

## Exploring the potentials of microalgae as an alternative source of renewable energy

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### Abstract

The world's energy needs highly depend on fossil fuels, which were formed over several million centuries. The price of petroleum increases daily and unfortunately, its exploitation is currently at an alarming rate for such essential non-renewable energy. Also, the recent clamour for safe and cheap alternative means of energy generation to mitigate global warming and its detrimental effects is drawing attention towards biofuel production to supplement and possibly, substitute fossil fuels. To this effect, many plant materials have been tested and employed in the past decades for biofuel production. However, a good number of plants used in biofuel production as feedstock are crop plants, which have more economic value as food. Therefore, it is imperative to explore the possibility of biofuel production from non-food sources, hence, we examine the potential of microalgae as an alternative source of renewable energy. Microalgae are of great interest in biofuel production for its high productivity, cosmopolitan nature, easy culturing on waters and land, and noncompeting with conventional agriculture for resources. In view of these, this article focuses on the potentials of microalgae in biofuel production and mitigation of environmental pollution by its considerably low greenhouse gas emissions.

**Keywords:** Biofuel, bioethanol, clean energy, greenhouse emission, microalgae, hydrogen

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### Introduction

Macroalgae and microalgae are distinguishable, the two groups differ considerably in their morphology. Macroalgae are multi-cellular and large (reaching several inches) most often found in ponds with a plant-like morphology (Patel et al., 2016). A good example of macroalgae is the seaweed, which is the largest yet known. A seaweed called the giant kelp can grow well beyond 100 feet in length. In contrast, microalgae are tiny (only a few micrometers with average cell diameter between 5-10  $\mu\text{m}$ ), unicellular algae often found suspended in water bodies. They are known to

be the most primitive plant species occurring singly or in colonies, usually as not specialized cells. There are several thousands of microalgae species, but only about half are yet discovered and studied (Li et al., 2008a).

Microalgae are autotrophic organisms, their photosynthesis process is similar to higher plants, but their simple cellular structure makes them more efficient solar energy converters. Microalgae release a high amount of oxygen to the atmosphere and help considerably in the removal of carbon dioxide and nitric oxides, which they harvest for their metabolic processes; thus, they are indispensable in maintaining a

healthy ecosystem (Peters et al., 2010). Microalgae are highly prolific, during the exponential growth period, they can double in a short period, usually within 24 hours (Metting, 1996). Chisti (2007) reported some microalgae, which double every three and a half hours at their peak growth period. In general, microalgae contain large amounts of lipids which make them important candidates for biofuel feedstock. The oil content of microalgae ranges between 20-50% (dry weight), some strains are found to have about 80% (Metting 1996; Spolaore et al., 2006; Sushchik et al., 2009; La Russa et al., 2012; Su et al., 2012). In relation to terrestrial oilseed crops, microalgae produce more quantity of oil per land area covered (Chisti, 2007). They are well adapted to grow in aqueous suspension due to their efficient water, CO<sub>2</sub> and nutrients utilization. Biomass generated by microalgae is homogenous, and lacks a vascular system making it cheaper to process; they are better suited for biofuel production (Seehan et al., 1998).

The discovery of biofuel and other renewable energy sources are fostered by the rapid depletion of oil reserves, increasing global concern on climate change, and greenhouse gases emission. Thus, there is a need for an eco-friendly, economically rational and renewable means of energy production; a gap biofuel promises to fill adequately. In this regard, microalgae are potential economical feedstock in biofuel production and other important products (Meng & Bentley, 2008; Peter et al., 2010). The advantages of integrated micro-algal production systems are numerous; it provides a viable substitute to the much-debated first-generation biofuel systems, however, the predicted consequences may be beyond reality (Borowitzka, 1999, Ugwu et al., 2008).

#### *Definition, Classification and Characterization of Biofuels*

Plants can convert energy trapped from sunlight into chemical energy through photosynthesis, the energy thus generated is stored as carbohydrates, oils, proteins, etc., this residual energy in plants is harvested as biomass and convertible to biofuels (Peter et al., 2010). Therefore, biofuels are said to be a secondary form of energy obtained from a biomaterial. Biofuels simply refer to fuels derived

from biomass such as plants, animals, microorganisms or other organic matters. For biofuels to be produced at a sufficient quantity to serve as an alternative or to replace fossil fuels, feedstock needs to be made adequately available (Meng & Bentley, 2008). Several crops and plant materials such as cereals, oil plants, sugar cane molasses, agricultural and forest residual waste, as well as the household wastes may be sources of raw materials for biofuel production. Owing to their large biomass, sugar beet and cereals have been the major feedstock for biodiesel production in the USA and Brazil (U.S EIA, 2020).

Biofuels can be solid, liquid or gaseous in nature. Solid biofuels include; hay, sawdust, straw (pellets), some tree species e.g basket willow, *Sida hermaphrodita*, certain shrubs and herbs (Patel et al., 2016). Liquid biofuels are produced by fermenting carbohydrates to ethanol, biomass to butyl alcohol, or esterifying vegetable oils e.g., soya, castor, rapeseed and *Jatropha* oils to produce biodiesel (Sushchik et al., 2009). Gaseous biofuels or simply biogases are produced from the anaerobic fermentation of wastes generated from livestock farming/animal production e.g farmyard manure (Demirbas, 2007). Biogas could also be a product of biomass gasification (such as wood gasification), a process through which gas (wood distillation gas) is produced (Demibras, 2009). Biofuels can also be grouped into different generations based on the nature of feedstock used for its production, namely; the first, second and third-generation biofuels (<http://biofuel.org.uk/types-of-biofuels.html>).

