liquefied under heat and pressure to a point where its properties consist of both liquid and gas, acting as the oil extracting solvent. This method utilizes high temperatures and pressures in order to rupture the algal cells (Demirbas and Demirbas, 2011; Singh and Gu, 2010).

Canela et al. (2002) reported that the pressure and temperature of supercritical fluid extraction (SFE) does not influence the yield of the extracted compounds, but instead it influences the extraction rate. Andrich et al. (2005) reported that over the temperature range of 45-55°C and the pressure range of 40 000-70 000 kPa there was no impact on the extraction of bioactive lipids (polyunsaturated fatty acids) from the species *Nanochloropsis*. However, they also reported that SFE system resulted in a similar yield when utilizing hexane as the solvent.

Demirbas (2009) and Demirbas and Demirbas (2011) noted thatthe supercritical fluid is capable of extracting 100% of oils. Andrich et al. (2005) reported a higher polyunsaturated fatty acid (PUFA) extraction yield from the species *Spirulina platensis* using supercritical fluid extraction system as oppose to solvent extraction method. Couto et al. (2010) extracted 50 % of the total oil from *Crypthecodinium cohnii* using supercritical carbon dioxide at 30 MPa and 323 K. Halim et al. (2011) extracted 7.1 % of the lipids present in *Chlorococcum* using supercritical carbon dioxide.

7.2.4. *Ultrasound*

Ultrasound is another method that can be used for oil extraction. In this method, algae cells are exposed to high intensity ultrasonic waves, creating tiny cavitation bubbles around the cells. The desired compounds are released into the solution when the bubbles collapse and emit shockwaves that shatter the cell walls (Singh and Gu, 2010).

Wiltshire et al. (2000) reported a 90 % extraction efficiency of fatty acids and pigments from the species *Scenedesmus obliquus* using ultrasound extraction. Pernet and Tremblay (2003) concluded that the ultrasonic method for oil extraction from *Chaetoceros gracilis* increased the extraction rate which intern affected the recovery of lipid extracts.

Hu et al. (2008) reported an oil extraction yield of 93 % in adlay seeds using ultrasound assisted supercritical fluid extraction. Araujo et al. (2013) achieved a 52.5 % oil extraction from C. vulgaris using ultrasound method. Latheef (2012) reported an oil lipid extraction efficiency of 69.53 % from *Nannochloropsis oculata* using ultrasound-assisted solvent extraction.

7.2.5. *Liquefaction*

A more practical and effective method for algal oil separation, is liquefaction of the algal cells with high moisture content. Hydrothermal liquefaction is done in an aqueous solution consisting of alkali or alkaline earth salts at a temperature of 302°C and a pressure of 10 MPa (Demirbas, 2011). These conditions result in the formation of supercritical waterwhich enhances the reaction rate. In this method, the algal cells are liquefied and the product is then extracted with dichloromethane

(CH_2Cl_2) to separate the oil fraction. This method is performed utilizing a stainless steel autoclave with mechanical mixing (Demirbas, 2010; Chen et al. 2009; Demirbas, 2011). Liquefaction can be done using two methods, direct liquefaction and indirect liquefaction (Demirbas, 2010).

7.2.5.1. Direct Liquefaction Method: In this method, rapid pyrolysis takes place resulting in liquid tars and oils (Demirbas, 2010). One of the major advantages of algae cell liquefaction is that it does not require the drying of water (Chen et al. 2009). Liquefaction is done using hexane in order to obtain the primary oil (Demirbas, 2011). This process for conversion of wet algal biomass into liquid fuel is convenient because it would eliminate the heating costs associated with drying the wet biomass. The liquefaction of these cells results in an oil-like product by the reaction of carbon monoxide/hydrogen in the presence of sodium carbonate. In this process, the algal biomass is changed into the liquefied products by alteration in the physical structure and undergoing chemical change. The biomass is broken down into smaller unstable and reactive molecules that repolymerize into oily compounds (Demirbas, 2000).

Jena et al. (2011) achieved an oil yield of 39.9 % using liquefaction of *Spirulina plantensis* at 350°C in 60 min. Minowa et al. (1999) reported a 37 % oil yield from *Dunaliella tertiolecta* using direct liquefaction which consists of a moisture content of 78.4 % at 302°C and 10 MPa. Jazrawi et al. (2013) used hydrothermal liquefaction on *Chlorella* and achieved a bio crude yield of 41.7% (wt) at 350°C in 3 min. Yu et al. (2011) obtained an bio-crude oil yield of 39.4 % at 200°C in 120 min using hydrothermal liquefaction of *C. pyrenoidosa*. Biller and Ross (2011) achieved a bio-crude yield of 5-25 % (wt) which is higher than the lipid content of the microalgae species *Chlorella vulgaris* and *Nannochloropsis occulate*.

