Production of Biodiesel from Marine and Freshwater Microalgae: A Review

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

| Review Article |

ABSTRACT

Aim: 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 emphasize 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.
Findings: 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 transesterification 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

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atmosphere, it is biodegradable and nontoxic and can be utilized in existing diesel engines without modification.

Conclusion: 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 extracting proteins, 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 lipid 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.

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 [1]. 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 [2]. In 2011, the total sales of gasoline and diesel in Canada amounted to 40.4 billion litres and 17.8 billion litres, respectively [3].

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 [4]. Carbon dioxide contributes about 63 % of the greenhouse gasses emitted into the atmosphere [5,6]. A change in global warming would increase the atmospheric temperature and negatively impacts all living organisms [7]. An increase in the earth's temperature results in a decline in the Adelilie penguins spices, melting of glaciers, increased precipitation causing floods [8,9] and increased sea level causing loss of property and productive agricultural lands [10]. In Canada, global warming effects are already felt across the nation. Forest fires, floods, insect infestations and drought are the results of global warming [11-13].

The environmental concerns of greenhouse gas emissions emphasize 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 [14]. Using biofuels over the traditionally used fuels offers the benefits of greater energy security, foreign exchange savings and reduced environmental problems [15-17]. 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 [14,18].

Algae, which are abundant in nature, can be used as an alternate fuel source [19,20]. 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 [21]. The majority of lipids produced by microalgae have a low degree of unsaturation, making them a good source of biofuels for replacement of the current fossil fuels [14]. 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 space as 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 [22-28].

Biodiesel, as a liquid fuel, can be produced by transesterification of oil extracted from algae [23,29,30]. 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) [18,28]. The waste generated from the algal biomass can be further processed to 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 [25,27,28,31]. It is important to note that different types of microalgae contain different cellular compositions (as percent of dry weight). Algae with higher lipid composition areappropriate for production of bioethanol [25].

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 [3,28]. The biggest advantage of biodiesel, compared with other alternative transport fuels, is that it can be utilized in existing diesel engines without much modification [14]. Other advantages of using biodiesel generated from microalgae include: no release of harsh compounds (So_x, NO_x and HC) into the atmosphere [32], it is biodegradable and nontoxic, a much cleaner energy source and far more environmentally friendly than the current energy sources being used [18,32-34]. In addition, microalgae can generate 44.9 billion litres of biodiesel in 345-764 thousand hectares of land per year (depends on algal composition) which is far less than the land required for the same amount of biodiesel to be generated using other terrestrial crops (Table 1). 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 accumulation in the cells, various oil extraction techniques and the methods of conversion of algal lipids into biodiesel.

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 [37]. 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 [25,28,37,38]. Both macroalgae and microalgae are found in marine and freshwater environments [37]. They form the base of the food chain by providing nutrition and an accommodating environment for other organisms [31,39]. 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 [40].

Table 1. Biomass feedstock oil yield comparison [19,35,36]

Crop	Oil Yield (L/ha)	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

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

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 [38]. Based on pigmentation, macroalgae are broadly classified to three main groups (Fig. 1): brown seaweed (Phaeophyceae), red seaweed (Rhodophyceae) and green seaweed (Chlorophyceae). In order to provide buoyancy, some of these species have gas filled structures [37,38]. Macroalgae are mainly used for food production and hydrocolloid extraction [25,38]. 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 [41]. 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 [31]. Also, their anticoagulant, antiviral, antitumor and brinolytic properties make them a great asset for use in medicine and pharmacology [42].

(a) Phaeophyceae

(b) Rhodophyceae

(c) Chlorophyceae

Fig. 1. Main groups of macroalgae [43,44]

Table 2. Chemical composition of macroalgae species [45,46]

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

Boopathy and Kathiresam [42] 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 proteins in 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 [31]. 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 [47]. Van Ginneken et al. [47] 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. [32] 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 [32,48]. However, they can be used for the production of biogas and bioethanol and also as a source of medicine [48].

2.2 Microalgae

Photosynthetic microscopic organisms are known as microalgae [25,28]. They are single

celled organisms and are categorized into several groups on the basis of pigmentation, basic cellular structure and life cycle [25,41]. Microalgae can be further broken down into prokaryotic cyanobacteria (Fig. 2a) and eukaryotic algae (Fig. 2b). Table 3 lists a few of the divisions found in prokaryotic and eukaryotic algae. Eukaryotic algae are classified into 12 divisions [28]. 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), Fig. 3 [28,41]. 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 [28,32].

Table 3. Microalgae group classification [37]

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

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 [49]. 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 rapid growth rate under optimal conditions and under unfavourable conditions they alter their metabolic products to form natural oils (triaclyglycerols) [28]. These characteristics add to their increased appeal for utilization in biodiesel production.

Li et al. [54] reported 95% biodiesel yield from oil extraction from Chlorella pyrenoidosa. Johnson and Wen [55] achieved a biodiesel conversion efficiency of 95.9 % from the oil extracted (57 %)

of Schizochytrium limacinum. Miao and Wu [56] reported a biodiesel yield of 70% from oil extracted from Chlorella protothecoides. Stephenson et al. [57] reported that Chlorella vulgaris can produce a total biodiesel of 8200 L/ha/year. Rodolifi et al. [58] noted that Nannochloropsis species can produce 23000-34000 L/ha/year of biodiesel.
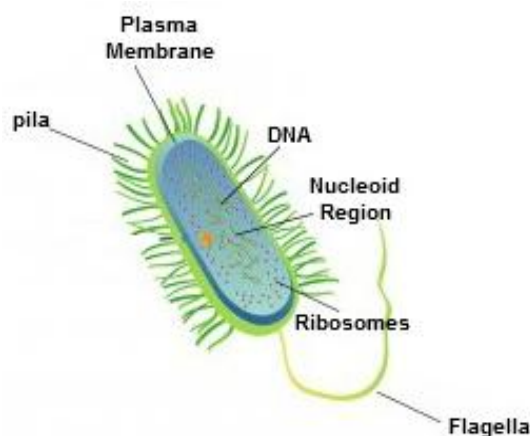
(a) Prokaryotic organism
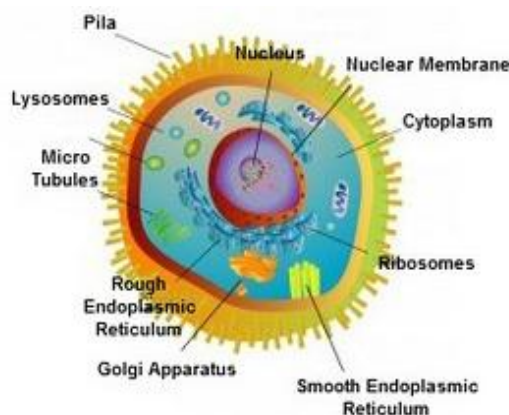
(b) Eukaryotic organism

Fig. 2. Cell structure of organisms [50]

After using microalgae for the production of biodiesel, the remaining biomass residues can be further used to produce numerous by-products (Fig. 4). These include: bioethanol via fermentation and hydrolysis [26], food ingredients, animal feed, nutraceuticals and pharmaceutical [59]. The glycerol can also be converted to several chemical products by fermentation including biofuels such as hydrogen and ethanol. Processing of algal biomass after the extraction of lipids would improve the economics of biodiesel [60].
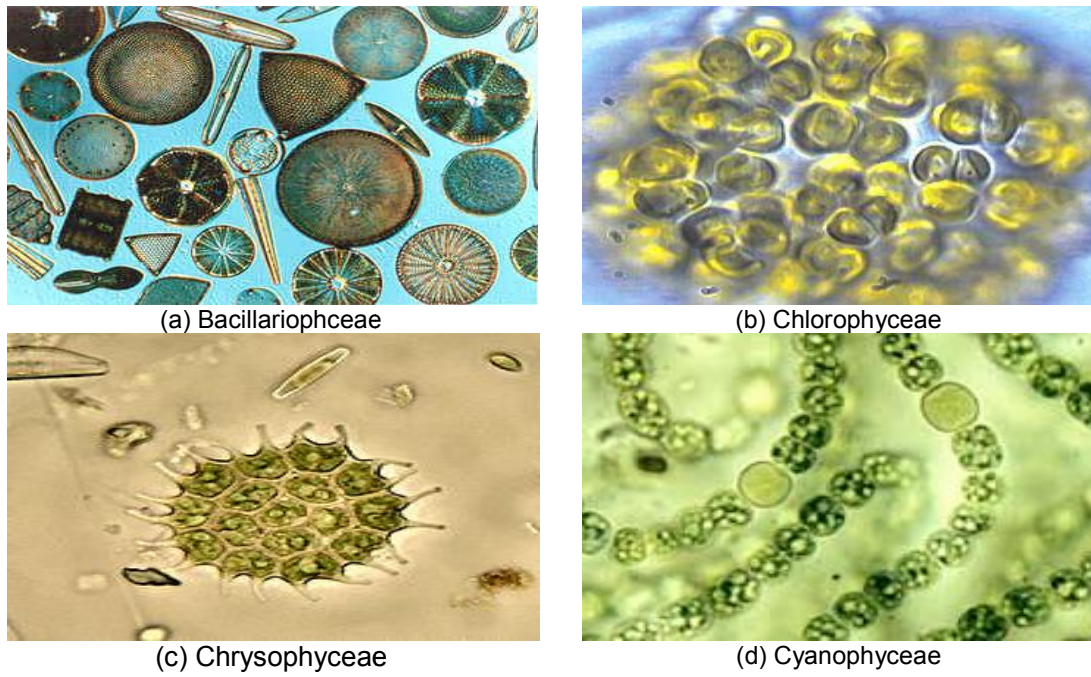
(a) Bacillariophceae