The first-generation biofuel came from earlier discoveries using primitive methods with little energy inputs; they are produced from organic materials, which could equally be used for food or fodder. The use of food materials as feedstock for the production of biofuel is a major concern, raw materials such as maize, sugar cane, wheat, sorghum, millets or sugar beet, which are useful as human food or animal feed are used. If more of these food materials are directed into biofuel production, this may result in food scarcity and a corresponding increase in food prices. This poses a challenge to global food security (Somerville, 2007; Brennan & Owende, 2010, Krishnamurthy et al., 2014; Patel et al., 2016).

The second-generation biofuels require a more advanced technique to produce compared to the first-generation biofuels, its processes also attract a higher cost; a major reason it remains unpopular (Mianda et al., 2016). They require similar feedstock compared to the first-generation biofuels but have the advantage of using the whole plant (including the stem, leaves and husks) not only a part such as grains as for the first-generation biofuels. Also, cereals having low grain yield, wastes from fruit or wood processing industries or plants with no edible part can be used for the biofuel production e.g., *Jatropha curcas*. The third-generation biofuels are primarily fuel cells, significantly different from the aforementioned, they may use hydrogen as the primary energy source (Peter et al., 2010). At present, algae serve as suitable raw material for the production of such biofuels at high-efficiency levels and low investment. Algae biomass is cost-effective with considerably high biofuel yield; besides, algae biofuel is eco-friendly and biodegradable.

#### *Renewable Energy Sources*

Power production from non-renewable energy sources has various impacts on the environment, which could be highly detrimental to the sustainability of the ecosystem; a more pronounced effect is global warming. Fossil fuels, although very useful, its combustion for power generation has exerted a damaging effect on the ecosystem, giving rise to air and water pollution, health hazards, wildlife and habitat loss, and greenhouse gases emissions (Asif & Muneer, 2007). The rate of release of greenhouse gases from the combustion of fossil fuels is alarming across nations, especially in developed countries where it is a major source of energy. In 2007, the United States alone generated about 6.0 billion metric tons of energy-related greenhouse gases, 40% of which was carbon (IV) oxide (CO<sub>2</sub>) (US Department of Energy, 2015). About the same amount of greenhouse gases was released in China from its energy-related activities. Currently, an estimated 50 billion tons of CO<sub>2</sub>e (carbon dioxide-equivalents) greenhouse gases are emitted globally each year, 40% higher than recorded in 1990, North America and Asia contributing more than 40% of the global emission (Ritchie & Roser, 2020). A good percentage of this

pollution can be prevented by employing renewable sources of energy.

Renewable energy sources utilize natural resources and can provide clean energies with very negligible emissions of pollutants compared to the non-renewable sources (Asif & Muneer, 2007). Renewable energy exploits natural energy sources, which includes; the sun, the wind, biomass, water bodies and geothermal energy. It provides an effective approach to avert the dreadful repercussions of climate change and other environmental issues of concern on the continuous use of fossil fuels. Renewable energy sources are much safer, having very low greenhouse gases emissions, and considerably reduces pollution, however, it has its subjects of environmental concern, although very negligible. Production and transportation of energy could produce some emissions and pollutants, technologies like hydrothermal consume a large amount of water; others could require much land area for installation. Presently, only about 14% of global energy demand is supplied by renewable energy sources and approximately 20% of global electricity is generated from hydropower energy plants (UI-Mulik & Reynaud, 2018). Renewable energy resources are more extensively distributed compared to fossil fuels and nuclear energy resources; however, environmental protection regulations are important for sustainable use.

In developing countries, especially in rural areas, constituting approximately 50% of world population, biomass (mainly wood) is used traditionally as fuel for cooking and heating. Globally, biomass provides approximately 14% of the energy demand, ranking fourth as an energy resource and increasingly gaining attention as an eco-friendly source of electricity generation, greatly reducing the amounts of greenhouse gases emissions (Larson & Kartha, 2000). Biomass use for energy generation is on a steady increase. Biomass quota in total energy consumption is only about 2 - 3 % in Europe, North America, and the Middle East, whereas in Asia, Latin America and Africa, where 75% of the world population resides, biomass contributes about 33% of total energy demands on the average, reaching as much as 80 to 90% in very poor countries (US Department of Energy, 2015).

Hydropower has been harnessed for energy generation for centuries. In fact, hydropower is one of the earliest discoveries for large-scale electricity production providing about 25 % of the global electricity supply and well above 40% of the electricity used in developing countries. Technically, large-scale hydropower could generate over 2,200GW of power globally (U.S EIA, 2020). Hydropower could also be used on the small-scale, there are two variants of small-scale hydropower systems based on their power generation capacities: first is the Micro-Hydropower Systems (MHP), which generates below 100 kW of power, and the Small Hydropower Systems (SHP), generating between 101 kW and 1MW (U.S EIA, 2020). While developing countries still have notable hydropower potential, the major set-back to its development has been inadequate funds, environmental and social constraints.

