Application of Microalgae in Biodiesel Production

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ABSTRACT

The constant depletion of fossil fuel reserve and environmental deterioration occasioned by the activities of greenhouse gases emissions from the same fossil fuel are the major challenges confronting the world today. As possible replacement to this unstainable and nonrenewable petroleum-based fuel first and second feedstocks for biofuel generation were formed from food crops and plant seed oils respectively. In addition to somewhat ethical issue as 'food vs fuel' dichotomy associated these feedstocks, they have equally not satisfactorily addressed the problems of climate change and economic development. Therefore, microalgae as third feedstock for biofuel production was developed and this technology has proved to be sustainable and renewable alternative source of energy. These highly efficient photosynthetic microorganisms with extraordinary metabolic apparatus, utilize very cheap materials to form versatile useful products, example lipids for biodiesel production which is the focus of this talk. Microalgae are ubiquitous organisms with fast growth rate and an amazing short harvesting cycle of about 10 days. These remarkable features of these novel cells made them preferred choice for biodiesel production. Three key areas in this review includes: Microalgal strain selection, biomass-bioproducts production process and the process of separation and purification of fatty acids for ultimate biodiesel production.
Keywords: Microalgae, Biodiesel, Lipids, Renewable, Greenhouse gas.

INTRODUCTION

The sustained depletion of fossil fuel reserve and emission of greenhouse gases have placed the world on alert. It was predicted that decades to come most of the world petroleum deposits will dry up because of the pressure exerted by incredible energy consumption rate of the world's population [1], [2]. This high level of greenhouse gas (GHG) emissions which translate to air pollution and global warming is mainly attributed to the large-scale use of fossil fuels for transport, electricity and thermal energy generation [3]. More concerns in Nigeria are the Niger Delta crisis which has led to drastic drop in oil production and the dwindling global oil price. With the global climate change and aforementioned problems in Nigeria and the world at large, it has become increasingly important to develop efficient and effective technology that will reduce the use of fossil fuel. Efforts in past to mitigate the effects of global temperature rise and provide carbon-neural energy sources were unsuccessful until the emergent of this robust greentechnology that provides sustainable alternative source of energy. This novel technology utilizes microalgae among other biomass as substrate for biofuel (biodiesel) production [4]. The first-generation biofuels are the liquid biofuel production based on sugar and starch crops (for ethanol) and oilseed crops (for biodiesel) [5]. But it is restricted due to the negative impact on global food markets like competitive consumption of crops or other food and competitive requirement of arable land [6]; [7]. Thus, the currently developed technology that uses lignocellulosic biomass for biofuel production is referred

to as the second-generation of biofuels, which can avoid the competition and negative impact on food supply. The cost of cellulosic feedstock itself is lower than the first-generation feedstocks, however, the technology for converting cellulosic biomass into liquid fuel is difficult and has not yet reached the scales for commercial exploitation [8]; [9]. Thus, with benefits like fast growth rate, large oil content (up to 75% of dry weight) [10], less farm land occupation and efficient photosynthesis, microalgae have been considered to be an alternative energy resource that can undoubtedly overcome the problems associated with production of first and second generation of biofuels, and the liquid biofuels derived from algae is specifically defined as the third generation of biofuels [11]. Especially when combining microalgae oil production with carbon dioxide and wastewater elimination, there is a great potential that an efficient and economical system can be built for biodiesel production [12].

Microalgae characteristics

Microalgae are microscopic algae that undergo photosynthetic metabolism with aid of sunlight or cool white light to make useful metabolites. Unlike conventional plants, algae do not possess roots, stems and leaves but thalli. Microalgae are diverse with over hundred thousand known species living in both aquatic and terrestrial environments. There are evidences that some species are among the suspensions in the atmosphere qualifying microalgae as ubiquitous organisms with extraordinary metabolic versatility. They are broadly divided into two major groups of prokaryotes and eukaryotes based on their organelle arrangement with the former also known as cyanobacteria. Cyanobacteria are somewhat like bacteria except that they possess distinctive chlorophyll *a* which gives the background color, blue green [13]. These novel organisms are distinguished by their remarkable rapid growth rates, unique and easy to manipulate metabolic pathway and very efficient and effective photosynthetic apparatus. Basically, the immense interest

on microalgae stems from their ability to accumulate variety of important products such as carbohydrates, proteins, lipids, vitamins etc. Microalgae can be a preferred choice substrate for biofuel generation [14].

Microalgae as substrates for biodiesel production which this review focuses on, rely heavily on their active role to produce neutral lipids in form of triglycerides. Biodiesel or fatty acid methyl esters (FAMEs) can be obtained from triacylglycerol via transesterification reaction catalyze by acid or base catalyst [15]; [16].

Lipids Metabolism in Microalgae

The production of biodiesel from microalgae is one of most studied application of algae because of the promise of renewable and sustainable energy source. Microalgal cell factories store energy and carbon inform of triglycerides and hydrocarbons respectively and use them not just for metabolic activities such as biomass multiplication and DNA replication but also as survival tactics during adverse conditions [17]. Complete breakdown of lipids generates more energy for the cell when compared to that of carbohydrates. In addition to triacylglycerols (TAGs), algae also accumulate polar lipids that are component of the organelle and membranes.Photosynthetic microalgae trap and utilize solar energy for biomass production and storage of metabolic products such as carbohydrates and lipids. These important metabolites can be generated from 3-carbon molecules (glyceraldehyde 3-phosphate (GAP) and 3-phosphoglycerate (3PG)) [18].
The synthesis of both triglycerides and carbohydrates strives for carbon through common C3 precursors, resulting in carbon sharing or what is known as carbon partitioning. However, the mechanism behind carbon sharing together with the change from carbohydrate towards the generation of triglycerides has not been fully comprehended, although the glyceraldehyde route in glycolytic pathway is a possibility. When the route towards carbohydrate generation is