(b) Chlorophyceae

(c) Chrysophyceae

(d) Cyanophyceae

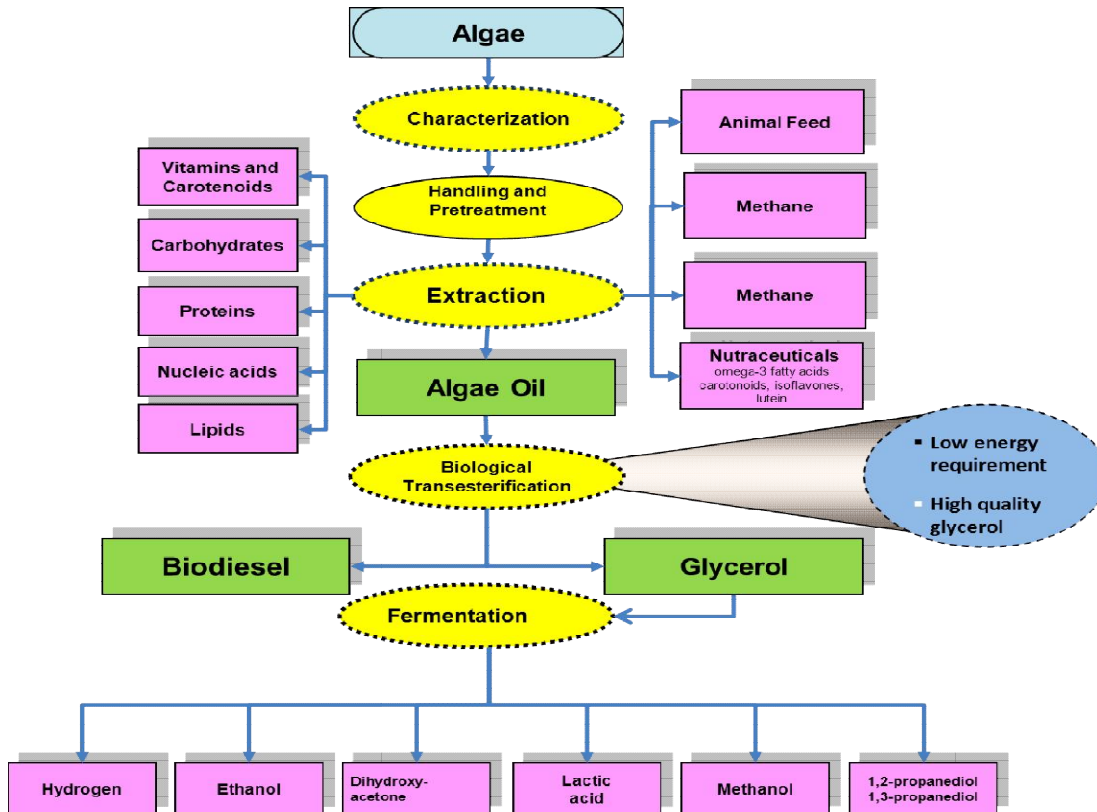**Fig. 3. The four most important groups of microalgae [51-54]**

Fig. 4. Algae biorefinery

Harun et al. [61] reported a bioethanol production of 3.83 g/L, using 10 g/L of lipid-extracted microalgae (Chlorococum sp.) debris via yeast (Saccharomyces bayanus) fermentation. Kim et al. [62] used hydrothermal treatment to fractionate Schizochytrium sp. for the production of bioethanol and achieved 11.8 g/L of ethanol form 25.7 g/L of glucose. Chisti et al. [18] illustrated that in order for an economical balance to be reached, the biomass that remains after lipid extraction needs to be transformed into methane via anaerobic digestion which can also recycle back nitrogen and phosphorus. Becker [63] noted that nutritional and toxicological analyses of algae biomass protein illustrated that it is highly suitable as a supplement feed similar to soybean meal, fish meal and rice Bran.

Currently 30% of the world's algae production is used as feed supplements. Soletto et al. [64] noted that the microalgae Arthrospira possess a high protein content, and it is for this reason that they are used in human nutrition today.

3. MICROALGAE ENVIRONMENT

Microalgae have the ability to withstand various environments. Their main requirements for biomass production include water, light and carbon dioxide. Microalgae can grow in salt, fresh and wastewater environments [26].

3.1 Freshwater

Freshwater (Table 4) contains a few or no salts compared 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 [65]. 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 [28].

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

[67]. Utilizing marine water for microalgae growth would minimize the need for additional nutrients into the production system.

Table 4. Composition of freshwater [66]

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	31.90
(HCO_3^-)	14.51
Silica (SiO_2)	0.74
Iron (Fe)	1.11
Nitrate (NO_3^-)	

Table 5. Composition of marine water [67]

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

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 [68,69]. Growing algae on municipal wastewaters has the advantage of removing certain elements such as phosphorous and nitrogen in addition to the removal of CO2 from the atmosphere, which if left undealt with are harmful to the environment [28].

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 [14,30,70].

Table 6. Composition of major constituents in wastewaters [69]

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

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 and play an important role in 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 [37,71].

Proteins extracted from algae biomass can be used in industrial, therapeutic and diagnostic applications [72]. The incorporation of algae protein in food processing is still in its trail phase [63]. Algae proteins have been noted to possess antioxidant peptides that play a vital role in the human health [73,74]. Toxicological and nutritional evaluation of algae proteindeem it suitable as a feed supplement or a substitute for current protein sources such as fish meal, rice bran and soybean meal [63,72].

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 in microalgae. They are formed by CO_2 fixation and can be in the form of sucrose, paramylon and 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 [37]. Some algae 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 [75-77]. This process involves the conversion of carbohydrates by yeast (Saccharomyces cerevisiae) under anaerobic conditions into ethanol and carbon dioxide [78]. Methane production can be achieved through anaerobic digestion of carbohydrates [19,79]. This process takes place under anaerobic conditions. The anaerobic microorganisms degrade and stabilize organic materials into methane and carbon dioxide [80].

4.3 Lipids

Another form of energy storage for microalgae is triacylglycerol (TAG) lipids (Table 7). The main building blocks of TAGs and all other cellular lipids are fatty acids. The enzyme acetyl CoA carboxylase is the enzyme responsible for regulating the rate of fatty acid synthesis, which is synthesized in the chloroplast [20]. Small amounts of spherical lipid droplets are contained in the chloroplasts between the thylakoids, which play a role in the growth and synthesis of lipoprotein membranes within the chloroplast [81]. Additionally, these lipids function as a cell structural support and as metabolic organelles in photosynthesis [28]. Surfactant lipids work to stabilize the structure of the mitochondria and in photosynthesis metabolism [70]. Algae typically have 5-20 % of dry cell weight (DCW) lipid oil when grown under optimal conditions, while growth in unfavourable conditions can increase the lipid content to 20-50 % of dry cell weight (DCW) [20,26].

Lipids extracted from microalgae can be used for biodiesel production. Lipids are converted into biodiesel through transesterification technology which works by using an alcohol and an acid or base. The alcohol plays the role of both the solvent (extracting the lipids from the biomass) and the reactant (converting the lipids into fatty acid methylesters) [25,28]. The alcohol most commonly used is methanol and the reaction catalyst can be an acid or base [23,30].

4.4 Nucleic Acid

Nucleic acid makes up to 1-6 % of the cell and plays a vital role in cell growth and cell repair. During the growth of algae, the accumulation of nucleic acid and other cellular constituents take place and the RNA and protein synthesis are

formed in a fixed ratio [82]. Nucleic acid contains high amounts of phosphorous and nitrogen which can be recycled back by the anaerobic digestion process. Also, the biomass containing nucleic acid can be used as fertilizer [27,28].

4.5 Vitamins

Microalgae have been noted to contain numerous essential vitamins such as vitamin A, B1, B2, B6, B12, C, E, nicotinate, biotin, folic acid and pantothenic acid. The amount of vitamins varies with species and cultivation techniques. The range of vitamins present in a few species ranges from 0.4 to 554 mg Kg^{-1} as shown in Table 8 [83]. These vitamins have important antioxidant properties and play vital roles in immune function, vision, reproduction, preventing against kidney disease, birth defects, depression and asthma [84-86]. Extractionof these vitamins from microalgae would provide an additional renewable source for vitamin extraction.