Geothermal energy; also, renewable, clean and affordable, it can be used for both industrial and domestic purposes. Geothermal energy is obtained from the natural heat residual in the core of the earth. It occurs both in high forms of enthalpy e.g., volcanoes and low forms of enthalpy, especially heat trapped in rocks in the crust of the earth (Banks, 2008). Geothermal energy has been harnessed for commercial electricity production since 1913, more energy providers subscribed to its use in the past few decades. More than 80 countries were listed globally to possess geothermal resources in the year 2000, however, only 58 countries have recorded its utilization (Fridleifsson, 2001). In 2016, about 3,812 MW

of geothermal electricity plants were operating in the United States, which is the highest reported in any country. Meanwhile, many more countries have subscribed to the use of geothermal for energy generation in 2019, 72% of the global geothermal power capacity installed are located in the U.S., Turkey, New Zealand, Philippines, Indonesia, Mexico. The United States produced about 16 billion kWh electricity from geothermal plants in 2019 and this is projected to rise to about 52.2 billion kWh in 2050 (U.S. DOE, EERE 2019; IREA, 2020; U.S EIA, 2020). Unfortunately, thermal energy has not been well harnessed in Africa even though the African Development Bank in 2017 projected that Africa has the potential of producing 15 GW of geothermal energy.

#### *Politics of renewable energy technology development*

The need for CO<sub>2</sub>-neutral sources of fuel arose from the damaging effect the CO<sub>2</sub> laden atmosphere exerts on the planet and the rapid depletion of fossil fuel reserves (Fig. 1). Thus, a cheaper and safer alternative became imperative. Climate change is a major time-constraining drive for the development of renewable energy technologies. In order to keep the increase in global temperature to below the 2 °C agreed on at the Copenhagen Climate Change Summit in 2009, the global CO<sub>2</sub> emissions must be reduced by 25–40% by 2020 and 80–90% in 2050. To achieve this, the development of renewable energy technology as a substitute for fossil fuels is an effective means.

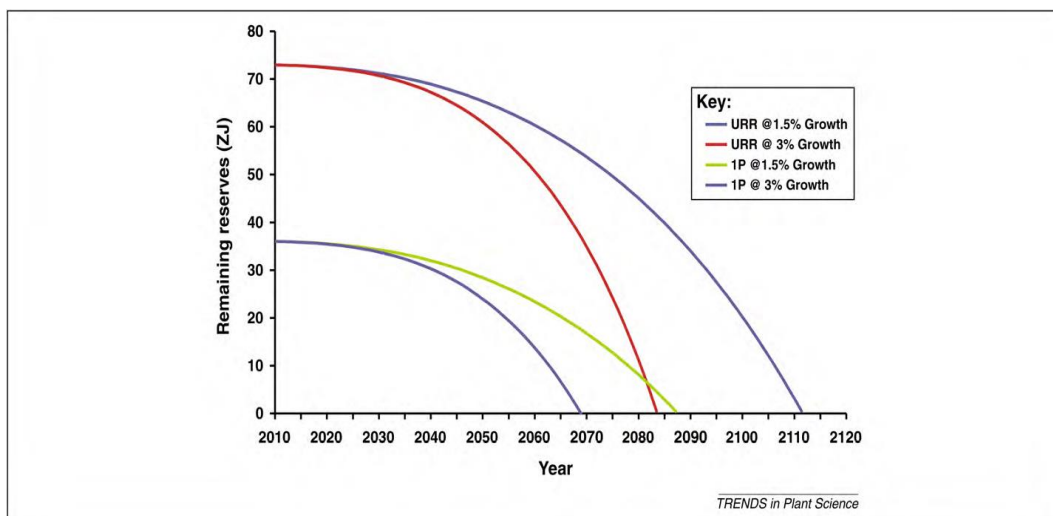


Fig.1-Predicted rates of global fossil-fuel depletion: The depletion of total fossil fuels (coal, oil, gas and uranium) determined based on acceptable international projection (1P) and the Ultimate Recoverable Reserves (URR), on the assumption of 1.5 and 3% economic growth with energy efficiency improvements of 1% per annum (which may be difficult to achieve). The world population is projected to reach 9.2 billion by 2050. The rate of depletion is based on 100% of fossil fuel use. Supplementing renewable energy sources will extend the supply. Data sourced from (Bentley et al., 2007; Meng & Bentley, 2008; Kjarstad & Johnson, 2009; Mohr & Evans, 2009)

#### *Can microalgae come to the rescue?*

Microalgae are photosynthetic lower plants that are characterized by rapid growth. They are ubiquitous unicellular or simple multicellular organisms which can survive under harsh environmental conditions (Amir & Singh, 2018). Several thousand algae and cyanobacteria species have been discovered to be very rich in oil content. An example is Cyanobacteria (*Cyanophyceae*), a prokaryotic green alga (*Chlorophyta*), and the diatoms (*Bacillariophyta*); eukaryotic microalgae (Li et al., 2008a; Li et al., 2008b). Microalgae have amazing growth rates; they double every few hours at their exponential growth phase, almost every 24 hrs, some can double its size every 3.5 hours at the peak of their growth phase. Besides, their cell wall is embedded with a high amount of lipids which makes them a potential candidate for biofuel feedstock (Chisti, 2007; Arun & Singh, 2012). Also, microalgae can grow in ponds once there is adequate sunlight and CO<sub>2</sub>; they can grow even in wastewater yet maintain a high lipid production. Furthermore, microalgae are capable of producing more oil compared to most terrestrial oilseed crops per

unit area of land (Table 1). The advantages of microalgae over other feedstock materials in biodiesel production has been highlighted by researchers (Chisti, 2007; Khan et al., 2009; Huang et al., 2010; Amir & Singh, 2018).