7.2.5.2. Indirect Liquefaction Method: This method utilizes catalysts for the conversion of non-condensable, gaseous products of pyrolysis or gasification into liquid products (Demirbas, 2010). Chen et al. (2009) obtained 57 % petroleum like product from *Botvyococcus braunii* using liquefaction with sodium carbonate as the reaction catalyst at a temperature of 300°C. Sawayama et al. (1995) reported a maximum oil yield of 64 % by liquefaction of *Botryoccus brunii* at 301.9°C with sodium carbonate as the reaction catalyst. Shuping et al. (2010) used sodium bicarbonate as the reaction catalyst for hydrothermal liquefaction of *Dunaliela tertiolecta* and achieved a bio-oil extraction of 25.8 % at 360°C and 50 min. Yang et al. (2004) obtained a 33 % oil yield from *Microcystis virdis* using liquefaction with sodium bicarbonate catalyst. Aresta et al. (2005) reported that biodiesel production from microalgae is more effective using the hydrothermal liquefaction method for the extraction as compared to the supercritical carbon dioxide method.

8. TRANSESTERIFICATION

Unlike microemulsion and thermal cracking which are problematic, transesterification has become the preferable method for the production of biodiesel (Ma and Hanna, 1999; Akoh et al., 2007; Robles-Medinaet al., 2009; Ranganathan et al., 2008). The transesterification reaction occurs when alcohol reacts with triglycerides to give esters and glycerol as a by-product (Figure 11). The stepwise transesterification reaction is shown in Figure 12. Short chain alcohol like methanol, ethanol, octanol and other branched alcohols are widely used in the transesterification process (Fukuda et al., 2001). Alcohols and esters are likely to produce fatty acid methyl esters (FAME's) (Robles-Medina et al., 2009). The alcohol plays the role of both the solvent (it extracts the lipids from the biomass) and the reactant (converts the lipids into FAME) (Wahlen et al. 2011; Demirbas, 2010). The alcohol in this case is methanol and a base or acid is used as the catalyst to speed up the reaction (Demirbas and Demirbas, 2011; Wahlen et al. 2011). The transesterification of algae oil is depicted in the following equation (Johnson and Wen, 2009; Demirbas, 2010).

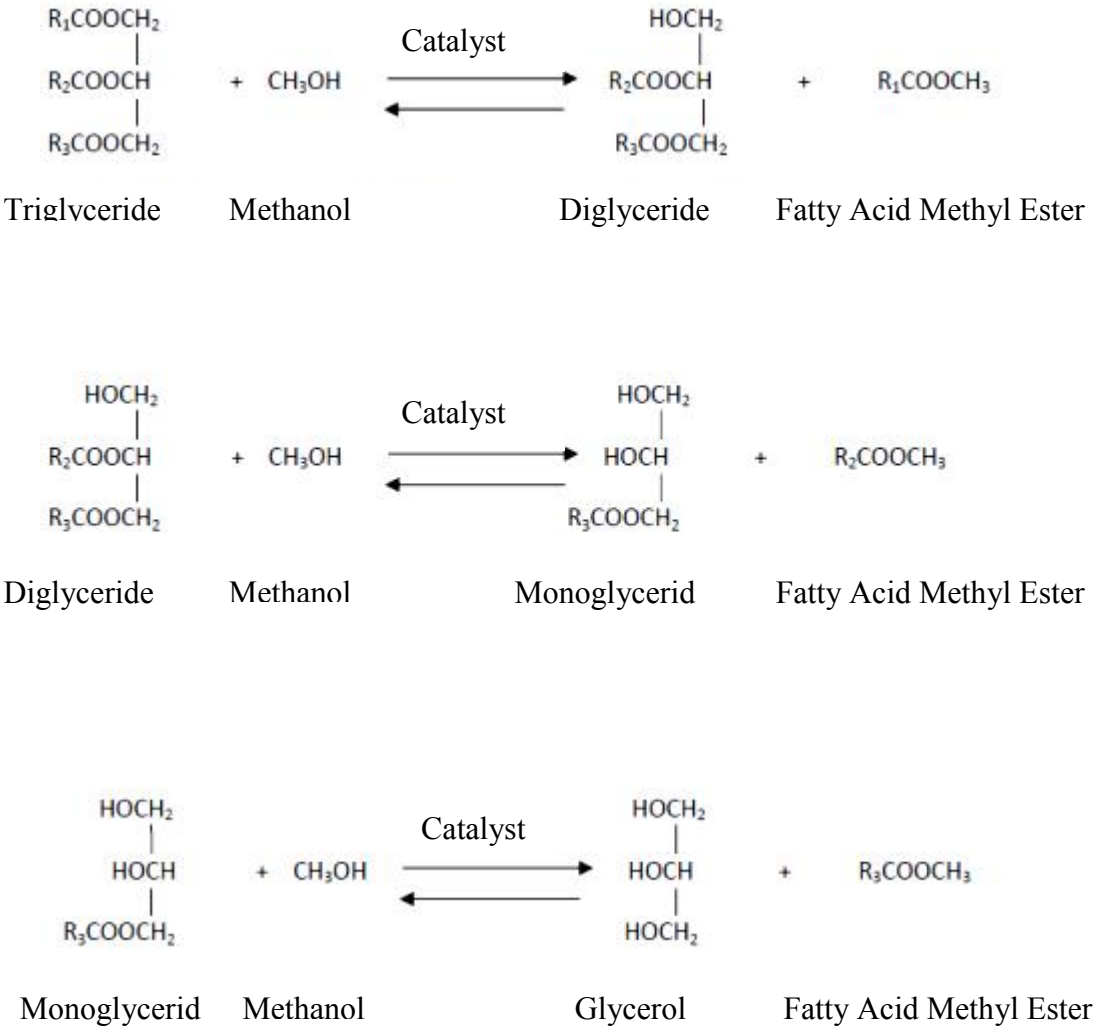
$$\text{Triglycrides} + 3\text{methanol} \xleftrightarrow{\text{Catalyst}} \text{Glycerince} + 3\text{methylesters (Biodiesel)} \qquad (1)$$