4.6 Cartenoids

Microalgae contain numerous carotenoids (β-carotene, zeaxanthin, lutein, violaxanthin, diadinoxanthin, pyrrhoxanthin, peridinin, neoxanthin, fucoxanthin, echinenone, canthaxanthin and astaxanthin) which play important roles in photosynthesis. Carotenoids typically make up 25 % of the microalgae cells [87]. They are 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 [88]. Caroteniods have important anti-inflammatory, antioxidant and anti-tumor activity [89,90], 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 [88,91].

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 [28,30,54,58,92]. 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 [20]. Oleaginous diatoms showed similar results indicating that under stress conditions the algal lipid content increases significantly.

Certain species such as Chlorella [93,94], Dunaliella [95], Nannochloropsis [58] and Neochloris [96] 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 [58].

Rodolfi et al. [58] 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. [97] 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. [20] 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 [58]. These conditions can be either chemical or physical environmental stimuli [20,98,99]. Physical stimuli include temperature and light intensity and chemical stimuli include pH and depravation of nutrients [20].

5.1 Temperature

Temperature plays a major role in the lipid yield and composition of fatty acids in algae [20]. The optimal temperature for algal growth is in the range of 18-20ºC. Temperatures that do not fall within this range are found to affect the rate of growth or result in the death of the organisms [28,30]. However, increases in lipid content have been noted in microalgae grown at temperatures both above and below the optimal range [28,100-104].

Renaud et al. [103] tested the temperature effect on the growth rate and lipid content of three algal species Isochrysis sp., Nitzschia closterium 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 of 20.1 % was noted at 20°C. Finally, Nitzschia paleacea was tolerant to low 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. [100] 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 [101] reported that the lipid content of Chaetoceros cf. wighamii increased with lower temperatures over the range of 20-30°C. Jiang and Gao [102] 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. [103] 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 [104] reported a 3 fold increase in lipid content of Spirulina species when exposed to temperatures below the optimal range.

5.2 Light

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 proteins that have a high sensitivity to light [37].

Through the process of photosynthesis microalgae are capable of taking photons from a light source and converting them into algal biomass [105]. The absorption of light photons depends on the algal cell pigmentation, culture density and the cells specific position [28,37]. 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 [28].

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 [28]. Scott et al. [92] 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 [28].

The pigments (chlorophylls) in charge of light absorption have the best absorption at wavelengths of 440 and 680 nm [28,37]. 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 [28].

Rodolfi et al. [58] 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. [106] noted that flashing light enhanced the photosynthetic process in Chaetoceros calcitrans. Nedbal et al. [107]

reported higher rates of growth using flashing light as opposed to continuous light. Rai and Gaur [108] noted that the photosynthesis in culture environments undergoing nutrient depravation is utilized for the formation of reduced storage products (fats). Pal et al. [109] noted that by varying the light intensity and salinity, the total fatty acid content increased to 47% DCW. Jacob-Lopes et al. [110] 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 [111] 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 pH due to carbon dioxide removal from the medium [108].

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 [112]. Increasing the pH decreases the competition between the metal ion and the H+ at the cell surface [112,113]. Therefore, the pH must be maintained at the appropriate level for a sufficient nutrient uptake.

Skrupski et al. [114] reported an increase in Chlorella lipid content from 15 to 45% (DCW) with incremental pH increases. Wang et al. [115] 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. [116] 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. [113] noted that Cu2+ 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. [58] 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 [30] which can naturally be obtained from the atmosphere [27]. Microalgae are capable of tolerating high levels of CO_2 as shown in Table 11 [27]. One ton of algal biomass can fix 1 ton of CO_2 by either autotrophic or heterotrophic metabolism [28]. 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. [117] reported that in Chlorella zofingiensis 51.1% lipid was observed under heterotrophic conditions and only 25.8 % lipid was observed under photoautotrophic conditions. The use of heterotrophic conditions would eliminate the need for light, bringing forward the possibility of increased productivity and cell density [122]. However, growing microalgae in heterotrophic conditions is difficult in large scale systems because bacteria thrive on sugars (unless this is overcome) and may result in biomass contaminations [123].

Sugars such as glucose can provide the carbon source required by algal cells for growth (Table 12). However, they are not as productive as the lignocellulose based materials such as rice straw, corn powder and sweet sorghum in their hydrolysate forms. Table 13 compares biomass and lipid productivity using various carbon sources. Amongst these various hydrolysate materials, rice straw has been noted to result in the highest cell lipid content of 56.3 % while running at the shortest culture time of 48 hours [54].

Table 7. Microalgae chemical composition [27]

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	-

Table 8. Vitamin content of various microalgae, values are mg Kg^{-1} [83]

	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

Table 9. Microalgae species screened for biomass productivity, lipid content and lipid productivity [58]

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

Table 10. Oil content of algae species and optimal conditions [27,82,118,119]

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

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. [120]
S. platensis	9	146.3	Sydney et al. [120]
D. tertiolecta	7.2	126.5	Sydney et al. [120]
C. vulgaris	7.2	128.6	Sydney et al. [120]
S. obliquus	6.5	156.5	Tang et al. [121]

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. [128]
	9	70	Blake et al. [128]
S. obliquus	7	126	Blake et al. [128]
	9	50	Blake et al. [128]
N. oculata	8	45.8	Merrett et al. [129]

Table 13. Impact of various carbon sources have on the biomass growth and lipid content of algal cells [54]

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

Liu et al. [117] noted that Chlorella zofingiensis grown under heterotrophic conditions and fed glucose produced a higher lipid content as opposed to phototrophic conditions, which resulted in a lipid yield of 79.5 % of which 88.7 % are made up of TAGs. Qiao and Wang [124] noted that Chlorella sorokiniana exhibited an increase in lipid content (from 0.053 g/L to 0.272 g/L) when glucose was used as the carbon source under heterotrophic conditions.

Liang et al. [125] reported that Chlorella vulgaris produced the highest amount of lipid when grown with glucose and light, and the fastest growth rate was achieved using 1% glucose concentration. However, the highest lipid content and lipid productivity were noted at 2% glycerol concentrations. Johnson and Wen [55] noted that the microalgae specie Schizochytrium limacinum grown in heterotrophic conditions resulted in the production of lipids suitable for biodiesel production.

Liu et al. [117] reported that the cell biomass rate of Chlorella zofingiensis under photoautotrophic conditions was 1.9 g/L while that under heterotrophic was 9.7 g/L. They also noted that the cells grown under heterotrophic conditions consumed nitrate much more rapidly. In addition, the algae cells grown under heterotrophic conditions produced natural lipids, oleic acid and TAGs which are better for biodiesel production as appose to the oils produced by the cells grown under photoautotrophic conditions.

During phototrophic conditions, algae photosynthesize and use CO_2 as the carbon source [126]. During this process the CO_2 is converted into algal biomass, which releases oxygen into the atmosphere [127]. In seawater, low CO_2 concentrations is a result of: (a) differenthabitats, (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 [108]. 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 [108] and (d) damage to the photosynthetic apparatus which is irreversible [130]. Some photosynthetic organisms have developed various pathways for controlling the amount of light allowed to be trapped in order to avoid damage to the photosynthetic apparatus [130,131]. These stress the importance of avoiding low CO_2 concentrations [108].

Huntley and Redalje [132] reported that Haematococcus pluvialis capable of taking up 16-34 % carbon dioxide. De Morris and Costa [133] noted that Chlorella kessleri was capable of taking up 18 % CO_2. Sobczuk et al. [134] stated that the species Phaeodactylum tricornutum was 63 % efficient in the uptake of CO_2. Wahlen et al. [23] reported that CO_2 is introduced into the culture by aeration with air at 1 % (v/v) of CO_2. Rodolfi et al. [58] 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 [105]. Algae are also capable of using bicarbonate as a carbon source (Table 9).

Blake et al. [128] 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. [135] noted that bicarbonate uptake by Nannochloropsis gaditana was activated by light. Merrett et al. [129] 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 [20,136]. Large scale production of microalgae for oil requires 8-16 tons/ha/year of nitrogen fertilizer [27]. 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 [137]. However, within the same genus Chlorella was found to produce starch under nitrogen stress [99].

In nitrogen deficient environments, the photosynthesis products shift from protein to carbohydrate and then to lipid production [58]. However, the accumulation of lipid results at the expense of biomass production [58,92]. 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 [108]. 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 [138].

Rodolfi et al. [58] noted that the marine species Nannochloropsis accumulated 60% DCW lipid content under nitrogen starvation. Rai and Gaur [108] noted that Synechococcus showed signs of protein destruction in nitrogen limited environments. Li et al. [96] 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 5 mM, respectively.

The nitrogen source nitrate maybe reduced by the process illumination in green algae, through which the hydrogen donors are, generated photochemically [69]. Round [105] 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 [139,140].

Lewin [69] 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. [141] 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 [122] 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. The effect of various initial nitrogen concentrations were also tested and the 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 [58].