Microalgae effectively transform solar energy into chemical energy through photosynthesis, thereby increasing the biomass within a few days. Although microalgae photosynthesis follows the same mechanism with higher plants, they can thrive under certain growth conditions that are unsuitable for other biodiesel plants like soybean, rapeseed etc. More also, microalgae require a considerably smaller space for growth. Recently, they have been used successfully in the production of energy fuels, which include biomethane from the anaerobic digestion of algal biomass (Spolaore et al., 2006); biohydrogen (Fedorov et al., 2005; Kapdan & Kargi, 2006); biodiesel (Roessler et al., 1994; Banerjee et al., 2002; Gavrilescu & Chisti, 2005; Deng et al., 2009); and bioethanol (Fortman et al., 2008; Mata et al., 2010) among others.

**Table 1:** Comparison of oil yield and the required land area of some biodiesel sources (Adapted from Chisti, 2007)

Crops	Oil yield (L/ha)	Land area requires (M ha)
Maize	172	1,540
Soybean	446	594
Canola	1190	223
Jatropha	1892	140
Coconut	2689	99
Oil palm	5950	45
Microalgae <sup>†</sup>	136,900	2
Microalgae <sup>††</sup>	58,700	4.5

<sup>†</sup>70% oil (by wt) in biomass; <sup>††</sup>30% oil (by wt) in biomass

#### *Biofuel from microalgae*

Algal biofuels are poised as a renewable energy source with potentials to replace fossil-

fuels, yet eco-friendly and sustainable. Biodiesel is renewable and ideal for an internal combustion engine. It can reduce global

warming and related health hazards significantly due to its very low greenhouse gases emission and biodegradation potential. Algae is converted to biodiesel by trans-esterification, the biodiesel enhances lubricity and improves engine performance. Besides, the advent of biofuels production has provided employment opportunities, and thereby contributes to the global economy (Frac et al., 2010; Amir & Singh, 2018). Microalgae are preferred to higher plants for biofuel production for some major reasons: First, for their higher biomass yield per unit land area. Secondly, arable land or freshwater is not required to cultivate microalgae as they can be successfully grown in ponds on hardpan soils, brackish water or even saline water. Some microalgae species, such as *Dunaliella*, grow in seawater as long as it has access to CO<sub>2</sub> enriched air. Also, microalgae are non-food plant material, thus, their use as biofuel feedstock does not result in food scarcity. Furthermore, algal oils are rich in unsaturated fats; triglycerides and free fatty acids, a good proportion of the total lipid content can be processed into biodiesel. When compared with Straight Vegetable Oil (SVO), algal oil is highly unsaturated therefore not advisable for direct combustion in sensitive engines, therefore, trans-esterification is necessary to produce biodiesel and glycerol (Figure 2).

*Lipid production from microalgae*

Algae could contain as many lipids as 50% of its dry weight (Table 2). Lipids content include natural lipids, polar lipids, sterols, wax esters, hydrocarbons and, phenyl derivatives such as carotenoids, tocopherols, terpenes, quinines and some phytylated pyrrole derivatives e.g., chlorophyll (Amir & Singh, 2018). Algal oils contain higher phospholipids and glycolipid concentrations than obtainable in higher plants (Table 3). These classes of lipid contain nitrogen, phosphorus and sulfur which hinders proper engine performance, when present in fuels. During esterification, approximately 30% of the lipid mass is lost to the polar phase, the lipid constituent significantly affects the fuel yield by trans-esterification. Triglycerides give more than 99% biodiesel yield while phospholipids give less than 70% yield. Some species of microalgae under certain conditions such as nutrient deficiency and environmental stresses accumulate neutral lipids particularly triglycerols (TAGs) which can be directly converted into biodiesel. In addition, nutrient stress for example; nitrogen deficiency adversely affected their lipid production. Shifrin & Chrisholm (1981) reported a slight decline in lipid content of *D. tertiolecta* when starved with nitrogen. A similar observation was reported in another study when *D. viridis* cells were grown

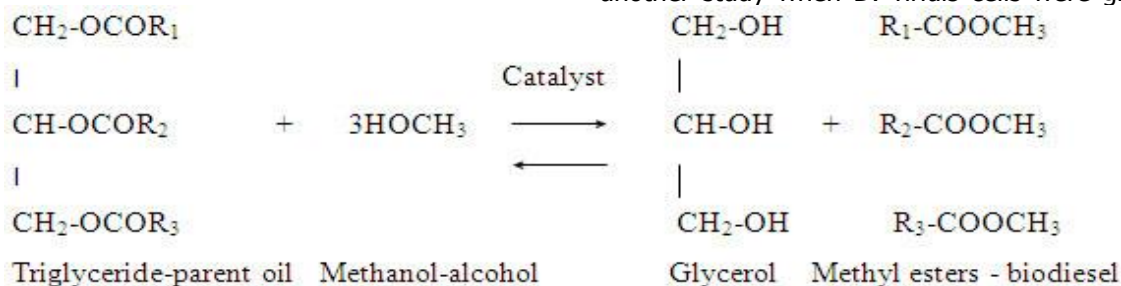


Fig.2- Transesterification of oil to biodiesel. R<sub>1</sub>-R<sub>3</sub>-hydrocarbon groups (Seehan et al., 1998)

in a nitrogen deficient culture of (0.035% CO<sub>2</sub>), but trend discontinues at 1% CO<sub>2</sub>.

**Table 2:** Lipids contents of some microalgae (Adapted from Becker 1994; Chisti, 2008; Miranda, 2012; Su et al., 2012).