blocked, the route towards the synthesis triglycerides molecules is enhanced [19]. If the pathway for carbohydrates accumulation in microalgal strains are somewhat inhibited by mutation, alternative pathway for lipid production will almost always be opened [19] (fig.1).
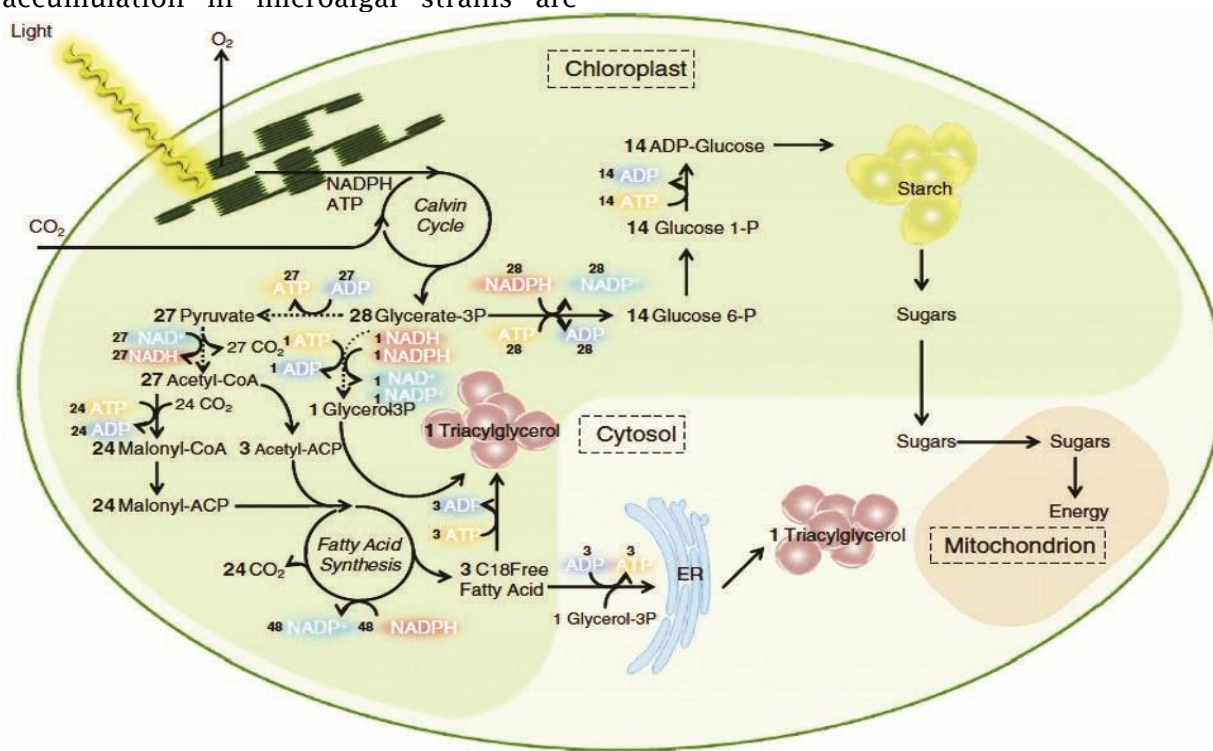

Fig.1: Metabolic pathways in microalgae that influence the storage of triacylglycerol by common C3 precursors [20].

SELECTION OF SUITABLE MICROALGAL STRAINS

The collection of an appropriate microalgal species to be used in the production vessel, is key to obtain the highest lipid yield. Also, strain collection guides the bioreactor setup, culture conditions, harvesting approach, choice of site for algal mass culture and product size [21]. Now, there is nearly 30 000 described microalgal species [22]. Algae that exhibit the high quantity of oil comprise *Schizochytrium*sp*.* which generates between 50 and 77 % oil per dry weight, *Botryococcusbraunii*that stores up to 75 % oil and *Nannochloropsis*sp*.* that accumulates between 31 to 68 % oil [23].

The strains selected as biodiesel precursor should possess rapid growth rates and high intracellular oil [24]. The capacity of the strain to cluster (flocculate) should also be considered when picking an isolate.Harvesting algae from broth is expensive, flocculation would be helpful in the culture vessel,

due to the settling qualities that it would generate consequently assisting in the algal filtration. Many algae have been noticed to clump. Flocculation, notwithstanding, is detrimental in that it forms a three-phase system (gas-liquid-solid) that reduces mass transfer rates and consequences in the obstruction of filters from harvesting [25].

Factors that Affect Lipid Metabolism

In as much as biochemical compositions of microalgae may not be viewed as intrinsic constant as they rely hugely on some environmental factors for good performance, metabolites accumulation in photosynthetic microalgae are however, unarguably species-specific. Lipids metabolism in microalgae can be influenced by the following external cultivation conditions, such as light intensity, temperature, carbon dioxide, nutrient deficiency and salinity stress.

Light intensity

Irradiance plays a critical role in the growth and production of photosynthetic microorganisms. Light energy influences algal biomass yield and biochemical composition [26] It has been revealed that proper exposure to suitable irradiance enhances algal oil storage as a result of the accumulation of much photons which can be transformed into chemical energy [27]; [28]. For instance, it has been observed that when *Nannochloropsis*sp (green microalga) was cultured at 700 µmol s^{-1} m^{-2} over 46% lipids (of dry weight) was produced. Further investigation also showed that *Chlorella* species (*C. sorokiniana*, *C. viscosa*, *C. emersonii, and C. vulgaris*) increased their lipids content with enough light intensity of 600 µmol m^{-2} s^{-1}.*Scenedesmus abundans* also maintained the trend of higher oil accumulation when exposed to the range of 42 to 84µmolm^{-2}s^{-1}irradiance [29] The oil content of 32.77% was stored at 84µmolm^{-2}s^{-1}irradiances while the records were 27.10 and 21.20% lipid content under the culture irradiance of 70 and 42µmolm^{-2}s^{-1} respectively. According to a study, *Botryococcus*sp. grown under the highest light intensity of 84µmolm^{-2}s^{-1} accumulated the highest oil content of 35.9%. Albeit, investigation has revealed *Nannochloropsis. oleoabundans*HK-129 culture under very high irradiance of 207µmolm^{-2}s^{-1} only mustered 33.0% lipid (of dry weight). This is a clear indication that different algal species response different to various levels of irradiance, it could be concluded that thecapacity to utilize light energy is algal-specific [30]; [31].