5.4.3 Phosphorous

Phosphorous as a nutrient plays an important role in the building blocks of nucleic acids,

phospholipids, complex carbohydrates [108] and normal algal growth [142], it also plays a central role in catabolic and anabolic pathways and in the conversion of energy through the energy rich phosphoanhydride bonds [108]. It has been reported by several researchers that phosphorous limitation enhances the lipid accumulation in microalgae cells [20,58,142,143].

Xin et al. [142] 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. [58] 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 [143] noted that Monodus subterraneus lipid content increased from 6.5 to 39.3 %, mainly TAG, due to phosphorus limitation over the range of 0-175 µM.

6. UTILIZATION OF ALGAE

Algal biomass can be used to produce non fuel products such as food supplements and livestock feed. They may also be used to produce biofuel products including biodiesel, biomethane and bioethanol.

6.1 Non Fuel Products

Algal biomass possesses high protein content and other health beneficial ingredients. The algal biomass can be used as feed for human consumption or animals (fish, pets and farm animals) [28].

6.1.1 Food supplements and other compounds

Microalgae are a source of Omega 3 fatty acids, eicosapentaenoic acid (EPA), chlorophyll, and docosahexaenoic acid (DHA) [144]. Harun et al. [61] reported that microalgae naturally contain omega 3 fatty acids, which can be extracted via purification to provide a high value food supplement. These are a major group of high value chemicals that contain polyunsaturated fatty acids. These chemicals are important source of nutrients for humans and animals.

The Crypthecodinium and Schizochytrium species are capable of producing DHA, which is helpful in the development of infant brain and eyes as well as improving the cardiovascular

health of adults [28]. DHA is also in clinical use for a cure and prevention of cancer, AIDS, heart disease to control or lower cholesterol, boost immune system as well as a body detoxification [14]. Pyle et al. [145] reported on DHA production form *Schizochytrium* fermentation on glycerol crude. Burgess et al. [146] noted that *Isochrysis galbana* produced 5.4 mg/g DHA in a closed photobioreactor. Jiang et al. [147] reported on *Crypthecodinium cohnii* consisting of 51.12 % DHA and a biomass concentration of 2.04 g/L. Kyle and Gladue [148] reported on *Crypthecodinium cohnii* biomass consisting of 15-30 % oil of which DHA is 20-35 %.

EPA is a compound known to play a role in the prevention and treatment of various human diseases and disorders. EPA is used in the medical industry as a source of treatment for inflammatory and heart diseases such as asthma, arthritis, migraine headache and psoriasis [149]. This compound is found in the microalgae species *Nannochloropsis*, *Phaeodactylum,* and *Nitzschia* [28]. Ohta et al. [150] noted a high EPA portion in *Porphyridium propureum.* Renaud et al. [151] noted an EPA content of 8.7 and 12.0 % in *Rhodomonas* sp. and *Chromonas* sp., respectively. Wen and Chen [152] reported an EPA content of 19.1 % in *Nitzschia laevis*. Yongmanitchai and Ward [153] reported that over the pH range of 6.0-8.8, the maximum EPA was achieved at a pH of 7.6 in *P. tricornutum*.

Chlorophyll is a product that can be obtained from microalgae [14]. Chlorophyll is of great importance in the pharmaceutical industry and can be used as ointment treatment, liver recovery and ulcer treatment. It is capable of repairing cells, increasing haemoglobin in the blood stream and increases cell growth [154]. Ferruzi and Blakeslee [155] noted that algae chlorophyll can be used in food products and pharamaceuticals because of their anti-inflammatory properties and that it makes up 0.5-1.5% of dry matter. Danesi et al. [156] reported that the chlorophyll amount present in *Spirulina platensis* was not affected by the addition of urea as the nitrogen source. Henriques et al. [157] noted that chlorophyll content achieved from *Nannochloropsis gaditana* was three times less than the concentration achieved using methanol solvent.

6.1.2 Livestock feed

Halama [158] and Phang [159] reported that algae are an important source of nutrients for animals, fish, and humans. After the extraction of oil from the algae, the remaining biomass, which is high in protein, can be utilized for livestock feed, thus reducing the amount of waste generated by algal biomass [30]. Da Silva and Barbosa [160] reported an increase in shrimp growth rates when their feed diets consisted of algae because algae are rich in protein. Algae present in chicken feed reduced the cholesterol level of egg yolk by 10 % and the color of the egg yolk became darker, indicating a high carotenoid content [161]. Schlichting [162] reported that the brown marine algae (*Ascophyllum*) has been used as livestock feed in Great Britain and Ireland.

6.1.3 Biofuel products

Microalgae strains that are selected for biofuel production must yield a high lipid content [58]. Microalgae are capable of producing 20000-80000 L oil per acre per year, making algae oil production 7-31 times greater than palm oil crops, which are the next best producing crops [25]. Various types of algae contain high percentages (by weight) of oils which is suitable for production of biodiesel, while other species possess high sugar content which is suitable for production of bioethanol [25]. Algae appear to offer a viable alternative for biofuel production due to their abundance and cellular structure. The waste generated from the algal biomass can be further utilized to produce biofuels such as methane and ethanol via fermentation or used as animal feed or organic fertilizer [25,27,28].

6.1.4 Biodiesel

Biodiesel is a fuel form that generates the same amount of energy as that generated from petroleum diesel without the release of harsh compounds (NOx, SOx and HC) into the atmosphere, it is biodegradable and nontoxic and is a much cleaner energy source and environmentally friendly [18,25,33,34]. The biggest advantage of biodiesel, compared with other alternative transport fuels, is that it can be utilized in existing diesel engines without modification [14]. Table 14 lists the advantages and disadvantages associated with utilizing algae for biodiesel production.

Biodiesel as a liquid fuel can be produced by transesterification of oil found in algae [23,30]. 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 [28].

Mata et al. [126] reported that microalgae have a much higher oil content than vegetable crops. Table 15.11 lists the oil content achieved by various microalgae species [19].

Sheehan et al. [41] noted that for biodiesel production, the algae strain should be cable of producing high lipid in nutrient sufficient or deficient environments, although these two are mutually exclusive to one another. The cell wall and the amount of polyunsaturated fatty acids present in algae strains must also be considered when they are desired for biodiesel production. The main substrate desired for biodiesel production is saturated and monounsaturated fatty acids that are stored in the organism in the form of TAGs [26,58,55]. The extracted algal oils (TAGs) are converted into fatty acid methyl esters by mixing them with an alcohol (usually methanol) and an acid or a base as the catalyst for the reaction [30].

Biodiesel product form microalgae must meet the international standards which include a concentration of phosphorous and sulfur no more than 10 ppm. Thus, the removal of phospholipids must be achieved before conversion of oil to biodiesel because it promotes water accumulation, increases the consumption of the catalyst during alkaline-catalyzed transesterification and increases the phosphorous content in the oil. The glycolipids are noted to increase the sulfur content of the biofuel [24].

Miao and Wu [56] reported a biodiesel yield of 70% from *Chlorella protothecoides* at 50°C using acid as the catalyst. Li et al. [54] reported 95% biodiesel yield using *Chlorella pyrenoidosa* with rice straw hydrolysate as the lignocellulose-based carbon source. Johnson and Wen [55] achieved a biodiesel conversion efficiency of 95.9 % from the oil extracted (57 %) from *Schizochytrium limacinum* using an extraction-transesterificaiton method. Stephenson et al. [57] reported that *Chlorella vulgaris* are capable of producing 8200 L/ha/year of biodiesel. Rodolfi et al. [58] reported that *Nannochloropsis* can produce 23000-34000 L/ha/year of biodiesel.

Table 14. Advantages and disadvantages of using algae for biodiesel production [14,20,26,27,30,55,58,117]

Advantages	Disadvantages
• No land space competition with other crops	• Open pound culture systems are prone to contamination
• Capable of growing in water with very high salt levels, no need for freshwater use	• Many polyunsaturates make biodiesel unstable
• Require less water than oilseeds	• In comparison with its mainstream alternative, biodiesel has poor performance
• Require less land for growth than terrestrial plants	• Difficult to maintain certain outdoor cultures
• Utilize CO_2 for growth, removing the common industrial pollutant from the atmosphere	• High energy inputs for mixing the culture, CO_2 transfer, harvesting/dewatering
• Rapid growth rate	• Concentration of biomass is low due to poor light penetration
• High intracellular lipid content	• Require more nitrogen fertilizer (8-16 tons/ha/year) than plants which could damage the environment
• High volumes of algal biomass can be achieved yielding higher oil content than other sources	
• Algal oil has limited competition in the market	
• Consume resources that are otherwise considered waste	
• Algal biofuel contains no sulfur, is non-toxic, and highly biodegradable	
• Algal biodiesel consisting of high levels of polyunsaturates is suitable for cold weather	
• Algal fertilizers can be obtained from wastewaters	
• Reduce nitrous oxide release	
• Can be used in diesel engines without much modification	

6.1.5 Biomethane

Anaerobic digestion of algal waste results in the production of methane, carbon dioxide and ammonia. The residues produced in the anaerobic digestion process maybe further used to produce fertilizers [14]. Nitrogen and phosphorous compounds are rich in algal fertilizer [25]. Methane production form algae can be enhanced by pre-treating the cells in order to break down the cell wall order to make organic matter more accessible to microbes [25]. The methane produced from the anaerobic digestion can be converted into electricity or used as a fuel gas [163].