Figure12 shows the transesterification process which consists of three continuous steps: (a) the conversion of triglycerides to diglycerides, (b) the conversion of diglycerides to monoglycerides and (c) the conversion of monoglycerides to methyl esters and glycerin (Freedman et al., 1984;Noureddini and Zhu, 1997; Marchetti et al., 2008). One fatty acid alkyl ester (FAAE) molecule is produced from each conversion of fats/oils by alcohol (Leung et al. 2010). Several catalysts (acids, alkali and enzymes) were used to increase the rate of transesterification reaction for the production of biodiesel (Bacovsky et al., 2007; Murugesan et al., 2009; Leung et al., 2010). McNeff et al. (2008) suggested that using the catalyst may affect the rate of reaction, purity of the feedstock, and the purification process of the product. Factors such as mixing intensity, alcohol to oil ratio, concentration of catalyst and temperature can also affect the reaction rate considerably (Marchetti et al., 2007).

Direct transesterification is a one-step method whereby the oil does not need to be extracted before conversion into biodiesel. However, it has been noted that the addition of a good solvent to extract the oil, dramatically increases the biodiesel yield. Johnson and Wen (2009) noted that direct transesterification produced a biodiesel yield of 12.7 % and emphasised the importance of the presence of a solvent for more efficient biodiesel yield. Although a solvent is required to increase the biodiesel yield, it can still be done in one step (direct transesterification), which decreases the overall

987 Triglycerid Methanol Glycerol Biodiesel

988 **Figure 11.** Overall reaction of the transesterification process (Leung et al., 2010).



989

990 **Figure 12.** Stepwise reaction of transesterification (Murugesan et al., 2009).

991

reaction time. Patil et al. (2011) reported 84 % fatty acid methyl ester content by means of direct transesterification of*Nannochloropsis sp*. Umdu et al. (2009) achieved a biodiesel yield of 97 % in direct transesterification of *Nannochloropsis oculata* with the aid of CaO/Al_2O_3 catalyst. Johnson and Wen (2009) reported of a biodiesel yield of 67 % (dry biomass) in *Schizochytrium limacinum*.

Extraction-transesterification requires the extraction of algal lipids from the cells first before transesterification. If solvents are used to extract the lipids, the solvent must then be evaporated by distillation before the transesterification step. The draw back with extraction followed by transesterification is the long time required for the reaction (Chen et al. 2009). Johnson and Wen (2009) reported on an extraction-transesterification process which resulted in 59.7 % biodiesel yield from the microalgae *Schizochytrium limacinum* using solvent chloroform. Nagle and Lemke (1990) used an extraction-transesterification process and achieved 81 % oil yield from *M. minutum* using 1-butenol as the solvent.Wahlen et al. (2011) extracted 27.3 mg of TAG from *Chaetoceros graciis* using solvent system chloroform:methanol (2:1) of which 82 % were transesterified. Krohn et al. (2011) reported a total lipid extraction of 19 % (total biomass) using hexane solvent and a 1.2 % FAME yield (total biomass).

8.1. Chemical Transesterification

Both acids and alkalis are used as catalysts in chemical transesterification. Alkali catalysts are commercially used because of their cost effectiveness, minimum reaction time and low temperature and pressure environment (Bacovsky et al., 2007; Leung et al., 2010). Acid catalysts are not widely used as alkali catalysts.

The major acids used in the transesterification process are sulfuric acid, hydrochloric acid and sulfonic acid. Acid catalysts can achieve a high yield without the formation of soap. The disadvantages of using acids as catalysts are that corrosion can occur in the reaction and the rate of reaction is slow compared to alkali catalysts (Freedman et al., 1984; Bacovsky et al., 2007).

Alkali catalysts have higher conversion yields but the major disadvantage of these catalysts is their effect on the purification process of biodiesel as saponified products are produced. Figure 13 shows the alkali process of transesterification for biodiesel production (Ranganathan et al., 2008). Many free fatty acids and water in the reaction mixture reduce the efficiency of the transesterification process (Leung and Guo, 2006; Marchetti et al., 2008). The purification step removes water from the transesterification process (0.2 ton of waste water per ton of biodiesel is produced) which makes the process expensive and not environmentally friendly (Fjerbaek et al., 2009). The major alkali catalysts used commercially are sodium hydroxide (NaOH) and potassium hydroxide (KOH) (Schuchardt et al., 1998; Marchetti et al., 2008; Robles-Medina et al., 2009).