Temperature

It is another key factor that affects microalgae productivity. Temperature impact the performance of photosynthetic microorganisms by altering their metabolic functions in near similar manner like irradiance. Increase in the yield of biochemical composition (e.g lipids) of microalgae could occur when there is a rise in the temperature of the culture. Metabolites continue to accumulate until the optimal performance temperature is reached beyond which the growth and activities of organisms are negatively affected [32]. Like light intensity, optimal temperatures for microalgal production are species-specific. For example, 25°C is the maximum temperature in which *Chlorella vulgaris* culture synthesizes the highest levels of its lipids below this temperature; the organism demonstrates a decline in lipids storage [33]. Also, the culture of *Scenedesmus* sp. was observed to produce its highest concentration of lipids at 20°C [34]. while *S. obliquus* showed a steady increase of lipid accumulation from 18 to 40% of dry weight at the temperature of 20 to 27.5°C [35].

*Nannochloropsis*oculata climaxes its lipids accumulation at 25°C from 20°C with the lipid values of 14.9 from7.9% respectively [36]. It was shown that *Chlorella minutissima* attains maximum lipid production at the optimum temperature of 20°C.

Although, the above trend could work for total lipid content, however it does not apply for all the classes of lipids. For instance, *Tetraselmis*subcordiformis and *Nannochloropsis*oculata cultures were discovered to respond to increase in temperature by declining neutral lipids and polyunsaturated fatty acids accumulation whereas saturated and monounsaturated fatty acids appreciated. Again, *Chlamydomonas reinhardtii* was seen to have yielded more unsaturated fatty acids at temperature lower than 25°C but drastically reduced total content of stored fatty acids synthesized as fatty acids profiling study revealed [37].

Carbon dioxide

The role of CO_2 in the metabolism of photosynthetic algae cannot be overemphasized. Microalgae utilize CO_2 as an inorganic carbon source to build energy reserve and make versatile products of commercial values. Although a high level of CO_2 can be injurious to metabolic apparatus and algal growth whereas very lower level CO_2 will hinder photosynthetic reactions and growth [38]. Alternatively, algae can take up HCO_3- in place of CO_2 in the absence or very low concentration of CO_2 to meet

photosynthetic needs. The presence of intracellular carbonic anhydrase (enzyme) in diatoms engineer them to utilize HCO_3^- which is later transformed into CO_2 by the protein or uptake HCO_3^- straight via C4 mechanism for carbon sequestration. When algae fix correct amount of carbon dioxide during the period of active photosynthesis, triose is formed which serve as a precursor for the synthesis of macromolecules (carbohydrates, lipids and proteins). By implication, pumping CO_2 into microalgal culture increases the chances of accumulating high concentration of triacylglycerol which is a precursor for biodiesel production [39].

Nitrogen

A significant amount of microalgal dry (about 10%) weight is nitrogen which constitutes an important part of algal proteins. Basically, photosynthetic microalgae have a very lower capacity to synthesize nitrogen storage substance when cultivated in nitrogen-rich culture such as blue-green algae that store pigments (cyanophycin and phycocyanin) rich in nitrogen. [40] Phycobilin proteins are actively consumed in a culture that is nitrogen-deficient thereby forcing the photosynthetic rate to drop in such condition. An alternative pathway to protein biosynthesis is turned on leading to increase lipid or carbohydrates production from active photosynthesis carbon fixation. It has been proved that storage of biodiesel precursor of algal origin is largely favored in a nitrogen-limiting culture across the microalgal class. Accumulation of triacylglycerol become the major target in growth media with insufficient nitrogen sources [41]. Although the trend varies from one species to another species. For instance, under the same condition of nitrogen-limiting while some species of *Chlorella* produce large amount of carbohydrates others were found to accumulate triacylglycerol [42].

Salinity

Microalgal cultures require salinities range between 20 and 30 PSU (practical salinity unity) for optimal growth and productivity. Although, marine algae may tolerate salinity above this range because of the peculiar nature of their habitat.

Studies have demonstrated that agellinated microalgae will flourish in salinities between 25-30 PSU while diatoms maximum performance lies between 20-25 PSU. It is highly recommended that the salinity of microalgal samples is determined before and after the experiment to guarantee to ensure maximum output and above ranges must be viewed as a guide. Monitoring culture salinity is of immense importance especially growing microalgae in open vessels that are prone to evaporation which can lead to increased salinity. The negative effect of salinity surge in the culture can be under mind with the addition of water.

Culture mixing

Effective nutrients distribution, CO_2 uptake, sustenance of suitable pH concentration, salinity and expulsion of oxygen are guaranteed by good culture mixing. Appropriate irradiance, temperature and preventing unnecessary and premature cell flocculation are also possible with continuous culture aeration. It is worthy of note that many species may not tolerate vigorous aeration and must be avoided in other to prevent the loss of culture.

Microalgal Cultivation Systems

After isolation and screening of microalgae strains for lipids accumulation, it is vital to figure out the suitable production system in order to maximize biomass production. A good photobioreactor system is needed to actualize growth and storage of useful algal metabolites at large-scale. Natural ponds, circular ponds, raceway ponds and inclined systems are some of the open ponds that are utilized in the cultivation of microalgae for economic benefits [43]; [44].