Vergara-Fernandez et al. [164] stated that algae exhibit a good process stability and high conversion efficiency for anaerobic digestion because of the absence of lignin and lower cellulose. The biogas production by the anaerobic process is affected by the organic loading, pH, temperature and the retention time. High methane yields are achieved with long solid retention times and high organic loading rates [165].

Chynoweth [165] reported that the *Macrocyotics* and *Laminaria* sp. have a methane yield of 0.39-0.41 and 0.26-0.28 m^3/kg, respectively. Bird et al. [166] noted a biomethane yield of 0.28-0.4 m^3/kg from *Gracilaria* sp. Morand and Briand [167] reported on the algae L. *Digitata* producing a methane yield of 0.5 m^3/kg. Morand and Briand [167] noted that *Ulva* sp. produced a methane yield of 0.2 m^3 /kg solids.

Table 15. Various microalgae oil content [19]

Microalgae	Oil Content (% Dry Weight)
Botryococcus braunii	25-75
Chlorella sp.	28-32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16-37
Dunaliella primolecta	23
Isochrysis sp.	25-33
Monallanthus salina	>20
Nannochloris sp.	20-35
Nannochloropsis sp.	31-68
Neochloris oleoabundans	35-54
Nitzschia sp.	45-47
Phaeodactylum tricornutum	20-30
Schizochytrium sp.	50-77
Tetraselmis suecica	15-23
B. braunii	25-75

6.1.6 Bioethanol

Ethanol is produced from crops via fermentation, in which the sugars and starch are converted into ethanol [27,28]. Algae have high degrees of carbohydrates and proteins which can be utilized as carbon sources for fermentation [14]. In algal biomass, ethanol production can be achieved by firstly releasing starch from the cells using mechanical equipment or enzymes. The fermentation begins by the addition of yeast (*Saccharomycess cerevisiae*) into the biomass, resulting in ethanol, water and CO_2 as the by-products as follows [14,25,27].

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \qquad (1)$$
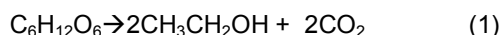
Harun et al. [61] reported a 38 % (by weight) ethanol using the yeast species *Chlorococum* sp. Harun and Danquah [168] reported an ethanol yield of 53 % (by weight) from *Chlorococcum humicola*.

7. 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 [27]. Current microalgae production systems include: open pond (circular and raceway), enclosed photobioreactores (tubular and plate), and hybrid systems.

7.1 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 [25,27]. 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 [28]. Open pond productivity is assessed based on the biomass production per day per unit surface area

available [25]. There are various types of open pond systems which include circular and raceway ponds.

7.1.1 Circular pond

Agitation in circular ponds is provided by the rotation arm as shown in Fig. 5 [25,28]. 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 [169,170]. The productivity of microalgae grown in circular ponds can range from 1.5-16.5 g dry weigh/ m^2 /d [28]. 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$_2$ [28].

Henrikson [171] reported on mass cultivation of Spirulina and Chlorella in Taiwan using circular ponds producing hundreds of tons of algae per year. Borowitzka [172] 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. [173] 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. [41] cultivated Oscillatoria in an open circular pond and achieved a biomass productivity of 15 g/m^2/d. Kanazawa et al. [174] achieved a biomass productivity of 2.43-13.52 g/m^2/d from Scenedesmus sp. grown in a circular pond.

7.1.2 Raceway pond

The raceway pond (Fig. 6) consists of a paddlewheel, propeller or air lift pumps which function to circulate and mix the algae and nutrients around the pond. Agitation and circulation are produced by the paddlewheel which operates continuously in order to bring the algae to the surface of the water and to prevent sedimentation. Shallow ponds are necessary for algal exposure to sunlight due to limited light penetration in the water, which is typically 15-25 cm deep [25,27,28]. Raceway ponds can be made of concrete or more simply earth dug and lined with a plastic liner. The plastic liner prevents the water from penetrating into the ground. In this system the water and nutrients are fed into the pond continuously, while the

water containing the algae is removed from the other end [27].

Blanco et al. [176] reported of lutein rich cells from Muriellopis sp. cultivated in a raceway pond operated with a wheel peddle. Moheimani and Borowitzka [177] achieved a 33 % lipid content and a productivity of 0.19 g/L/day using Pleurochrysis carterae in a raceway pond. Jimenez et al. [178] cultivated Spirulina in raceway pond and obtained a productivity of 10.3 g/m2 per day over the duration of 9 months. Olguin et al. [179] noted a productivity of 9-13 g/m2/d of Spirulina platensis grown in a raceway pond. Moreno et al. [180] achieved a productivity of 9.4-23.5 g/m^2/d for Anabaena sp. grown in a raceway pond. Garcia et al. [52] achieved a biomass productivity of 1.6-3.5 g/m^2/d of Dunaliella salina in raceway pond.

7.2 Enclosed Photobioreacters

Although the cost of enclosed production systems is much higher than open ponds, they require less land area for cultivation. Cultivation of algae in photobioreacters (PBR) does not only function to grow the algae but also to remove nutrients from wastewaters as well as reducing the amount of gases released into the atmosphere by power plants and transportation industry [27]. PBR are closed bioreactors consisting of a light source. An open pond may also be considered as a PBR if it is enclosed within a greenhouse, in which all the required nutrients are introduced into the system for cultivation purposes [27]. Enclosed PBR mediums can achieve high cell densities and are easily maintained because of their enclosed structure [28].

Tubular and plate PBR are the major types. Their advantages over open pond systems include: their narrow light path (1.2-1.3 cm) which allows for more cell concentration, large illuminating area and less contamination. The enclosed PBR disadvantages include: wall growth, fouling, formation of dissolved oxygen and CO$_2$ along the tube, pH gradients, hydrodynamic stress and the high cost [28]. Singh and Gu [14] stated that although enclosed photobioreacters produce higher fuel per hectare as appose to open ponds, the start-up cost is much greater.

7.2.1 Tubular photoreactors

Tubular PBR are made up of several horizontal tubes running parallel to one another (Fig. 7) and

made of transparent glass or plastic [27,28]. 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 [27]. 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 [28]. 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 [180].

Kong et al. [181] achieved a productivity of 2.0 g/L/day of Chlamydomonas reinhardtii grown in a vertical coil reactor. Ugwu et al. [182] reported on Porphyridum cruentum grown in an airlift tubular photobioreactor achieving a productivity of 1.50 g/L/day. Barbosa et al. [183] achieved a biomass productivity of 0.5 g/L/d from the species P. cruententum grown in a vertical tubular reactor. Alias et al. [184] achieved a productivity of 0.25 g/L/d from S. Platensis grown in a horizontal tubular reactor. Lee [185] reported on Chlorella pyrenoidosa grown in an inclined tubular PBR that achieved a biomass density of 2.90 g/L/day.

7.2.2 Plate photobioreactor

Plated PBRs (Fig. 8) are made up of a transparent plastic material. The large surface area allows for more illumination which increases photosynthetic activity [25,27]. Plate PBRs also possess low concentrations of accumulated dissolved oxygen [27]. The plate PBR can be horizontal, vertical or inclined [28].

Ugwu et al. [182] reported on Nannochloropsis sp. achieving a productivity of 0.27 g/L/day when grown in a flat plate reactor. Lee [185] noted that

Spirulina platensis grown in an inclined plate achieved a productivity of 4.30 g/L/day. Zhang et al. [186] noted a biomass productivity of 1 g/L/day using the species Synechocystis aguatillis in an outdoor flat plate photobioreactor. Cuaresma et al. [187] cultivated Chlorella sorokiniana in a flat panel photobioreactor and achieved a productivity of 12.2 g/L/d. Meiser et al. [188] noted a productivity of 1.37 g/L/d for Phaeodactylum tricornutum grown in a flat panel airlift reactor.

7.3 Hybrid Systems

A hybrid system is a combination of both an open pond and a closed bioreactor as shown in Fig. 9 [27,28]. Since open ponds are very proficient but are easily contaminated, a mix ofboth is most likely the most cost effective algae cultivation method [27].

Putt et al. [191] 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. [58] described a two stage hybrid production plant that was dedicated to producing 22 % biomass and 78 % oil production. Huntley and Redalje [132] 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 [192] noted that Haematococcus grown in acombination of closed and open systems for use in astaxanthin production resulted in a productivity of 50 tons/acre. Kizilisoley and Helvacioglu [193] reported a productivity of 0.35 g/L using Spirulina cultivated in a closed pond system.