Both acid and alkali catalysts consume energy due to the complexity of the purification process (Xu and Wu, 2003). A separation process is required after completion of the transesterification process to separate biodiesel from impurities, monoglycerides, diglycerides, triglycerides, catalyst, glycerol, monoacylglycerols and diacylglycerols. The separation process involves few steps including gravitational settling or centrifugation (to separate glycerol from the end product), deodorization and pigment removal (Antczak et al., 2009; Banerjee and Chakraborty, 2009).

8.2. Enzymatic Transesterification

The use of enzyme catalysts eliminates the requirement of excess energy by reducing the downstream processing and the problems associated with both alkali and acid catalyst (Roberts, 1989; Arnold, 1998).There are other advantages of using lipases as biocatalysts (compared to acid or alkali) including:(a) separation of high quality glycerol as by-product, (b) no washing step is required toesterify both free fatty acids and triglycerides, (c) no limitation in raw material, (d) less energy for conversion of free fatty acids to FAAE's is requiredand (e) lower molar ratios are required than chemical transesterifcation (Narasimharao et al., 2007; Tamalampudi et al., 2008; Fjerbaek et al., 2009). The disadvantages of enzymatic transesterification process are: (a) slower rate of reaction, (b) lengthy reaction time, (c) high dosage of catalyst is required and (e) high production cost (Bacovsky et al., 2007; Jeong and Park., 2008; Fjerbaek et al., 2009).

Jegannathan et al. (2008) stated that enzyme transesterification has proved to be more effective in reducing feedstock limitations and the separation of glycerol from biodiesel. Harding et al. (2007) and Fjerbaek et al. (2009) indicated that contrary to alkali catalysts, enzyme catalysts do not allow formation of soap in the reaction and hence the presence of free fatty acids in the reaction is not a problem. Dizge and Keskinler(2008) reported that the waste water produced with enzyme catalysts are lower in volume and strength than that produced with acid catalysts. Fukuda et al. (2001) stated that unlike chemical catalysts (which do not convert insoluble feedstock in the reaction), enzyme catalysts converts the entire free fatty acids in the reaction to product allowing waste oil and fats from all sources to be used as the feedstock.

Enzymes can be used in immobilized forms so that the separation process of enzyme catalysts from the FAAE's is simplified and the enzyme can be reused (Akoh et al., 2007; Robles-Medina et al., 2009). Table 15 illustrates the differences between the alkali and enzyme catalysts.China is the first and major producer of biodiesel using lipase as the catalyst, producing 20,000 tons of biodiesel per annum (Du et al., 2008). The schematic diagram of producing biodiesel using enzymes is shown in Figure 14.

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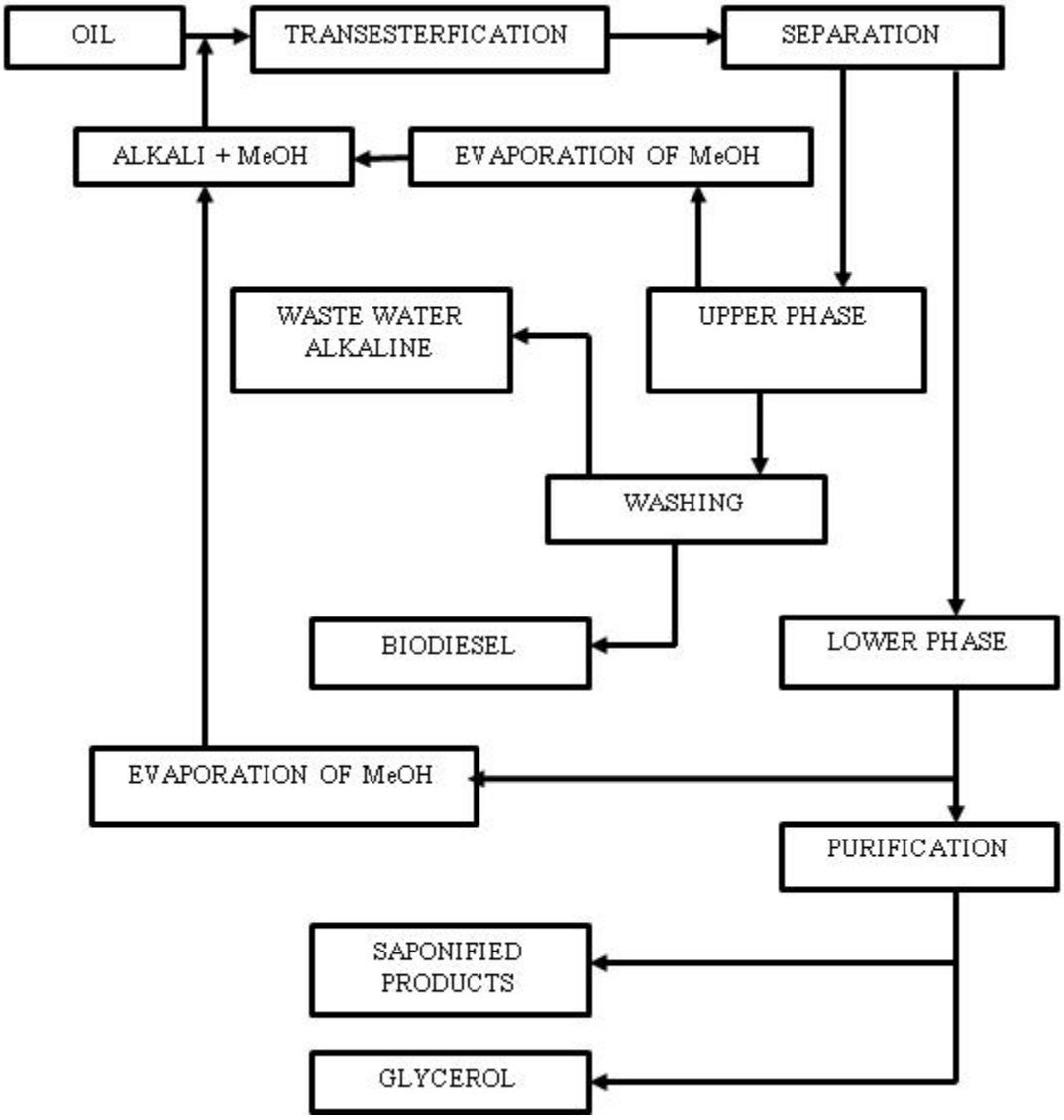