Microalgae	% of Lipid	Microalgae	% of Lipid
<i>Ankistrodesmus</i> sp.	29 - 40	<i>Nannochloris</i> sp.	30 - 50
<i>Amphora</i> sp.	21	<i>Nannochloropsis</i> sp.	44
<i>Amphidinium</i> sp.	8 - 10	<i>Nannochloropsis</i> sp.	31 - 68
<i>Botryococcus braunii</i>	25 - 80	<i>Nannochloropsis salina</i>	22

<i>Chlorella protothecoides</i>	57	<i>Nitzschia</i> sp.	45 - 37
<i>Chlorella pyrenoidosa</i>	46	<i>Parietochloris incise</i>	30
<i>Chlorella zofingiensis</i>	51	<i>Phaeodactylum</i> sp.	20 - 30
<i>Chlorella emersonii</i>	28 - 32	<i>Neochloris oleoabundans</i>	35 - 54
<i>Chlamydomonas</i> sp.	21	<i>Navicula jeffreyi</i>	6
<i>Chlorella vulgaris</i>	14 - 22	<i>Pavlova pinguis</i>	3 - 7
<i>Chlorococcum</i> sp.	7	<i>Pleurochrysis carterae</i>	30 - 50
<i>Cryptocodinium cohnii</i>	20	<i>Proteomonas sulcata</i>	8
<i>Cylindrotheca</i> sp.	16 - 37	<i>Rhodomonas salina</i>	5
<i>Cyanobium</i> sp.	8	<i>Prymnesium parvum</i>	22 - 38
<i>Dunaliella salina</i>	6	<i>Scenedesmus obliquus</i>	12 - 14
<i>Dunaliella tertiolecta</i>	35	<i>Skeletonema</i> sp.	3
<i>Dunaliella primolecta</i>	23	<i>Scenedesmus dimorphus</i>	16 - 40
<i>Euglena gracilis</i>	14 - 20	<i>Schizochytrium</i> sp.	50 - 77
<i>Heterocapsa</i> sp.	6	<i>Spirulina platensis</i>	4 - 9
<i>Hormidium</i> sp.	38	<i>Spirulina maxima</i>	6 - 7
<i>Isochrysis</i> sp.	25 - 33	<i>Thalassiosira</i> sp.	8
<i>Monallanthus salina</i>	>20	<i>Tetraselmis suecica</i>	15 - 23
<i>Monoraphidium</i> sp.	20	<i>Tetraselmis</i> sp.	12-14

**Table 3:** Fatty acid methyl esters (FAMES) yield of soybean (*Glycine max*), Cotton (*Gossypium hirsutum*) and microalgae. (Adapted from Ma & Hanna, 1999; Yoo et al., 2010; La Russa et al., 2012)

Fatty acids	% of FAMES				
	Cotton	Soybean	Microalgae-1	Microalgae-2	Microalgae-3
Stearic (C18:0)	0.89	3.15	2.7	5.09	1.3
Palmitic (C16:0)	28.33	11.75	36.3	32.9	2.1
Palmitoleic (C16:1)	-	-	4	1.7	3.4
Linoleic (C18:2n6)	57.51	55.53	31.1	17.7	47.8
Linolenic (C18:3)	-	6.31	-	9.1	-
Oleic (C18:1n9)	13.27	23.26	25.9	18.3	24.8

Saturated fatty acids; C16:0 (palmitic acid), and C14:0 (myristic acid), are the major components of fatty acids in most of the microalgae classes, whereas, a specific distribution of the unsaturated fatty acids occur among the algal groups (Léveillé et al., 1997; Reuss & Poulsen, 2002; Sushchik et al., 2009)

### Methane production from microalgae

Anaerobic digestion involves the use of microbes in the conversion of organic or other biodegradable materials into biogas, in the absence of oxygen. Biogas is a compound molecule, which is a mixture of carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), traces of hydrogen sulphide (H<sub>2</sub>S) and moisture. Biomass and waste of several sources have been employed as feedstock to produce methane gas. Anaerobic digestion of lingo-cellulosic biomass yields a very low quantity of biogas whereas, more putrescible materials such as food wastes, animal wastes, wastewater slugs, etc. yields considerably (Borowitzka & Borowitzka, 1988). Golueke et al., (1957) when comparing biogas yield from digested domestic wastewater sludge and the green microalgal (*Scenedesmus* and *Chlorella*) biomass collected from wastewater ponds demonstrated that the algae yielded as much as 0.25-0.50 L CH<sub>4</sub>/g VS input at 11-day retention time when incubated at 35-50 °C. The least value was 32% lower than the yield from the wastewater sludge.

Digesting *Spirulina maxima* anaerobically yielded 0.3–0.37 m<sup>3</sup> biogas Kg<sup>-1</sup> VS, with 70% methane and conversion was about 48 % efficient. Mixing algal biomass which is proteinaceous with sewage sludge or waste paper which have high carbon content further increases biogas productivity (Yen & Brune, 2007), the digester feeding C/N ratio is also increased. Algal biomass has been subjected to both mechanical and thermochemical pretreatments to make the algae more biodegradable. Thermochemical pretreatment of algae biomass at 100 °C for approximately 8 hours without NaOH yielded good results, this helps to improve the methane fermentation efficiency by 33%, up to 0.32 m<sup>3</sup> kg<sup>-1</sup> VS (Chen & Oswald, 1998). Twice as much methane yield and productivity were reported for an equal amount of *Spirulina* biomass and wastewater sludge digested together.

### Bioethanol production from microalgae

In 2007, 49.5 billion liters of ethanol was produced for energy, this represents 4.4% of total gasoline consumption all over the world. This increased to 27.05 billion gallons in 2017, despite the little decline in 2012 and 2013 (Renewable Fuels Association US, 2018).