Fig. 5. Circular algae ponds in Yaeyama, Japan [28]

Fig. 6. Raceway pond, for algal cultivation [175]
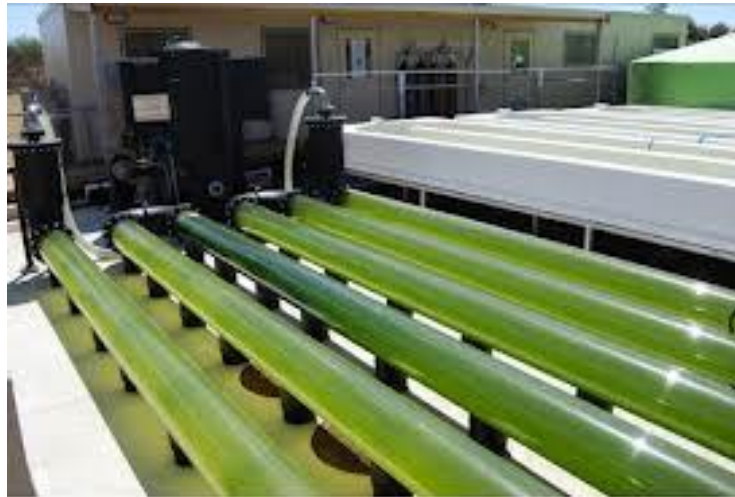
Fig. 7. Tubular photobioreactor [189]

Fig. 8. Plated microalgae photobioreactor [190]

Fig. 9. Hybrid microalgae production system (enclosed pond) [194]

8. 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 [92].

8.1 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.

8.1.1 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, areverse-flow vacuum filter method is utilized to move the liquid upward across the membrane. Separation of large amounts of algal cells from the water by filtration is very energy consuming, thus making it less cost effective for large scale produciton [28].

Tsukalhara and Sawayama [195] used membrane filtration on B. braunii to achieve high cell density. Sawayama et al. [196] filtered Botryococcus braunii through filter paper of 0.2 µm pore size to recover the algal cells grown in treated sewage from domestic wastewaters. Grima et al. [197] noted that microfiltration is cost effective for small volumes of algae (less than 2 m³/d), than centrifugation.

8.1.2 Chemical flocculation

Algal cells possess a negatively charged surface which allows the separation from one another upon suspension. The surface charge of the cells may be disrupted by the addition of iron, alum, lime, cellulose, salts, polyacrylamide polymers, surfactants and chitosan. These chemicals result in cell flocculation and settling. Filtration through this method has been reported to achieve 95% recovery of the algal cells from the culture media [28]. Flocculation of microalgae is much more economically feasible on large scale production, since algae will be concentrated together with less water, making it much easier to retrieve the cells for further processing [198].

Bilanovic and Shelef [199] noted that microalgae flocculation was effective at salinity levels less

than 5 g/L. Sukenik et al. [200] reported on the marine microalgae Isochrysis galbana and Chlorella stigmatophora requiring 5-10 times more flocculent dosages than those required by freshwater microalgae. Morales et al. [201] noted a 100 % flocculation efficiency using chitosan concentration of 40 mg/L for 20 L batch cultures of Chlorella sp. and Thalassiosira nordenskoldii.

8.1.3 Air flotation

Air flotation method for filtration of algal cells is performed by the generation of fine air bubbles. Air is then adhered to the cells, causing them to float to the surface of the column in a form of foam. The foam of cells formed on the surface may be removed or the water may be drained from below the cells. Different types of flotation methods may be performed including air floatation, dissolved air flotation and suspended air flotation. These methods work by generating small air bubbles that adhere to the algal cells and float to the surface [28,202]. Suspended air flotation differs from dissolved air flotation in that the bubbles generated are coated with a surfactant [202]. This method is expensive and impractical on large scale because air compression requires a tremendous amount of energy [28].
McMahon [203] achieved a microalgae harvesting efficiency of 95% using dissolved air floatation method. Elder [204] optimized dissolved air floatation method for algae harvest and noted a capture efficiency of 68-70 %. Wiley et al. [202] reported suspended air flotation and dissolved air flotation capture efficiencies of microalgae of 76.6% and 84.9%, respectively. Boussiba et al. [205] 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. [206] noted that removal of microalgae is more efficient by means of flotation as appose to sedimentation.

8.1.4 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 [28]. Centrifugation is a high energy consumption process which is not economically feasible for large scale production of microalgae. However, Dassey and Theegla [207] noted an 82 % reduction in energy

consumption by increasing the flow rate, thus this method can be made feasible.

Heasman et al. [208] reported a harvesting efficiency of 95-100 % and a cell viability of 88-100 % using centrifugation at 13000 g. Chen et al. [118] reported a 80-90 % microalgae recovery efficiency from the liquid media using centrifugation at 500-1000 g. Grima et al. [198] noted that the preferred method for microalgae biomass recovery is centrifugation, especially producing extended concentrates for shelf-life. Sim et al. [209] 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 [210] compared flotation, filtration and centrifugation for algal removal efficiencies and concluded that centrifugation is the only one of the three that is economically feasible.

8.1.5 Ultrasound wave

In this method, the algal cells agglomerate to the low pressure nodes of ultrasound wavesgenerated 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 [28].

Joen et al. [211] noted that increasing sonication time (10- 60 min) of algal biomass decreased the algal surface area from 75 to 28 %. Bosma et al. [212] reported efficiencies higher than 90 % using ultrasound separation of Mondus subterraneus. Zhang et al. [213] noted that ultrasonic irradiation improved algae settleability, but also change the structure of the algal cell. Yin et al. [214] reported that ultrasonication significantly hydrophilizes algal cells.

8.2 Oil Extraction

There are five common methods for oil extraction: oil press, solvent extraction, supercritical fluids, ultrasound and liquefaction [30]. Table 16 summarizes some of the advantages and limitations of the various methods for oil extraction.

8.2.1 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 [14,30]. 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 [215]. For maximum efficiency when utilizing this method, the algae should first be dried [14]. Despite its high extraction efficiency and simplicity, this method has been noted to be less desirable than other methods because of the long extraction time required [216].

Shah et al. [217] achieved an oil extraction of 115 ml from 500 g of Scenedesmes dimorphus using the expeller method. Topare et al. [218] experimented with filamentous algae obtained from an open pond and obtained an oil extraction efficacy of 75% using the expeller method. Demirbas [219] achieved a microalgae oil extraction efficiency of 70-75% using the oil press method. Popoola and Yangomodou [216] reported an oil extraction efficiency of 75% using the oil press method. Govindarajan et al. [220] achieved a 95 % oil extraction using the combined methods of expeller and solvent.

8.2.2 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 [14].

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 [28]. Despite the simplicity of this method, it is impractical for use on a large commercial scale because these solvents are environmentally destructive and costly [14,27].

Demirbas [219] and Serrato [227] 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 [30] stated that hexane is the most inexpensive chemical known for algal oil extraction. Xiou and Xu [228] 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. [229] 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 [230] reported that the mixture of chloroform-methanol provided the highest extraction efficiency of microalgal lipids (25-27%) from Nannochloropsis.

Li et al. [54] 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 chloroform and reported a 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 solubility of 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.

8.2.3 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 [28]. 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 [14,28,30]. This, results in a product of high purity [28,30]. 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 [14,30].

Canela et al. [231] 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. [232] 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 [219] and Demirbas and Demirbas [30] noted that the supercritical fluid is capable of extracting 100% of oils. Andrich et al. [232] 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. [233] extracted 50 % of the total oil from Crypthecodinium cohnii using supercritical carbon dioxide at 30 MPa and 323 K. Halim et al. [234] extracted 7.1 % of the lipids present in Chlorococcum using supercritical carbon dioxide.

8.2.4 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 [14].

Wiltshire et al. [235] reported a 90 % extraction efficiency of fatty acids and pigments from the species Scenedesmus obliquus using ultrasound extraction. Pernet and Tremblay [236] 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. [20] reported an oil extraction yield of 93 % in adlay seeds using ultrasound assisted supercritical fluid extraction. Araujo et al. [237] achieved a 52.5 % oil extraction from C. vulgaris using ultrasound method. Latheef [238] reported an oil lipid extraction efficiency of 69.53 % from Nannochloropsis oculata using ultrasound-assisted solvent extraction.

8.2.5 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 302ºC and a pressure of 10 MPa [27]. These conditions result in the formation of supercritical water which 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 [25,27,29]. Liquefaction can be done using two methods, direct liquefaction and indirect liquefaction [25].

8.2.5.1 Direct liquefaction method

In this method, rapid pyrolysis takes place resulting in liquid tars and oils [25]. One of the major advantages of algae cell liquefaction is that it does not require the drying of water [28]. Liquefaction is done using hexane in order to obtain the primary oil [27]. 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 [239].

Jena et al. [240] achieved an oil yield of 39.9% using liquefaction of Spirulina plantensis at 350°C in 60 min. Minowa et al. [68] 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. [241] used hydrothermal liquefaction on Chlorella and achieved a bio crude yield of 41.7% (wt) at 350°C in 3 min. Yu et al. [242] obtained an bio-crude oil yield of 39.4 % at 200°C in 120 min using hydrothermal liquefaction of C. pyrenoidosa. Biller and Ross [243] 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.