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1057 **Figure 13.** Alkali process of transesterification of biodiesel production (Ranganathan et al., 2008).

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1059 **Table 15.** Comparison of alkali catalyst and biocatalyst transesterification (Shah et al., 2003, Fukuda et
1060 al., 2001).
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Major factors	Alkali catalyst transesterification	Biocatalyst transesterification
Temperature	60-80ºC	20-60ºC
Presence of FFA's in feed stock	Soap formation	Complete conversion into the methyl ester
Presence of water	Soap formation is more likely as hydrolysis of the oil may take place	No effect on final product
Yield of biodiesel production	High, nearly 99%	Comparatively lower than alkali catalyst, around 90%
Downstream processing	Multi-step purification of end products	None
Biodiesel production cost	Cheap as catalysts comparatively cost less	Very expensive as biocatalyst are expensive
Commerialization	100% commercialized	China and Brazil
Waste water generation	Saline and alkaline effluent needs treatment before discharge	No waste water generation

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9.FACTORS AFFECTING ENZYMATIC TRANSESTERIFICATION

In the enzymatic transesterification process, the factors affecting the rate of conversion of biodiesel include the selection of alcohol, use of solvents, alcohol to oil molar ratio, water activity and reaction temperature.

9.1. Selection of Alcohol

Alcohols can be divided in two types namely long chain alcohols and short chain alcohols. Long chain alcohols can be used in the transesterification reaction but the conversion yield is lower than that obtained with the short chain alcohols because they inhibit the lipase activity (Coggon et al., 2007). Short chain alcohols like methanol and ethanol are widely used in the transesterification process for the enzymatic production of biodiesel. Other short chain alcohols can be used in the process including propanol, iso-propanol, 2-propanol, n-butanol and iso-butanol (Iso et al., 2001; Antczak et al., 2009; Varma and Madras, 2010). Salis et al. (2005) used different types of short chain alcohols with *Pseudomonas cepacia* without a solvent system and obtained a conversion yield of 40% with methanol, 93% with ethanol, 99% with propanol, 99% with 1-butanol, 83% with 2-butanol, 99% with 2-methyl-1-propanol and 99% with pentanol.

Short chain alcohols like methanol and ethanol are cost effective but are responsible for deactivation and inhibition of immobilized lipase (Chen and Wu, 2003; Samukawa et al., 2000). The deactivation of enzyme was reported by insoluble methanol present in the oil or fats (Salis et al., 2005; Al-zuhair et al.,2007). Glycerol also inhibits the immobilized lipase. Kumari et al.(2009) reported that glycerol deactivates and destabilizes the lipase because it has the tendency to get absorbed by the surface support matrix. Deactivation of the enzyme is determined by the decrease in carbon atoms in the alcohol (Chen and Wu, 2003; Ranganathan et al., 2008). Antczak et al. (2009) states that the rate of transesterification process is directly proportional to the length of alcohol carbon chain and indicated that ethanol is more favorable than methanol in some reactions.

Some researchers have suggested ways to avoid the inhibition of the enzyme by short chain alcohols including stepwise addition of alcohol or adding it in sequence (Shimada et al., 1997; Watanabe et al., 2002; Soumanou and Bornscheuer, 2003; Matassoli et al., 2009) and using a solvent system (Nelson et al., 1996; Mittelbach, 1990; Modi et al., 2007). Stepwise addition of short chain alcohol is applicable only for methanol because ethanol has less of an inhibition effect towards immobilized lipase. To prevent the methanol inhibition effect, the ratios of oil: fat should be maintained below 3 and for ethanol it should