Open pond systems

There are different types of open pond culture systems namely; shallow ponds, circular ponds, raceway ponds, inclined (cascades) systems and mixed ponds [45].

a) Shallow ponds (lagoons)
Chlorophytes are the class of microalgae that largely grow in lagoons. Mixing in these types of systems is by natural means such as wind and convection. They are relatively of low depth compared to

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other kinds of open pond culture systems.

b) Raceway ponds

This kind of culture systems is the most widespread in microalgae biotechnology. Raceway ponds are almost always oblong or rectangular in shape which paves way for the easy circulating of culture as shown in fig. 2. They consist of paddlewheel which applied in the mixing of cells in the system [46]. There is also CO_2 inlet and feeding point where nutrients are introduced into the system from. Cells from aged culture can be removed from a harvesting point. Raceway ponds can be constructed in concrete or channels with dug in the ground and may be covered with a plastic liner. These systems are used to produce *Arthrospira* by Earthrise Nutritionals, LLC (California, USA) and Hainan DIC Microalgae (China) and to produce astaxanthin from *Haematococcuspluvialis* by Cyanotech Co. (Hawaii, USA) and Parry Agro Industries Ltd (India) [47] and for commercial *Dunaliella* production [48]
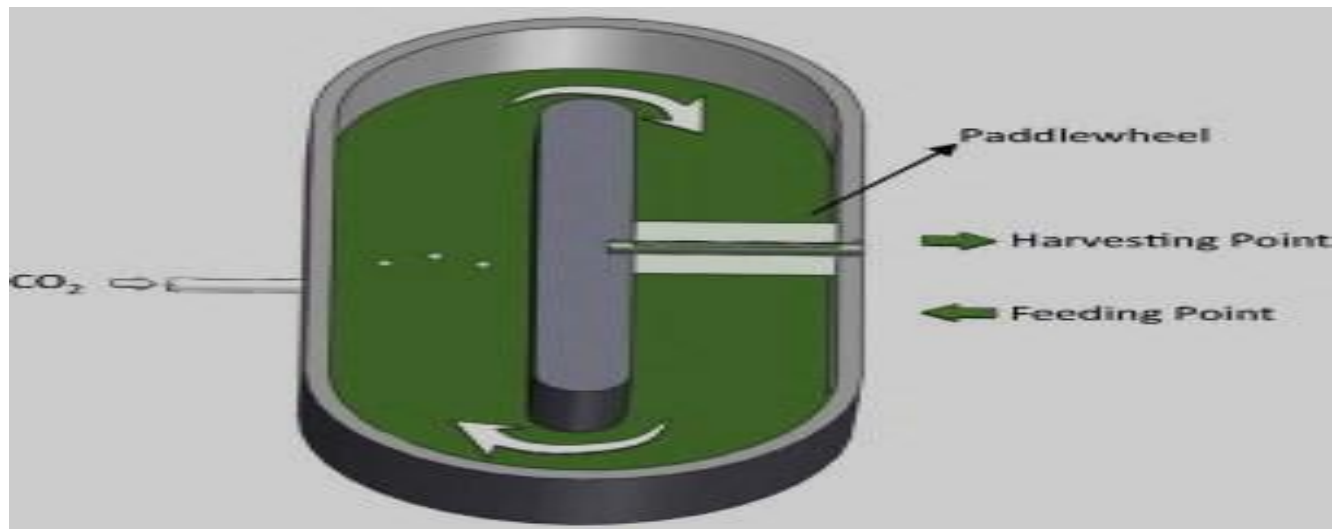
Fig.2: Open Raceway Pond [49]

c) Circular ponds

Fig.3 is circular ponds having agitator at the center are the most commonly used for microalgae cultivation today [50]; [51]. Albeit, the size of the pond is restricted to few tens of thousands meter due to the heterogeneous mixing and challenges associated with the bigger size. Although about 50 m size circular pond has been designed [52].

Fig.3: Circular Open Pond [53].

d) Inclined systems (cascades)
Microalgal culture in these systems are channeled in descending order and collected in a retention tank and again forced uphill [54]; [55]. Effective utilization of light because of its short optical distance of less 10 mL results in high cell density with very easy separation process is an advantage. Inclined systems also do not give room to culture lost due to unfavorable culture conditions [56]; [57].

e) Mixed ponds
Mixed ponds are commonly practiced in aquaculture where microalgae are cultivated as fish feed. The depth of a mixed pond is usually 50 to 80cm and aerated from the bottom of the tank which could lead to low productivity [21]. Microalgae cultivated in mixed pond have the capacity to flourish even in adverse conditions [29]. For example, *Dunaliella, Spirulina* and *Chlorella* strains grow in environments with extremely high salinity, alkalinity and nutrients, respectively grow and yield high biomass.
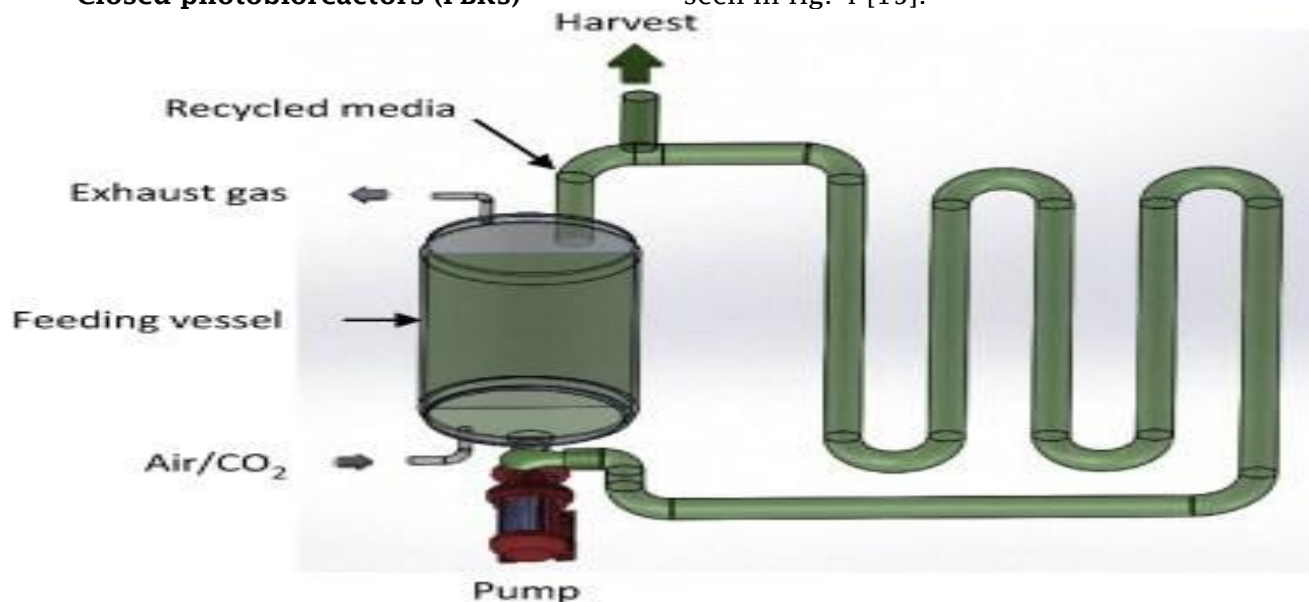
Closed photobioreactors (PBRs)