8.2.5.2 Indirect liquefaction method

This method utilizes catalysts for the conversion of non-condensable, gaseous products of pyrolysis or gasification into liquid products [25]. Chen et al. [28] 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. [244] 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. [245] 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. [246] obtained a 33 % oil yield from Microcystis virdis using liquefaction with sodium bicarbonate catalyst. Aresta et al. [22] 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.

9. TRANSESTERIFICATION

Unlike microemulsion and thermal cracking which are problematic, transesterification has become the preferable method for the production of biodiesel [247-250]. The transesterification reaction occurs when alcohol reacts with triglycerides to give esters and glycerol as a by-product (Fig. 10). The stepwise transesterification reaction is shown in Fig. 11. Short chain alcohol like methanol, ethanol, octanol and other branched alcohols are widely used in the transesterification process [251]. Methyl alcohols and fats are likely to produce fatty acid methyl esters (FAME's) [249]. The alcohol plays the role of both the solvent (it extracts the lipids from the biomass) and the reactant (converts the lipids into FAME) [23,25]. The alcohol in this case is methanol and a base or acid is used as the catalyst to speed up the reaction [23,30]. The transesterification of algae oil is depicted in the following equation [25,55].

Triglycrides + 3 methanol $\xleftarrow{\text{Catalyst}}$

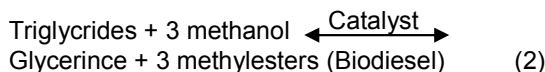Glycerince + 3 methylesters (Biodiesel)   (2)

Fig. 11 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 [252-254]. One fatty acid alkyl ester (FAAE) molecule is produced from each conversion of fats/oils by alcohol [29]. Several catalysts (acids, alkali and enzymes) were used to increase the rate of transesterification reaction for the production of biodiesel [29,255,256]. McNeff et al. [257] 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 [258].

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 [55] 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 solventis required to increase the biodiesel yield, it can still be done in one step (direct transesterification), which decreases the overall reaction time. Patil et al. [259] reported 84 % fatty acid methyl ester content by means of direct transesterification of Nannochloropsis sp. Umdu et al. [260] achieved a biodiesel yield of 97 % in direct transesterification of Nannochloropsis oculata with the aid of CaO/Al_2O_3 catalyst. Johnson and Wen [55] 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 [28]. Johnson and Wen [55] reported on an extraction-transesterification process which resulted in 59.7 % biodiesel yield from the microalgae Schizochytrium limacinum using solvent chloroform. Nagle and Lemke [261] used an extraction-transesterification process and achieved 81 % oil yield from M. minutum using 1-butenol as the solvent. Wahlen et al. [23] extracted 27.3 mg of TAG from Chaetoceros graciis using solvent system chloroform:methanol (2:1) of which 82 % were transesterified. Krohn et al. [262] reported a total lipid extraction of 19 % (total biomass) using hexane solvent and a 1.2 % FAME yield (total biomass).

9.1 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 [29,255]. 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 [252,255].

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. Fig. 12 shows the alkali process of transesterification for biodiesel production [250]. Many free fatty acids and water in the reaction mixture reduce the efficiency of the transesterification process [263,264]. 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 [265]. The major alkali catalysts used commercially are sodium hydroxide (NaOH) and potassium hydroxide (KOH) [249,253,267].

Both acid and alkali catalysts consume energy due to the complexity of the purification process [266]. 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 [267,268].

9.2 Enzymatic Transesterification

The use of enzyme catalysts eliminates the requirement of excess energy by reducing the down stream processing and the problems associated with both alkali and acid catalyst [269,270]. 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 to esterify 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 required and (e) lower molar ratios are required than chemical transesterifcation [264,271,272]. 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 [249,264,273].

Jegannathan et al. [274] stated that enzyme transesterification has proved to be more effective in reducing feedstock limitations and the separation of glycerol from biodiesel. Harding et al. [275] and Fjerbaek et al. [264] 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 [276] reported that the waste water produced with enzyme catalysts are lower in volume and strength than that produced with acid catalysts. Fukuda et al. [264] 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 [247,249]. Table 17 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 from Chlorella prothecoides [277]. Currently 11,000 L of biodiesel have been produced from Chlorella prothecoides [278], however biofuel facilities are being built in Dongying, Shandong which is projected at producing 33 million litres of algae derived transport fuels [279]. The schematic diagram of producing biodiesel using enzymes is shown in Fig. 13.

Table 16. Advantages and limitations associated with various microalgae oil extraction methods

| Extraction method	Advantages	Limitations	Reference
Oil Press	No solvent required Easy to use	Time consuming Large amount of sample required	Mata et al. [126]
Solvent Extraction	Inexpensive solvents Reproducible	Organic solvents are highly flammable or toxic Energy intensive solvent recovery Large volume of solvent required	Herrero et al. [221] Galloway et al. [222]
Supercritical fluid extraction	Non-toxic Non Flammable Simple operation	Often fails in large extractions of polar analyte Insufficient interaction between supercritical CO_2 and the sample	Macias-Sanchez et al. [223] Pawliszyn, [224]
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, [225] Martin [226]
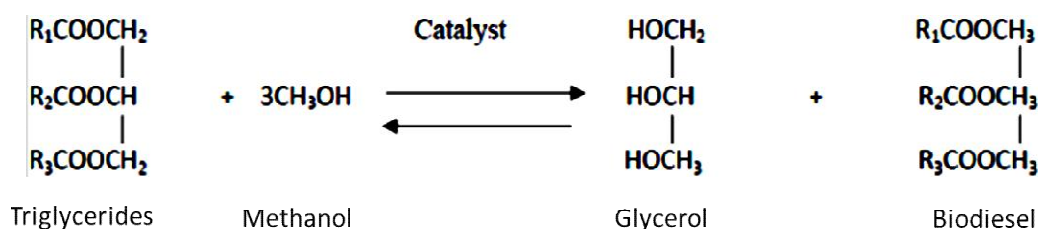
Fig. 10. Overall reaction of the transesterification process [29]
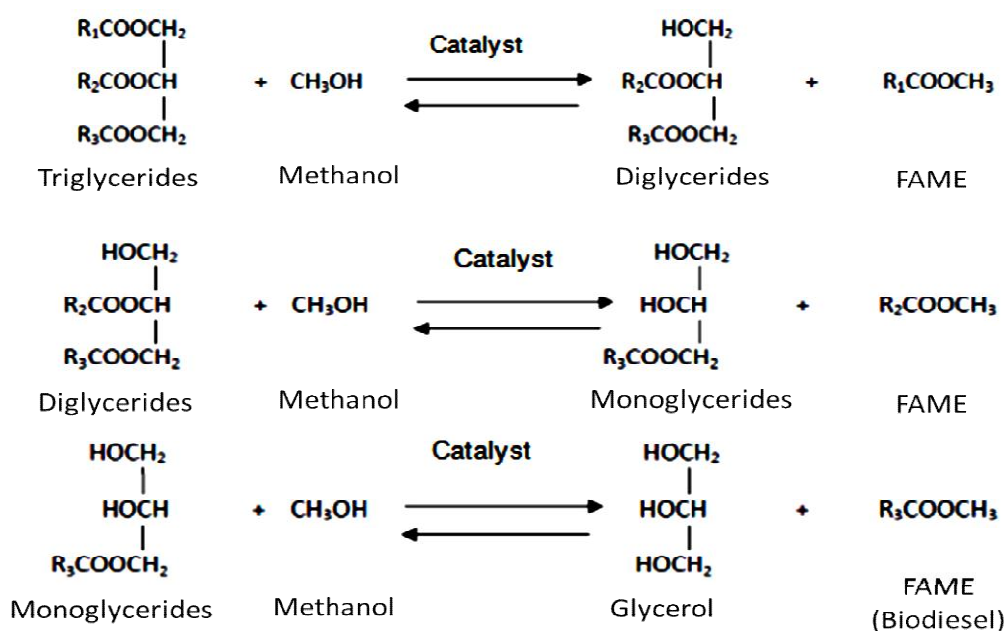
Fig. 11. Stepwise reaction of transesterification [256]

Table 17. Comparison of alkali catalyst and biocatalyst transesterification [251,280]