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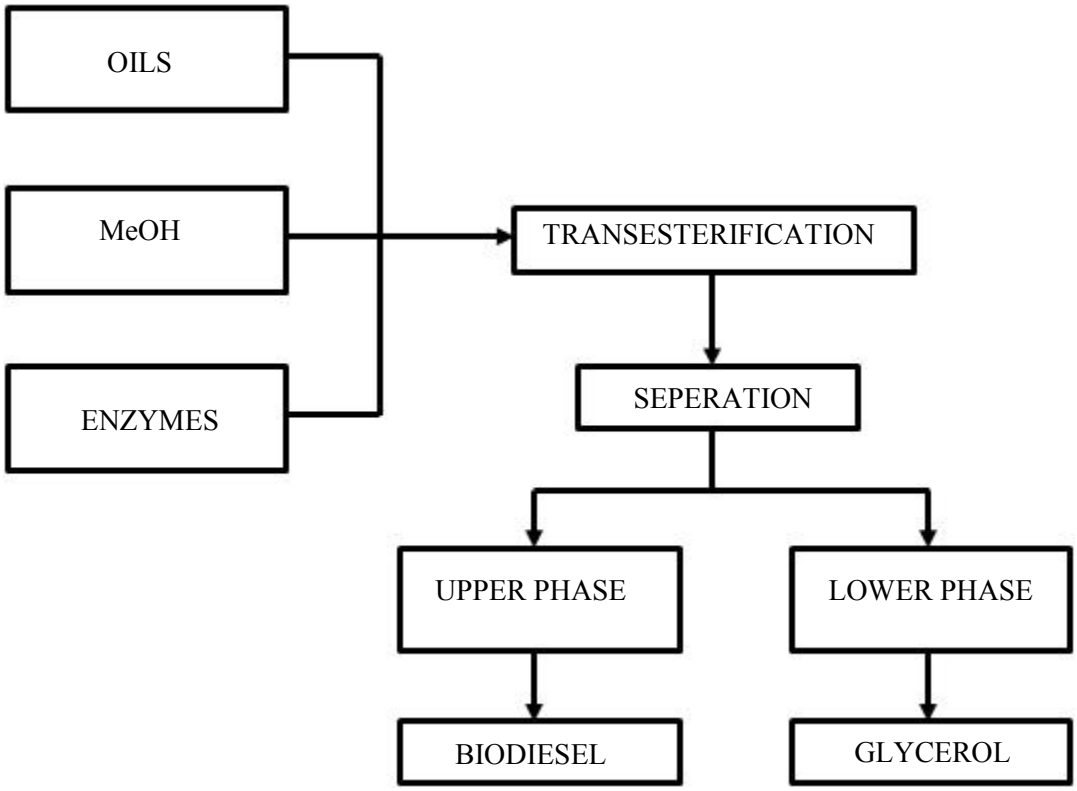

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1095 **Figure 14.** Enzymatic production of biodiesel (Ranganathan et al., 2008).

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maintain below 11 (Robles-Medinaet al., 2009). Lee et al. (2008) obtained a 98.92% conversion yield using stepwise addition of methanol and 65% conversion yield when methanol was added in a batch process. Every lipase has different inhibition level and lipases that are extracted from *Pseudomonas* are more resistant towards alcohol inhibition than lipases extracted from *Thermomyces lanuginosa* and *Rhizomucor miehei* (Fjerbaek et al., 2009).

9.2.Use of Solvents

Solvents are used to lower the inhibition effect of alcohol by increasing its solubility (Kumari et al., 2009). Solvents can also solubilize the by-product glycerol which can prevent the surface coating of the immobilized enzyme and the inhibition effect (Royon et al., 2007). Solvent systems provide a homogenous mixture between reactants and products which reduces the inhibition of enzymes and stabilizes the immobilized lipase in the reaction (Ranganathan et al., 2008; Fjerbaek et al., 2009). The homogenous mixture readily reduces the problems associated with multiple phase reactions and mass transfer reduction due to the high viscosity of the oil/fat substance (Fjerbaek et al., 2009). Vasudevan and Briggs (2008) stated that the rate of the transesterification reaction increases in the solvent system when compared to a solvent free system.

The solvents commonly used in the transesterification process are hydrophobic in nature and include hexane, n-heptane, petroleum ether and cyclohexane (Holmberg and Hult, 1990; Nelson et al., 1996; Soumanou and Bornscheuer, 2003; Ghamgui et al., 2004; Lara and park, 2004; Coggon et al., 2007). The most stable solvent commonly used is hexane which has a moderate polarity towards enzymes (Li et al. 2006; Fjerbaek et al., 2009). Tert-butanol and 2-butanol are alcohols which can also be used as solvent for regeneration of lipase (Robles-Medinaet al., 2009). Royon et al. (2007) showed that the *Candida antarctica* (Novozyme 435) conversion yield was higher when *tert*-butanol was introduced to the solvent system. In a methanolysis reaction, the enzyme catalyst *Thermomyces lanuginosa* showed a conversion of 10% in solvent free system but when *tert*-butanol was added, a conversion yield of about 75% was obtained (Li et al., 2006). Qin et al. (2008) investigated the methanolysis of soybean oil using an enzyme from *Rhizopus chinensis* as a catalyst with different solvents and found n-heptane to be the best solvent with respect to efficiency. The conversion yields were 84.2, 73.5, 73.4, 71.1 and 65.8% for the solvents n-octane, iso-octane, petroleum ether, acetone and cyclohexane when used with *tert*-butanol as alcohol in the reaction, respectively.