In closed systems, the light is collected on the wall of culture vessels before transmitting to microalgal culture. In addition, the direct exchange of gases, liquids and particles between the cultures and atmosphere is restricted [11]. Closed culture systems can be in tubular form, plates or bags made of plastics, glass or other forms that can easily allow the passage of light from a source [17]. Basically, two major types of closed cultivation systems exist; flat plate and tubular photobioreactors [19].

a) Tubular photobioreactors
These forms of bioreactors are the most common designed, available and the preferred ones for large scale microalgal cultivation and they are mainly made of either glass or plastic tubes in which the culture is aerated with pumps or by means of airlift systems. Horizontal straight tubes linked by U-bends type of photobioreactors-coiled tubular, a cross tubular form, vertical and inclined arrangement-forms of photobioreactors are being designed for consideration as seen in fig. 4 [15].

Fig.4: Tubular Photobioreactor With Parallel Tube [9]

b) Flat panel (plate) photobioreactors
Flat photobioreactors have been employed in various microalgal production. For instance, a flat-cuvette bioreactor (Fig. 8) that can basically control culture conditions with real-time monitoring by a build-in fluorometer and

densitometer and tested its performance with nitrogen fixing blue-green algae *Cyanothece*sp.*Pleurochrysiscarterae* has been successfully cultivated in a uniquely constructed flat reactor with the V-shape of the base of the bioreactor [32]; [36]. Flat plate photobioreactors were also used

for growing some Cyanobacterium *Spirulina platensis*, *Isochrysis*sp., *Nannochloropsis*sp., *Porphyridium*sp. and *Chaetocerosmuelleri*var. *Subsalsum* [38]. Closed photobioreactors have shown

more successful microalgae productivity than open systems for obvious reasons. Albeit, they aremore expensive to operate than open ponds.
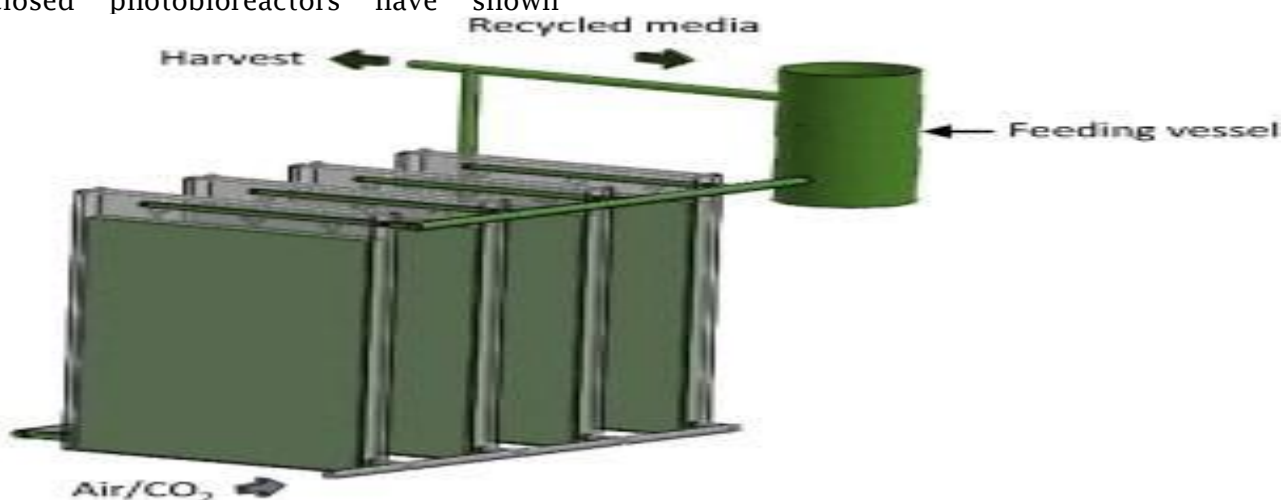

Fig. 5: Flat-plate Photobioreactor [37]

Biodiesel

Lipid accumulation proficiencies of microalgae and reported promising efficiency of lipids from microalgae perhaps are reasonably higher and better than in another biodiesel feedstock [13]. Today, the initial step in research is to find microalgal strains and optimize their growth conditions as well as manipulate them to yield highest oil productivities [12]; [13]. The biosynthesis and storage of a large amount of triacylglycerols (TAG) together with substantial modifications in lipid and fatty acid profile, happens under stress induced by nitrogen limitations and other culture conditions [40]; [41]; [42]; [43]; [44]; [45]. Microalgae species such as *Chlorella protothecoides*, *Chlamydomonas reinhartii*, *Nannochloropsis*sp.*, Scenedesmus obliquus*, *Nitschia* sp., *Schzochytrium* sp., *Chlorella protothecoides*and *Dunaliellatertiolecta* were isolated in order to pick the highest

oil producers in relations to amount (cell yield and oil accumulation) and superior (fatty acid profile) as a lipid precursor for biodiesel production [8]; [9]; [10]; [11]; [12]; [13].

Generally, microalgal cell fatty acid content largely consist of a mixture of saturated fatty acids, such as palmitic (16:0) and stearic (18:0) and unsaturated fatty acids, such as palmitoleic (16:1), oleic (18:1), linoleic (18:2) and linolenic (18:3) acid [8].