Bioethanol is useful as an octane enhancer; it could also be blended with diesel to reduce the exhaust gases. Like other biofuels, bioethanol is biodegradable and less toxic (Pejin & Mojovic, 2009; John et al., 2011; El-Mekkawi et al., 2019). Microalgae has high carbohydrates content (11-50%) present as cellulose and starch which can be fermented to bioethanol, this makes it very useful as feedstock for bioethanol production (Doma et al., 2018, Silva et al., 2018). Compared to lignocellulosic materials, microalgal carbohydrates do not have lignin, making them easier to convert to monosaccharides (John et al., 2011; El-Mekkawi et al., 2019).

Ethanol production from microalgae follows two different pathways; first, by fermenting the stored carbohydrate products e.g., starch and glycogen in green algae and cyanobacteria respectively. Another process is endogenous, involving the self-fermentation of stored carbohydrate by the algal enzymes, this process occurs in anaerobic conditions. *Saccharomyces cerevisiae* (yeast) is commonly used, being able to ferment a good number of sugars, giving high ethanol yield and its tolerance to alcohol. Its efficiency and yield can be enhanced by immobilizing its cells (Lin et al., 2012). This also makes it easy to separate yeast cells from the fermentation medium (Domingues et al., 2000; Choi et al. 2010). Several factors determine the bioethanol yield obtainable from fermentation, this includes; the sugar concentration, inoculum size, agitation rate, pH, temperature, and fermentation time (Tofighi et al., 2014; El-mekkawi et al., 2019). Gfeller & Gibbs (1984) established that in the absence of light and oxygen, self-fermentation of intracellular starch occurs in *Chlamydomonas reinhardtii* to produce formate, acetate, ethanol, glycerol, and hydrogen.

### Hydrogen production from microalgae

Another promising renewable energy source is the biological extraction of hydrogen from microalgae, this is much-more eco-friendly than fossil fuels, releasing only water vapour as a by-product. Besides, it has a higher energy density than other hydrocarbon fuels (Oey et al., 2016). Microalgae can produce hydrogen through bio-photolysis or photo-fermentation (Razu et al., 2019). Hydrogen (H<sub>2</sub>) is becoming increasingly important as a CO<sub>2</sub>-free fuel that



can be harnessed to power the coming generation of H<sub>2</sub> fuel cells through various renewable technologies e.g., photovoltaic (PV), wind, and biological systems such as microalgae (Oey et al., 2016). Hydrogen production is becoming more important in the global energy economy, and certain microalgae can produce enzymes (hydrogenase and nitrogenase) required in H<sub>2</sub> production. This provides an attractive means of hydrogen production through a less risky microbial process. Many species of microbes, including strains of different physiological forms, produce H<sub>2</sub>. A typical example is *Chlamydomonas reinhardtii*, which is a green alga, commonly found as green pond scum. The alga produces 'hydrogenase' which splits water into its constituent atoms (hydrogen and oxygen), thus it is capable of producing large amounts of hydrogen. Gaffron & Rubin (1942) were the first to report H<sub>2</sub> production by green algae. They discovered that under anaerobic conditions, *Scenedesmus obliquus* which is a green alga could evolve H<sub>2</sub> in both dark and light conditions.

Evolution of hydrogen gas in green algae is preceded by anaerobic incubation in the absence of light in which the hydrogenase enzyme is induced. The hydrogenase enzyme then combines protons and electrons to produce H<sub>2</sub>. The processes involved in the biological production of H<sub>2</sub> from microalgae can be further differentiated into distinct approaches: (i) Direct biophotolysis (ii) Indirect biophotolysis (iii) Photofermentation (iv) Dark fermentation. Hydrogen production through direct or indirect biophotolysis uses microalgae to facilitate solar energy and H<sub>2</sub>O conversion into H<sub>2</sub> fuel, with O<sub>2</sub> released. Direct biophotolysis was observed in *Anabaena* strains (Yu & Takahashi, 2007). A good example is *Anabaena variabilis* (a Cyanobacterium) under light intensities of 45–55 Amol1m<sup>2</sup> and 170–180 Amol1m<sup>2</sup> in concurrent stages. Troshina et al., (2002) reported an indirect biophotolysis in the Cyanobacterium; *Gloeocapsa alpicola*, they established that maintaining the pH value of the medium between 6.8 - 8.3 yielded optimum hydrogen. Twice as much of its hydrogen production was yet realized when the temperature was increased from 30 - 40 °C. Dark fermentation occurs when microbial reduced substrates e.g., starch, glycerol, glycogen, etc. are converted anaerobically into

hydrogen, essential solvents, and mixed acids. This is facilitated either by introducing anaerobic heterotrophs (e.g., *Clostridia*) or, at times, by the microbe cell itself. Biological production of H<sub>2</sub> from renewable systems; such as microalgae and cyanobacteria are carried out at ambient temperature and pressure. Hence, requires less energy and could be carbon negative.

#### *Biofuel production system*

Macroalgae (i.e seaweed) and microalgae require different culture systems for biofuel production. Microalgae due to their small size (µm) are cultivated in special systems, this could be a Photo-bioreactor or an Open pond system.

#### *Photo-bioreactors*

A closed culture system is done in a photo-bioreactor (Fig. 2). Photo-bioreactor is a reactor used to grow prototrophs (e.g microbial, algal or plant cells) or carry out photo-biological reactions. Photobioreactors (PBRs) are classified based on design and their mode of operation. There are different PBRs based on designs (serpentine, flat, helical, and manifold), the major categories are: (1) flat or tubular; (2) horizontal, inclined, vertical or spiral; and (3) manifold or serpentine. Tubular reactors are useful for culturing outdoors, they are relatively affordable, and have an extensive surface area for illumination. and have good biomass yield. However, it has its disadvantages, which include; fouling, growth on walls, dissolved oxygen and carbon (iv) oxides along the tubes, and pH gradients (Dragone et al., 2010).