The solvents are used in the reaction to reduce the inhibitory effect of short chain alcohols but there are some disadvantages of using solvents in the reaction mixture including: (a) additional processing is

required to separate the biodiesel product from the solvents, (b) organic solvents are unstable and hazardous, (c) the volume of reactors must be increased and (d) using solvents increases the overall cost for the producing the biodiesel (Ranganathan et al., 2008; Fjerbaek et al., 2009).

9.3.Alcohol: Substrate Molar Ratio

In the transesterification process, the alcohol: oilmolar ratio is a vital part of the reaction. The rate of the reaction is directly proportional to the alcohol: oil ratio and the alcohol: oil molar ratio should be more than one to enable the process to proceed at specific rate (Antczak et al., 2009). Deactivation of the enzyme occurs when alcohol is insoluble in the reaction. Alcohol must be dissolved completely in the reaction mixture to prevent the deactivation of lipase and to increase the reaction rate (Jeong and Park, 2008). In solvent free methanolysis, the concentration of methanol is inversely proportional to the activity of lipase in the reaction (Iso et al., 2001; Kose et al., 2002; Chen et al., 2006).

The selected alcohol must have more than three carbons. If the carbons are less than three, the alcohol has a tendency to inhibit lipase in the reaction. The stoichiometric ratio of both methanol and ethanol are 1:3 and 2:3, respectively. The inhibition of lipase can be restricted by dissolving the alcohol completely in the reaction mixture within their stoichiometric ratios (Shimada et al., 2002; Robles-Medina et al., 2009). Matassoli et al. (2009) suggests that the ratios of methanol and ethanol to oil in solvent system should be 1:3 and 1:6, respectively.

In a solvent-free reaction, the inhibitory effect of lipase can be lowered when the addition of alcohol occurs in a stepwise manner (Selmi and Thomas, 1998; Kose et al., 2002; Vasudevan and Briggs, 2008). The molar ratios of short chain alcohols like methanol to oil must be around 3:1 (Antczak et al., 2009). In ethanol, the molar ratio of ethanol: oil can reach 11:1 (Robles-Medinaet al., 2009; Munio et al., 2008).

Salis et al. (2005) reported that in the butanolysis of triolein with the enzyme catalyst *Pseudomonas cepacia*, the molar ratios 3:1, 6:1, 9:1, and 12:1 were used and the optimum ratio was found to be in the range of 3:1 - 6:1. The conversion yield in that range was 100% after 4 hours of reaction but the ratios 9:1 and 12:1 showed 100% conversion yield after 5 and 6 hours, respectively. Jeong and Park (2008) reported that in the methanolysis of rapeseed oil using *Candida antartctica,* the optimum ratio was between 2:1 and 5:1, which gave a high conversion yield. The 6:1 ratio gave low yield due to inhibition effect of lipase in the reaction (). However, the optimum level of molar ratio depends on the alcohol, lipase and feedstock used (Shimada et al., 2002; Robles-Medina et al., 2009; Matassoli et al., 2009).

9.4. Water Activity

Water activity is one of the vital factors in enzymatic transesterification which sustains the three dimensional structure of the enzyme and determines the FAME yield and rate of reaction (Jegannathan et al., 2008; Lu et al., 2009). It can be expressed as water activity or percentage concentration (Antczak et al., 2009). The optimum water activity increases the activity of lipase and reduces the hydrolysis in the enzymatic transesterification process even with short chain alcohols (Noureddini et al., 2005; Akoh et al., 2007; Jegannathan et al., 2008). Optimization of water activity depends on factors such as the reaction system, alcohol type, lipase source, immobilization technique and stability of enzyme (Jegannathan et al., 2008; Antczak et al., 2009). Few lipases such as those from *Candida rugosa, Pseudomonas cepacia,* and *Pseudomonas fluorescens* do not react with alcohols if there is no water activity but they show high conversion yield with water activity between 1% and 20% (Akoh et al., 2007; Fjerbaek et al., 2009). The conversion yield of *Rhizopus oryzae* was high with water activity between 4% and 30%. The water activity for some lipase can lead to no reaction. For example, the lipase from *Candida antarctica* does not like water in the transesterification process (Deng et al., 2005; Fjerbaek et al., 2009). Robles-Medina*et al*. (2009)suggests that the water activity leads to flooding the pores which tends to lower the reaction rate. Li et al. (2006) stated that the optimum water activity must be 2% or less for transesterification process to give high conversion yield. He found that when *Thermomyces lanuginosa* and *Candida antarctica* were used in combination with tert-butanol as solvent, the water activity was maintained above 2% which gave low methyl ester yield.

9.5. Reaction Temperature

According to Marchetti et al. (2008), lipases are thermally stable within the temperature range of 20°C - 70°C. However, the rate of conversion is highly dependent on temperatures outside this range. Antczak et al. (2009) states that the optimum temperature of immobilized lipase depends upon stability of lipase, type of solvent and type of alcohol. Jeong and Park (2008) performed a transesterification process with reaction temperature between 25°C - 55°C and found the optimum reaction temperature to be 40°C. Lee et al. (2008) showed an optimum reaction temperature of 45°C using combination of *Rhizopus oryzae* and *Candida rugosa* with methanol as the alcohol.