Fatty acid content can also vary both in amount and value with their functionality and growth conditions [23]. In addition to growth factors,the stage of growth of the culture also influences the TAG content and fatty acid profile [5]. The aging of culture also enhances oil content of algal cells, with an outstanding increase in the saturated and mono-unsaturated fatty acids, and a decrease in polyunsaturated fatty acids (PUFAs) [1].

Biodiesel production

Biodiesel can be defined as the mono-alkyl esters of plant oils or animal fats per the American Biodiesel Standard ASTM D6751. The American Standard and the European Standard EN 14214 are used as benchmark for the production criteria anywhere across the globe. Biodiesel is

used as transportation and heating oil. Different principle (EN 14213 in Europe) when employing biodiesel as heating oil has been established. The American Standards ASTM D975 (diesel oils) ASTM D396 (heating oils) now cover blends

about 5% biodiesel meeting the stipulations of ASTM D6751 [17]

Biodiesel is strictly competitive with fossil diesel. Benefits of biodiesel include that it is produced from a renewable, local store, blends with fossil diesel at all blend points, positive energy balance, less or exhaust emission, biodegradability, less or no sulfur and aromatics composition, increased flash point, and inherent lubricity.The last property being of special interest in connection with modern ultra-low sulfur diesel fuels which possess poor lubricity. The key disadvantage of biodiesel is attributed to its poor cold flow and oxidative stability [29].

Transesterification reaction

The most effective and common way for transesterification is base catalysis in which lipids are converted to fatty acid methyl esters using alcohol as shown in Fig.6.
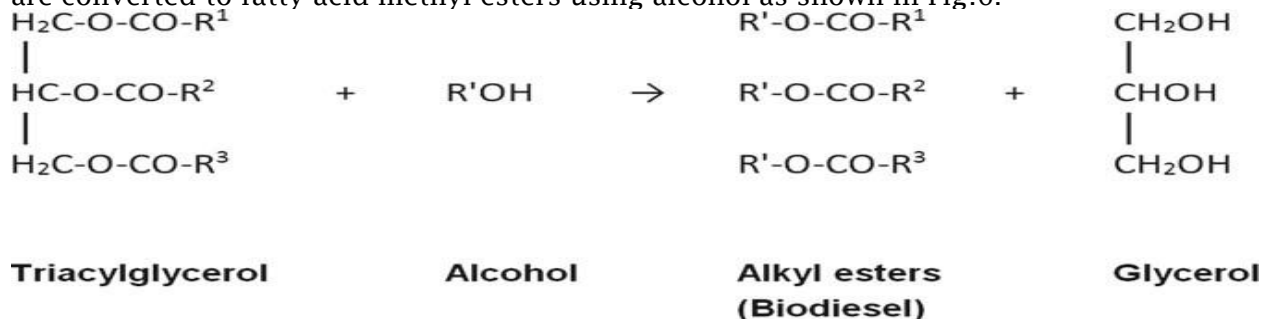

Fig. 6: The Transesterification Reaction.

The feedstock, be it a vegetable oil, animal fat or algal oil, is represented by the triacylglycerol (triglyceride; R^1, R^2 and R^3 may or may not be identical). The most commonly used alcohol is methanol ($R' = CH_3$) [24].

Generally, base catalysis is relatively faster than acid catalysis. Sodium or potassium hydroxide are usually utilized as catalysts. First benefit of base-catalyzed transesterification is the warm reaction situations, which for the generation of methyl esters characteristically are 60s at 65 °C and ambient pressure, 2 % catalyst and a molar ratio of 6 moles of methanol (alcohol) to 1mole of oil. Transesterification reaction mixture has two phases at the beginning (methanol and oil) and two phases at the end of the reaction (methyl esters, glycerol). The methyl ester phase at the completion of the reaction is quickly removed from the weightier glycerol phase. The methyl ester (biodiesel) is normally washed with water. The very high result is usually achieved from this method of transesterification reaction, above 96 % [51]; [52].

Characteristics of Biodiesel
a) Cetane number

The cetane number is a numerical label of the ignition quality of a diesel oil theoretically comparable to the octane number utilized in gasoline. It is associated with the ignition delay of the fuel observed upon injection into the combustion space of a diesel engine. Less time to the ignition delay, the higher the cetane number and vice versa. Higher value cetane numbers are usually linked with better ignition and burning features. The superior standard compound on the cetane scale is hexadecane (trivial name cetane, giving the cetane scale its name) with an allotted cetane number of 100. The inferior reference compound on the cetane scale is 2,2,4,4,6,8,8-heptamethylnonane with an allotted cetane number of 15, which means that branching in the hydrocarbon chain length diminishes the cetane number. Aromatics hydrocarbons also incline to hold low cetane numbers, thereforeincreasing length of an unbranched alkyl side chain increases the cetane number. Generally, straight-chain alkanes hold the utmost cetane numbers and brand the "ideal" constituents of a petrodiesel oil [56].

The cetane number of biodiesel oil is unarguably influenced by the compound structure. Usually, the cetane number (CN) increases with increasing chain length and reducing unsaturation. For instance, methyl palmitate and methyl stearate have high cetane numbers near hexadecane (100) while other long-chain alkanes have perfect CN (CN of methyl stearate almost 100, that of methyl palmitate almost 85, that of methyl laurate around 65) [45]. One double bond lowers CN significantly, accordingly the CN of methyl oleate is between 55 to 59.