Major factors	Alkali catalyst transesterification	Biocatalyst transesterification
Temperature	60-80°C	20-60°C
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
Commercialization	100% commercialized	China and Brazil
Waste water generation	Saline and alkaline effluent needs treatment before discharge	No waste water generation
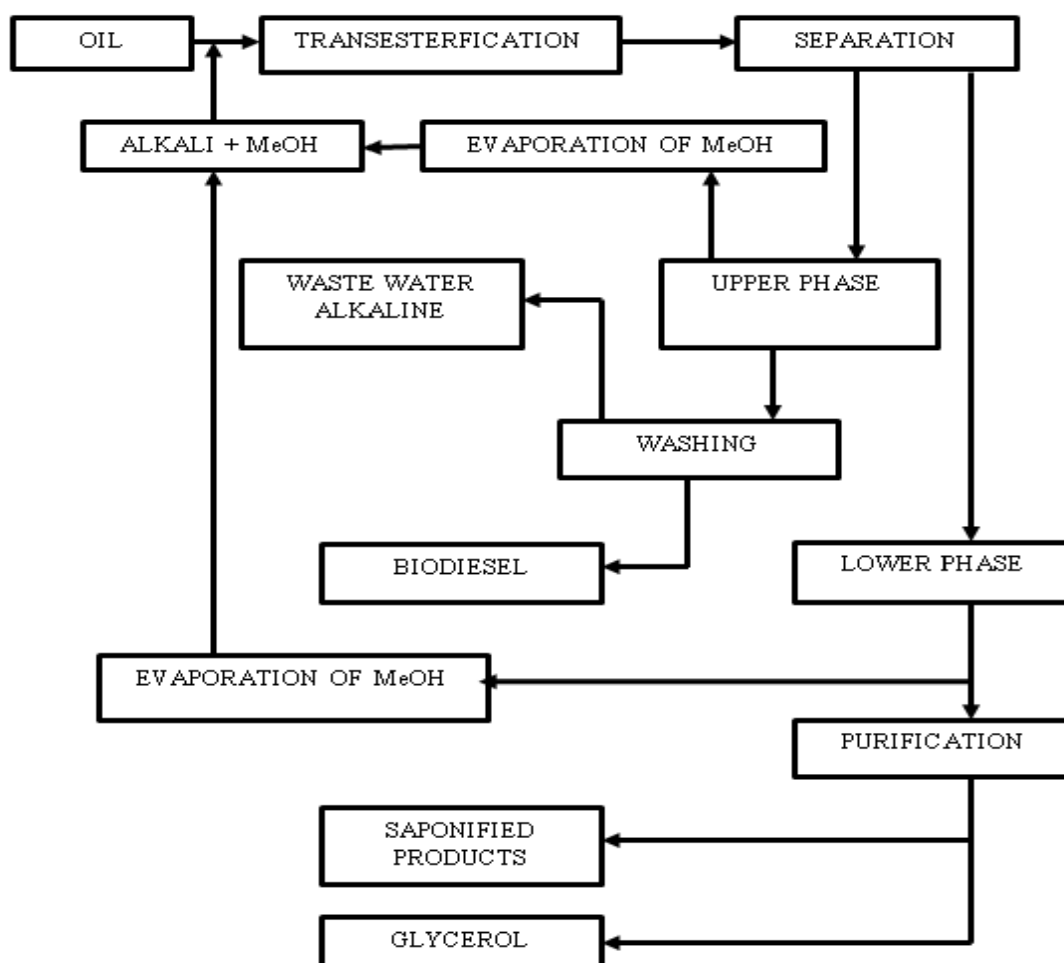
Fig. 12. Alkali process of transesterification of biodiesel production [250]
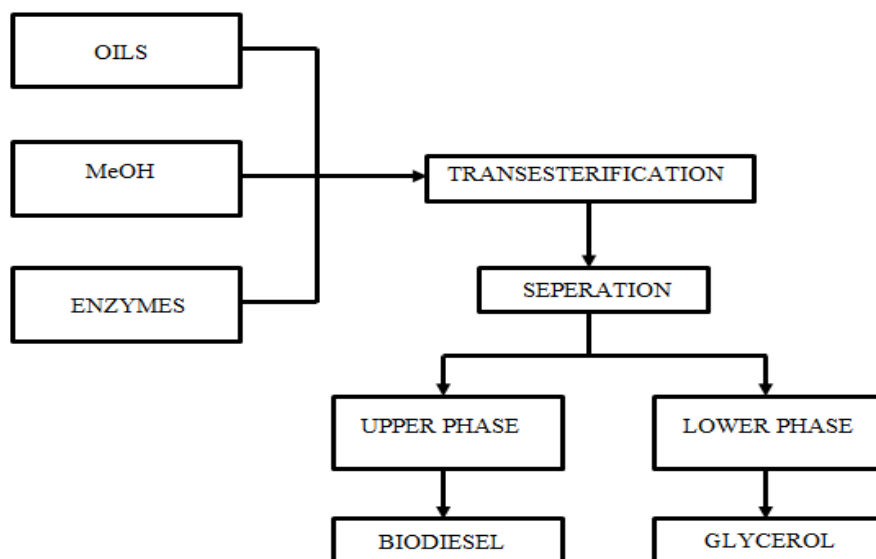
Fig. 13. Enzymatic production of biodiesel [250]

10. 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.

10.1 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 [280,281]. 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 [267,282,283]. Salis et al. [284] 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 [285,286]. The deactivation of enzyme was reported by insoluble methanol present in the oil or fats [284,287]. Glycerol also inhibits the immobilized lipase. Kumari et al. [288] 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 [251,286]. Antczak et al. [267] 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 [289-292] and using a solvent system [293-295]. 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 maintain below 11 [249]. Lee et al. [296] 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 [289].

10.2 Use of Solvents

Solvents are used to lower the inhibition effect of alcohol by increasing its solubility [288].Solvents can also solubilize the by-product glycerol which can prevent the surface coating of the immobilized enzyme and the inhibition effect [297]. Solvent systems provide a homogenous mixture between reactants and products which reduces the inhibition of enzymes and stabilizes the immobilized lipase in the reaction [250,264]. 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 [264]. Vasudevan and Briggs [298] 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 [281,291,293,299-301]. The most stable solvent commonly used is hexane which has a moderate polarity towards enzymes [264,302]. Tert-butanol and 2-butanol are alcohols which can also be used as solvent for regeneration of lipase [249]. Royon et al. [297] 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 [302]. Qin et al. [303] 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 [250,264].

10.3 Alcohol: Substrate Molar Ratio

In the transesterification process, the alcohol: oil molar 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 [267]. 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 [273]. In solvent free methanolysis, the concentration of methanol is inversely proportional to the activity of lipase in the reaction [282,304,305].

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 [249,306]. Matassoli et al. [292] 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 [298,304,307]. The molar ratios of short chain alcohols like methanol to oil must be around 3:1 [267]. In ethanol, the molar ratio of ethanol: oil can reach 11:1 [249,308].

Salis et al. [284] 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 [273] 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 [249,292,306].

10.4 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 [274,309]. It can be expressed as water activity or percentage concentration [277]. The optimum water activity increases the activity of lipase and reduces the hydrolysis in the enzymatic transesterification process even with short chain alcohols [248,274,310]. Optimization of water activity depends on factors such as the reaction system, alcohol type, lipase source, immobilization technique and stability of enzyme [267,274]. 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% [248,264]. 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 [274,311]. Robles-Medina et al. [249] suggests that the water activity leads to flooding the pores which tends to lower the reaction rate. Li et al. [302] 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.

10.5 Reaction Temperature

According to Marchetti et al. [254], lipases are thermally stable within the temperature range of 20ºC - 70ºC. However, the rate of conversion is highly dependent on temperatures outside this range. Antczak et al. [267] states that the optimum temperature of immobilized lipase depends upon stability of lipase, type of solvent and type of alcohol. Jeong and Park [273] 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. [296] showed an optimum reaction temperature of 45°C using combination

of Rhizopus oryzae and *Candida* rugosa with methanol as the alcohol.

11. GLYCEROL USES

Glycerol also known as glycerin or glycerine is produced as a by-product in the conversion of microalgae fatty acids into biodiesel. For every one fatty acid molecule being converted, one glycerol molecule is formed. Glycerol 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 [312,313]. Various chemicals can be obtained from glycerol as feedstock via fermentation including hydrogen, ethanol, dihydroxy-acetone, lactic acid, methanol, 1-2 propanediol and 1-3, propanediol. It can also be used as a carbon and energy source for production of antibiotics such as jadomycin. Biebl et al. [312] 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 [313]. 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 [314]. Table 18 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 [315]. 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 [313], 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 [313,315]. 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 [313].

Table 18. Usage of glycerol in various applications [313]

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

12. CONCLUSION

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. 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. Thus, biofuels produced from algae such as biodiesel are CO_2 neutral. Microalgae generate large amounts of oil in the form of triacylglycerols which can be converted into biodiesel, via chemical or enzymatic transesterificaition processes. 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. 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. Current methods used for harvesting of microalgae cells include membrane filtration, chemical flocculation, air floatation, centrifugation and ultrasound wave. Various oil extraction methods such as oil press, solvent extraction, supercritical fluid, ultrasound and liquefaction are used for oil separation. 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 extracting other value added products such as proteins, vitamins, carotenoids, nucleic acid, carbohydrates and lipids from the algal cells and processing the them 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 processing the algae biomass to produce these value added products in addition to the biodiesel, the economics of biodiesel production can be improved significantly. Improvements in harvesting, pre-treatment, and processing of microalgae and oil extraction techniques are also very important.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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