10. GLYCEROL USES

Glycerol is known as glycerin or glycerine. It is a simple alcohol with many applications in various industries such as cosmetics, paint, automotive, food, tobacco, pharmaceuticals, pulp and paper, leather

and textile industries (Biebl et al. 1998; Wang et al., 2001). Various chemicals can be obtained from glycerol as feedstock. Biebl et al. (1998) reported that glycerol can be used as a feedstock in the chemical synthesis of poly trilmethylene or polyterephthalate which can enhance certain physical properties (good resilience, stain resistance and low static generation) of fiber used in the textile industries. The conversion of (5 -15%) glycerol to (75 - 90%) dihydroxyacetone using *Acetobacter suboxidans* bacterium as the medium in submerged fermentation is an example of using glycerol as a feedstock for industrial fermentations (Wang et al., 2001). The dihydroxyacetone can be further converted from dihydroxyacetone kinase to dihydroxyacetone phosphate which is a substrate molecule for aldolases to produce optically active sugar derivatives (Itoh et al., 1999). Table 16 shows the usage of glycerol in various applications.

The annual production of glycerol was 600,000 tons in 2001. The production of glycerol from hydrolysis of fats has decreased, due to soap being replaced by detergents in the developing countries and industrial nations (Agarwal, 1990). Also, the production of glycerol can be obtained from the oxidation or chlorination of propylene. However, the cost of propylene is high and there are associated environmental concerns (Wang et al., 2001), thus the production of glycerol from propylene has been in decline. Glycerol can also be produced as a byproduct during the microbial fermentation of sugar to ethanol using *Saccharomyces cerevisiae* in a redox-neutral process (Agarwal, 1990; Wang et al., 2001). This method became more attractive and cost effective than the chemical synthesis from petrochemical feedstocks or the recovery as a byproduct of the soap manufacture process from fats (Wang et al., 2001).

11. CONCLUSIONS

The increase in the annual global energy consumption over the past century has relayed heavily on fossil fuels. Fossil fuel burning have accelerated CO_2 emissions on a global scale. Carbon dioxide makes up 63 % of the greenhouse gasses present in the atmosphere. The environmental concerns associated with greenhouse gas emissions emphasise the need for alternate energy sources that are more environmentally friendly. Microalgae are abundant in nature and can be used as an alternate source of energy. They are photosynthetic microorganisms that are capable of growing in marine and fresh water environments and convertorganic substances to oil. Their high growth rate, ability to produce large amounts of lipids which can be used for biodiesel production and the fact that they utilize CO_2 present in the atmosphere for growth, makes them a good alternative to current fossil fuel. Microalgae generate large amounts of oil in the form of triacylglycerols which can be converted into biodiesel, via chemical or enzymatic a

Table 16. Usage of glycerol in various applications (Wang *et al*., 2001)

Field of Use	Percent Use (%)			
	USA	Europe	Japan	China
Drugs	39.5	23.1	34.0	5.0
Tobacco	15.8	2.5	5.3	7.0
Glycerintriacetate	ND	14.4	ND	ND
Food	14.5	5.6	ND	ND
Polyether alcohol	10.5	13.1	11.6	5.2
Paints	9.2	13.1	19.5	49.0
Cellophane	2.0	4.4	3.8	1.5
Dynamite	0.6	3.1	1.9	3.1
Toothpaste	ND	ND	ND	16.0
Cosmetics	ND	ND	ND	6.0
Miscellaneous	7.9	20.7	23.9	7.2

*ND = No Data
USA Production = 160,000 tons/yr
Europe Production = 190,000 tons/yr
Japan Production = 50,000 tons/yr
China Production = 80,000 tons/yr

transesterificaition processes. Biodiesel is a renewable fuel that generates the same amount of energy as that generated frompetroleum diesel without the release of harsh compounds into the atmosphere. It is biodegradable and nontoxic and can be utilized in existing diesel engines without modification. Various production systems for microalgae growth have been reviewed and their advantages and disadvantages have been noted, these include open ponds (circular and raceway), enclosed photobioreactores (tubular and plate), and hybrid systems. Lipid synthesis (i.e. non-polar TAGs) are the best substrates for biodiesel production, which can be formed by altering some of the algal growth conditions. These conditions can be either chemical or physical environmental stimuli. Chemical stimuli include depravation of nutrients and pH and physical include light intensity and temperature. The various nutrients, obtained from the surrounding media of the microalgae, play a role in lipid accumulation. Altering the amount of these nutrients affects the rate of growth and the organisms' ability to synthesize lipids. Such nutrients include: carbon, nitrogen, and phosphorous. Currently, the use of microalgae for biodiesel production is not economically feasible because of the high harvesting and pre-treatment costs associated with the production process. This can be overcome by extractingproteins, vitamins, carotenoids, nucleic acid, carbohydrates and lipids from the algae and processing the algae biomass into various value added products such as ethanol, methane, animal feed and fertilizer. Additionally, the glycerol produced as a by-product during the conversion of lipids into biodiesel can be further fermented to produce products such as methanol, lactic acid, ethanol and hydrogen. By producing these value-added products in addition to the biodiesel, the economics of the harvesting, pre-treatment and processing of microalgae into biodiesel can be improved significantly.

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