Radial fluid movement is needed for enhanced light-dark-cycling, this is achievable using airlift cylinders and vertical bubble columns. These reactors are designed with a low surface/volume ratio, more chaotic gas-liquid flow, and could hold more gas than most horizontal reactors. Elevated reactors have an advantage, they allow orientation and tilting at desired angles, they use diffuse and reflected light; enhancing their productivity. PBR is designed to work with different light paths, and different pump types for effective mixing, mixing can also be done by air bubbling, these improve productivity and solar energy use efficiency. The kind of material selected for the photo-stage is very important for a suitable PBR construction. The materials, which could be plastic, glass materials, tubes etc. must not be toxic and must

be highly transparent. They must be affordable, durable, strong and stable. If plastic materials are exposed outdoors, they must be easy to

clean and not lose their transparency with time, these are important factors to consider.

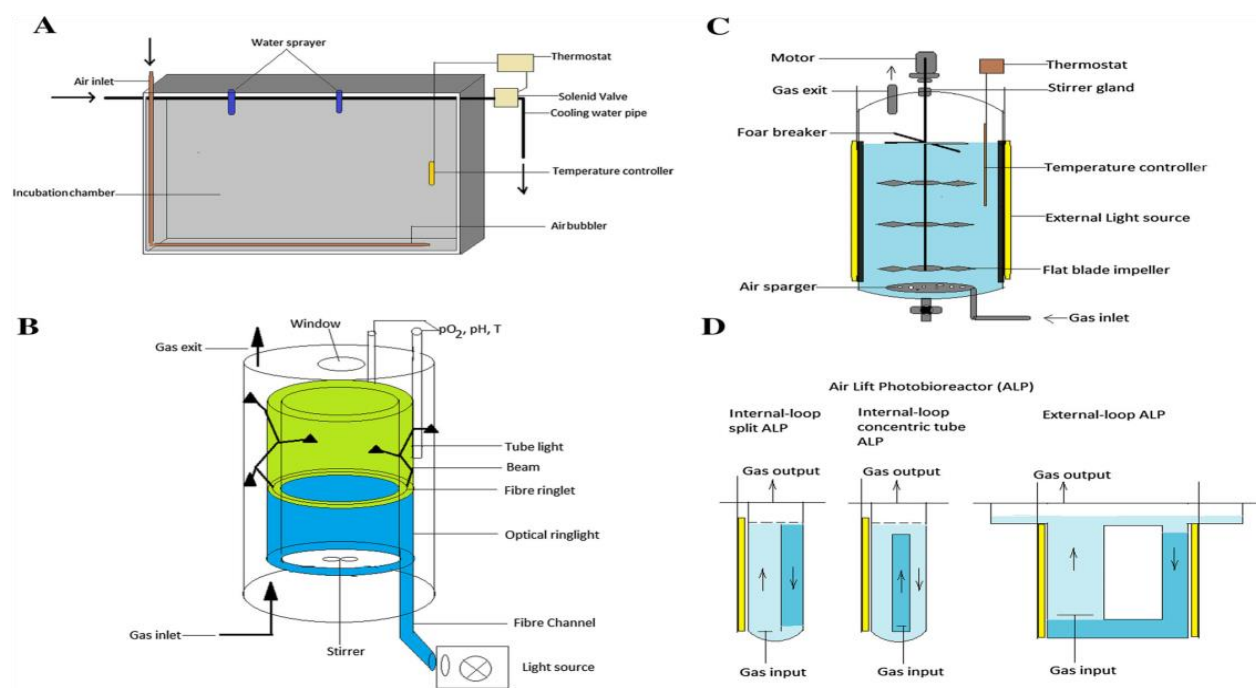


Fig. 2: a–d Diagrammatic representation of the different types of photobioreactors used for microalgal cultivation (Mondal et al., 2017)

### Open pond system

Micro-algae can be cultivated in open-culture systems which include lakes or ponds. Open-culture systems are cheaper to build and require little skills to operate, besides, they are more durable. Generally, ponds are open, thus, water temperature, evaporation and lighting are not controlled, making them very susceptible to changing weather conditions. Although micro-algae is produced in large quantities through this system, it occupies a more extensive area, thus contaminations from other microbes or micro-algae are common, owing to limited resources, interspecific competition could slow down the growth of the desired microalgae.

Micro-algae are very small organisms often between 3 – 30  $\mu\text{m}$  in diameter. They can be cultured in broths, dilute culture broths may be used (about 0.5  $\text{g l}^{-1}$ ), thus, a large volume is needed. Method of harvest varies with species, the density of cells, and the culture conditions. The cost incurred on harvesting makes about 20-30% of the total cost (Grima et al., 2003). Microalgae can also be harvested using

conventional processes, e.g., ultrasonic separation (Bosma et al., 2003), foam fractionation (Csordas & Wang, 2004), concentration by centrifugation, membrane filtration (Rossignol et al., 2000) and flocculation (Poelman et al., 1997; Knuckey et al., 2006).