The cetane numbers of the increased unsaturated esters methyl linoleate and methyl linolenate are even lower, between 38 to 40 and 22 to 25 respectively. The cetane number of a mixture such as biodiesel is associated to the cetane numbers of the different components that make up the mixture. Thus, most biodiesel fuels possess cetane numbers in the range of high 40 s to lower 60 s. The stipulations for smallest cetane number of biodiesels are 47 (ASTM D6751) and 51 (EN 14214). Basically, higher cetane numbers are more attractive [45].

b) Kinematic viscosity

The high thickness of vegetable oils, which is more gelatinous than petrodiesel, is the key aim of lipids transesterification to biodiesel. The high viscosity of vegetable oils, influencing permeation and atomization of the fuel in the burning compartment, results in operational challenge such as engine deposits. Biodiesel fuels enjoy viscosity values nearer to those of petrodiesel, which often display kinematic viscosity values between 2.0–3.0 mm^2 s^{-1}. Nevertheless, the range of kinematic viscosity in biodiesel criteria ranges outside this typical range witnessed for biodiesel fuels as most biodiesel fuels exhibit kinematic viscosity between 4.0–5.0 mm^2 s^{-1} at 40 °C. Careful observation, however, shows that the kinematic viscosity range recommended in the European biodiesel standard EN 14214 is tight and there seems to be no practical validation for the high lower limit (3.5

mm^2 s^{-1}) which is above the kinematic viscosity of most petrodiesel fuels. There seems to be no practical reason why the kinematic viscosity could not be the same for biodiesel and petrodiesel. The compound structure also meaningfully impacts viscosity. Viscosity rises with chain length and declining *cis* unsaturation. Conversely, compounds with *trans* double bonds exhibit higher kinematic viscosity than *cis* isomers. For example, the kinematic viscosity of methyl elaidate, the *trans* isomer of methyl oleate, is 5.86 mm^2 s^{-1}, almost identical to that of the corresponding saturated C18 compound, methyl stearate [7]. Hence the kinematic viscosity of a biodiesel fuel rest on its fatty acid outline with the individual constituents affecting viscosity roughly relative to their separate quantities. Kinematic viscosity is also strongly temperature dependent, rising significantly at lower temperatures [5].

c) Oxidative stability

Unsaturated fatty acid chains, particularly the polyunsaturated species (*bis*-allylic CH_2 position) that is, esters of linoleic and linolenic acids are prone to oxidation. It was noted that relative rates of oxidation are 1 for oleates (C18:1), 41 for linoleates (C18:2), 98 for linolenates (C18:3) and 195 for C20:4. Relative rate of oxidation for linoleate(C18:2) was set as 1, 2.1 for C18:3, 2.9 for (C20:4; arachidonate) and 5.1 for (C22:6; DHA) [20]. Therefore, other polyunsaturated fatty acid chains, such as octadecatetraenoate (C18:4; also known as stearidonate), eicosapentenoate (C20:5, EPA), and docosapentaenoate (C22:5) will

also be very susceptible to oxidation. Fatty acid chains oxidation is a multifaceted reaction, involving primarily the production of hydroperoxides followed by secondary reactions, during which yields such as acids, aldehydes, ketones, hydrocarbons and other compounds can be formed [27].

Oxidative strength is put in biodiesel standards mainly by the conforming order which recommends the use of a Rancimat device. This device allows an enhanced test to be directed with the goal of determining the oxidative stability of a sample. The decrease in induction time by

this method leads to the less oxidatively stable the sample. Smallest induction times approved in biodiesel standards by this assessment are three hours (ASTM D6751) and six hours (EN 14214) [30].

d) Cold flow

Poor low-temperature flow properties put together by relatively high cloud points (CP) and pour points (PP) is one of the main challenges associated with the utilization of biodiesel. The CP, which customarily happens at an increased temperature than the PP, is the temperature at which a fatty compound turns gloomy due to the production of crystals and solidification of saturates. Solids and crystals quickly grow and massed, obstructing fuel lines and filters and triggering key operability challenges. As temperature reduces, leading to increased solid formation and the material nears the pour point, the lowest temperature at which it will still flow. Besides CP and PP, other test approaches for the low-temperature flow properties of orthodox diesel fuels, specifically the low-temperature flow test (LTFT; used in North America) and cold filter plugging point (CFPP).

Saturated fatty materials have meaningfully higher melting points than unsaturated fatty mixes and within a homologous succession the melting points mostly increase with increased chain length. Therefore, in a blend the saturated esters form at a higher temperature than the unsaturated ones. The nature and quantity of saturated fatty mixtures is a decisive element of the cold flow properties of biodiesel. For example, the cloud point of the methyl esters of soybean oil is about 0 $^{\circ}C$ (with roughly 15 % saturated fatty acids, about two-thirds thereof C16:0, the rest largely C18:0) and that of the methyl esters of palm oil is about(40 % C16:0 and other saturated fatty acids). Negligible constituents of biodiesel can expressively affect low-temperature properties. Examples are monoacylglycerols (monoglycerides, specifically of those of saturated fatty acids) and sterol glucosides [36].

e) Iodine value

Iodine value (IV) estimates unsaturated fatty acid by measuring the quantity of iodine (grams) that can be added into 100 g of biodiesel [37]. The needed limit is 120 g I_2 100 g^{-1}(EN 14214). Most biodiesel from microalgae shows IV as the lowest limit reported; however, *Kirchneriellalunares* and *Isochrysisspharica* show a high IV due to the presence of unsaturated fatty acid (USFA). For example,*K. lunares* contain a high value of docosahexaenoic acid (C22:6), and *Lyngbyakuetzingii* contain DHA, EPA, and linolenic acid. Microalgal lipids overall,tend to contain higher unsaturated fatty acids than oilseeds, however, the IV is similar to vegetable oils. Thus, this biodiesel property is not a restrictive parameter for utilizing microalgal biomass as substrates for biodiesel generation [8].

Processing of Microalgae Lipid
Flocculation

The small size (0.5-30 μm) of microalgae make their separation from cultures very difficult. Cells in the aged culture tend to sediment at the bottom of culture vessels. Microalgae that were formally distributed around the photoactive zone when nutrients have not depleted, beginning to clog at bottom of vessels as soon as nutrients were used up. Chemicals known as flocculants could be employed to aid in cell sedimentation for easy harvest.