Most of the extraction methods used today are adapted from the method developed by Bligh and Dyer in 1959 (Lewis et al., 2000). Bligh and Dyer's method was designed for animal tissue, algal tissue is much different. Research shows that algae lipid extraction is difficult using this process (Ahlgren, 1990). Several direct trans-esterification reactions involve mixing solvent, alcohol and catalyst. Lipid is extracted by the solvent while the alcohol and catalyst work simultaneously to convert the extracted lipids to methyl esters. Application of heat, methanol and catalyst can also be used to separate fatty acids and transform them (Bo Liu, 2007), less solvent is needed compared to the extraction– trans-esterification process (Lewis et al. 2000). This is noteworthy since many of the organic solvents

used are very toxic, yet must be recovered. They reported a significant increase in the quantity of fatty acids extracted using the direct transesterification process. Upon the completion of the transesterification process, the biodiesel is separated. To remove glycerol, the separated biodiesel must be washed with water severally (Wen & Johnson, 2006). If a direct transesterification method was used, particles of the algal biomass appear in the mix, this must be removed, its best done by filtration. After proper washing, the biodiesel is ready for use. The major setback to algal biodiesel production is that it is too expensive for public use (Sheehan et al., 1998).

#### *Prospect of microalgae*

Algal biofuel is globally accepted, it has the potential to replace petroleum-based fuel in the nearest future. Algae has a high oil content, high biomass yield, requires less space for growth, and can grow in an aqueous environment, even in saline water etc. Algal-biofuel is increasingly gaining attention all around the world, nevertheless, the biofuel production cost is too expensive for commercialization. To make the biofuel from algae able to compete effectively with other fuels in the near future, especially in price, efforts must be made to reduce the extraction and processing cost of algae biomass to biofuel. Also, a cost-effective production method for algae production should be developed. This is achievable by enhancing algal yield (biomass and oil) and culture-system engineering. Besides, microalgae can be used to produce other value-added products besides the algal fuel. The integrated biorefinery will also help to reduce algal biofuel production cost. Microalgae have a high content of oil, proteins, carbohydrates, and other important nutrients (Spolaore et al., 2006), oil extraction residue is useful as animal feed or in the manufacture of other value-added products.

In recent times, attention is being drawn to renewable energy sources owing to the fast depletion of fossil fuel reserves and global increasing fuel demands. Presently, about 80% of global energy demand is met by fossil fuels, it is estimated the world will need 50% more of its current energy supply by 2030 (Daming et al., 2012; Yahaya et al., 2013). Thus, there is a need for an energy source that

is more sustainable, economically viable, safer, eco-friendly and relatively more affordable compared to fossil fuels, a gap biofuel promises to fill. The discovery of biofuel for commercial usage was embraced by developed countries. From the dawn of the 21st century, biofuel production in the United States, Brazil, Argentina and several European countries has increased geometrically. Every year, about 30 billion litres of biofuel is used globally, IEA reported that biofuel could reach 10% of global fuel use for transportation by 2025 (IEA, 2008; Ullah et al., 2014). Currently, about 84% of world total biofuel production comes from the United States and Brazil.

Major feedstocks used for commercial biofuel production across America and Europe. Include; waste cooking oil, animal fats and various oleaginous species such as sunflower, corn, oil palm, peanut, soybean, rapeseed, jatropha and sugarcane (Felizardo et al., 2006; Vasudevan & Birggs, 2008). The report shows 94% of US biofuel production is derived from corn, while sugarcane bagasse is a major feedstock in Brazil (EIA, 2019; DOE and EERE, 2020). However, research revealed Algae could produce 10 – 100 % more fuel per land area than other crops. Every year, a considerable amount of these food materials produced are diverted into biofuel production, creating scarcity thus increasing food prices. There is also a fall in food production as more farmland is dedicated each year to biofuel feedstock production. Chakravorty et al. (2009) reported a drastic increase in vegetable oil prices owing to its use in biodiesel production. This resulted in the debate "food vs. fuels". Microalgae, having a high oil content and biomass production have thus gained attention from researchers and government bodies, serving as a non - food material alternative, useful as biofuel feedstock (Deng et al., 2009). Besides, Microalgae can be grown virtually anywhere, as there is adequate water and sunlight, they can be cultivated even on the sea, thus, not competing for farmland. Growth requirement of algae is very minimal and will perform well in certain conditions no other crops could, besides having as much as 50 times more oil yield than most crops. Also, algae have a high carbon sequestration ability. Biofuel from algae is classified as a second-generation biofuel; coming from a non-edible source has a smaller greenhouse gas footprint.

The United Airlines made the first commercial flight in 2011, which was powered mainly by algae products. A unique structure in Hamburg, Germany, known as the 'BIQ House' fills bioreactors with algae biomass for heat generation without any external power supply. The major setback algae suffer from wider adoption is the price. In late 2014, Biofuels Digest estimated algae biofuel to be around \$7.50 per gallon, which is higher than other fuels. Experts projected that for algae fuels to compete effectively with other oil-based products, its price should not exceed \$3 per gallon (Renewable Fuels Association US, 2018).

In previous years, one could not tell if algae or other biofuels could replace oil, but in recent times, the alarming rate at which biofuel is produced and used commercially in developed countries convinces it could. Algae-produced biofuel is most promising in this regard, assuming it could be made available at competitive prices. If microalgae are grown under optimal growth conditions, microalgae biofuel can replace fossil fuels completely without interfering with the food supply of agricultural products (Richmond, 2004). The large expanse of arable land required to grow crops for biofuel production is a major barrier to the production of biofuel in large quantities, besides the unavoidable effect on food prices, if more farmland is devoted to fuel production, a challenge microalga promises to solve.

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