Different forms of flocculants include alum, lime, cellulose, salts, polyacrylamide polymers, surfactants and chitosan and *Moringaoleifera* extract are now emerging. These chemicals when added in the culture force down microalgal cells to settle at the bottom of the vessels making flocculation possible [33]. There could be autoflocculation, bioflocculation (aid by other organisms) and lastly electroflocculation.

Filtration

Filtration is the method of removing water from microalgal culture. It is one of the oldest techniques in downstream processing which depend on the size of algal strain. The problem associated with this technique as regard to biofuel generation is that most oil storage algal strains are small in size that they can easily pass through the pore size of most filters. The following must be considered in filter design. The filter pore size must be such that will allow only the passage of liquid while algal cells are collected on the surface. The efficiency of the process can be enhanced if cells do not clog the pores. The force of adhesion between microalgae and filter materials must not be such that will hinder the release of cells during collection. The filter must be designed with materials that will not react with the product during the extraction procedure.

Centrifugation

Centrifugation remains one of the commonly used microalgal harvesting technique in large scale production. The effectiveness of centrifugation relies heavily on the characteristics of the algal strain in terms of their size. This method must reflect on the cost per capita and be able to treat a large volume of algal culture

Drying

As soon as microalgal cells are concentrated, the next step should be to remove moisture content to avoid decomposition that may affect the chemical composition. Cell content is preserved for long-term use by drying and dehydration. Different forms of drying are sun drying, freeze dryers, low-pressure shelf and use of rotating drums. Although sun drying is unarguably the cheapest, however, it has a lot of disadvantages that makes it inefficient [8]. Drying plays a significant role in effective lipid extraction since moisture content if allow interferes with the process and thereby reducing the overall biofuel production [9].

Lipids Extraction Methods

An extraction process must be able to extract triacylglycerol from the biomass without simultaneously extracting polar lipids (phospholipids and glycolipids). The following methods are frequently used for microalgal oil extraction.

Mechanical disruption

Mechanically disrupting microalgal cells causes their walls to break and extraction of the intracellular substance happens [4]. Disruption approaches reduce further chemical contamination that would otherwise be produced from solvents while maintaining the quality of substances contained in the biomass. Frequently used methods include bead milling, homogenisation, and mechanical pressing [5]. Bead milling takes place as a result of the agitation of small glass beads inside a vessel being rotated at high speeds. As a result, disruption occurs as the biomass is swiftly stirred, causing cellular damage thereby releasing the cellular content [31]. Conversely, homogenisation forces biomass through an orifice and produces a rapid pressure change and high shear stress. The other method is the mechanical pressing. This process extracts oil by crushing the cell walls using a press. The impact of disruption caused on the cells is affected by the size, strength and shape of the microalgal cells [30].

Supercritical fluid extraction

Supercritical fluid extraction (SFE) is a technique that occurs with a fluid in a state like both a liquid and a gas as the temperature and pressure increase above the critical point [45]. The fluid's properties are between both states of matter with the viscosity being like that of a gas and the density like to that of liquid. This situation improves the solvating power and rises the diffusivity to produce faster extraction yields and separation. The most commonly studied solvent is carbon dioxide (CO_2), given its moderately low critical temperature (31.1 °C) and pressure (72.9 atm). Added

advantages of using CO_2 includes reaction inertness, less harmful, cheapness, readiness and its ability to be handled in large amounts [44]. Supercritical CO_2 has a low polarity; as a result, it is less efficient in extracting compounds with moderate to high polarity. To increase solvent polarity and thus lipid extraction, modifiers (co-solvents) such as ethanol are used in combination with CO_2. One possible drawback is the presence of moisture in the cell that acts as an additional layer over the cells and decreases the diffusion effectiveness of CO_2 [50].

Solvent extraction

Solvent extraction uses precise chemicals to release the lipids and separate them from the crude biomass. Possible solvents include benzene, ethanol, hexane or ethanol-hexane mixtures [12]. The most widely used solvent however, is hexane, due to its lower cost availability and low poisonousness, density and boiling point [40]; [41]; [42]. Solvent extraction of oil from algae cell is a process that transfers crude lipids from either a solid or a liquid phase to a second, immiscible phase. Extracted oils are dissolved in the solvent and form a solution separate to the cell debris [43]. This is due to high solubility of oil in the organic solvents used. Extraction effectiveness are improved when the solvent can penetrate algal cells, hence polarity similar to that of the crude oils being extracted. Non-polar solvents typically extract non-polar lipids whereas polar lipids are characteristically . extracted by polar solvents. For the hexane extraction, dried powder and wet paste derived from microalgae cell were used as feedstock. It was noted that the hexane extraction using wet algal paste produced a 33% lower yield than dried powder. The combination of n-hexane and isopropanol produced a three-fold increase in lipid yield when compared to hexane extraction from dried cell. This was because algae cell walls can prevent direct contact between non-polar solvents and their cell membrane from reducing the effectiveness of lipid extraction. The use of alcohol can disrupt the membrane-based lipid-protein interactions by forming hydrogen bonds with the polar lipids [14]. This permits hexane to extract more of lipids, and therefore, hexane: alcohol extraction is seen as the most suitable method for large quantity production

Thermal liquefaction

Thermal liquefaction can occur with microalgae biomass with high moisture content, thereby reducing the need for drying of the biomass. After harvesting, liquefaction of the microalgae cells in sub-critical conditions, converts wet biomass to bio-crude oil. The main benefits of liquefaction are that many different products can be produced and that the drying of the biomass is not required. The downsides of this method are the high energy cost of this process, which may make this an expensive technology, for a process aimed solely at biodiesel production. The preparation of extra products could help to improve process economics.

Ultrasonic-assisted extraction

This is the process of applying sound energy to stir the sample and disrupt the cell membranes of the algae biomass, causing them to release their cellular contents. The release of these cellular contents is enhanced using solvents. Typically, in ultrasonic-assisted extraction, a centrifuge is used to separate the residual algae biomass from the solvent and extracted lipid at the end of the process [10].

The main benefits of using the ultrasonic extraction process are the ability to increase the yield of algae oil and reduce the duration of the extraction process with moderate or low cost [10].

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