



A FUTURISTICS APPROACH FOR HARNESSING GREEN ENERGY: FROM POND SCUM TO POWERHOUSE ALGAE'S JOURNEY AS BIO-FUEL

Shiwangi Bharti¹, Varsha Gupta¹, Mansvi Yadav¹, Deepesh Kumar Neelam¹, Devki¹, Ravi Kant Rahi^{1*}

¹ Department of Microbiology, JECRC University, Jaipur, Rajasthan, India 303905

Corresponding Author: Ravi Kant Rahi*

Corresponding Author Maid Id: ravikrahi.20@gmail.com

Abstract

Bio-fuel is the fuel produced over a short period from biomass, rather than by the very slow natural processes involved in forming fossil fuels, such as oil. Bio-fuel can be produced from plants, agricultural, domestic, or industrial bio-waste. Algae is the term used for a large and diverse group of eukaryotic organisms that are photosynthetic and have chlorophyll as their primary photosynthetic pigment. Algal biomass contains a very high oil fraction and can be used for bio-fuel production. Various algal strains, *Botryococcus braunii*, *Chlorella*, *Dunaliella tertiolecta*, *Gracilaria Pleurochrysis carterae*, *Sargassum*, and others have been found to successfully and efficiently produce *biodiesel. Mechanism of biofuel production includes transesterification and esterification of oil with alcohol. This review mainly focuses on the various algal strains isolated from various sites that are responsible for bio-fuel production, production mechanisms, and various applications of bio-fuels.

Keywords: Biomass, Fossil fuels, Photosynthetic, Chlorophyll, Biodiesel, Transesterification, Esterification

1. Introduction

Algae are being used as a third-generation feedstock for the production of different types of renewable energy sources such as bio-ethanol, biodiesel, biogas, and bio-hydrogen (Anto, et al., 2020). Algae are the most notable resource of viable bio-fuels in renewable energy due to the depletion of fossil fuel sources, their sources, and their emissions (Menetrez, 2012). Algal bio-fuels obtained from algae depend upon the rich quantity of oil present in algae that can be associated with the ability to abundantly photosynthesize (Adeniyi, et al., 2018).



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Algae are aquatic organisms with over 30,000 to 1 million species (Guiry, 2012). Wastewater obtained from different sources like agricultural activities, industries, and municipalities provides a cheap and sustainable medium for the growth of algae (Pittman, et al., 2011). The advantage of algae apart from the highest oil concentration is the capacity to transform all the energy from raw materials to different types of useful products (Adeniyi, et al., 2018). Algae can digest different types of waste products and produce useful products with high compositions which include phospholipids, which can be further processed into bio-fuels; sugar, which can be further processed into alcohol; and protein, which can be used for consumption (Menetrez, 2012). Liquid bio-fuels are substitutes to decrease the outgrowing demand for fossil fuels, its cost of production varies widely by raw materials, the process of conversion, production scale, and region (Demirbas, 2010). This strategy must control a variety of problems before it can take part in the fuel market (Hannon, et al., 2010). That day is not far off when algae will become the most important source of bio-fuel because microalgae seem to be the only means of renewable biodiesel that can meet the growing demand for transportation fuels (Demirbas, 2010). Algal bio-fuels are capable of avoiding the disadvantages related to bio-fuels obtained from crops as algae do not compete with food crops, due to the presence of optimum conditions for growth in various countries has led to a great deal of supposition about their strength for decreased oil imports, vitalizing rural economies and challenging poverty (Adenle, et al., 2013). Cultivating microalgae as a substitute feedstock is anticipated to be a potentially crucial strategy for renewable bio-ethanol bio-fuel production and this procedure offers several environmental perks, including the effective use of land and good CO₂ sequestration without entering into the "food against fuel" dispute. The demand for ethanol alternative fuel has been increasing globally as a result of rapid population growth and industrialization, and this process offers several environmental benefits (Ruffing, et al., 2022). The ability of algae to address today's problems has been demonstrated by the uptake of nutrients from wastewater by algae cultivation and the production of biomass for use in energy supply (Chia, et al., 2022). Filamentous algae have higher resistance to grazer-predation and low-cost recovery in mass production (Zhang, et al., 2016). Because they coexist with bacterial communities and can maintain symbiotic relationships, photosynthetic microalgae can absorb solar energy and transform it into bio-energy and biochemical products, in addition, because bacteria can remineralize sulfur, nitrogen, and phosphorous to support micro-algal growth, dissolved organic carbon released by photosynthetic microalgae can also be available to bacteria (Yao, et al., 2019). Bio-fuels have unique importance to reduce gaseous emissions, greenhouse gases, climatic changes, global warming receding of glaciers, rising sea levels, and loss of biodiversity (Singh, et al., 2014). The key technologies for enabling algal bio-fuels are high-throughput strain engineering, omics-informed genome-scale modeling, and microbiome engineering where high-throughput strain engineering efforts produce improved traits, such as high biomass productivity and lipid content in various algal species, while genome-scale models built with the help of omics data offer insight into metabolic constraints and direct rational algal strain engineering efforts and by introducing beneficial species and removing detrimental ones, microbiome engineering aims to improve ecosystem health respectively (Ruffing, et al., 2022). As crude oil analyzing drops,

microalgae biological refineries are being researched to manufacture biologically active such as enzymatic agents amino acids, omega-3 fatty acids oils, pigments, regenerated products, and vitamins, to reduce the costs of bio-fuel production; although microalgae have been examined as an intriguing source for biodiesel production, algal bio-fuel extraction is not yet economically feasible, which reflects the high costs of energy linked with the cultivating, harvesting, and dehydrating of algae (Puri, et al., 2022). The long-term viability of microalgae-producing biodiesel can be achieved by creating an advanced photo-bioreactor, cheap methods for biomass harvesting, drying out, and oil extraction, and better genetic engineering techniques to manage environmental stress conditions, it can also be achieved by designing pathways of metabolism for high lipid production (Medipally, et al., 2015). The process was optimized, and it was combined with other biological and chemical procedures to produce efficiency, increasing the yield of bio-oil to up to 41.1% (Vo Hoang, et al., 2018). By using the photosynthetic process, which may be either oxygenic or an-oxygenic and can collect up to 183 tonnes of CO₂ for every 100 tonnes of microalgal biomass generated, these bio-fuels are created from sunshine, CO₂, and water, all of which are renewable resources (Razeghifard R., 2013).

Due to their high photosynthetic efficiency, lack of concurrence with land-based crops, and capacity to recycle nutrients and fix CO₂ from waste sources, algae are a beneficial substrate for bio-fuels, food, feed, bio-chemicals, and biomaterials as well as various types of cutting-edge technologies like *BICCAPS, *BECCS and *OMA, algae are also a promising solution to the energy crisis, food security issues, and pollution problems (Leong, et al., 2021). Although algae-based bio-fuels have numerous benefits, such as high area productivity, higher species including amoeba, protozoa, ciliates, and rotifers can feast on algae, especially in open pond systems, as a result, these higher organisms must be managed (Park, et al., 2016). The United States on-road petroleum fuel consumption was made up of 6% of the 67 million acres of soybeans grown in 2007, however, even considering modest algal productivity, the same amount of land used for algal culture would produce more than 100% of the petroleum fuel consumption (Duffy, et al., 2009). Nowadays, waste algal biomass is being thermo-chemically converted into solid fuels with a focus on employing hydrothermal liquefaction techniques (Chang, et al., 2015). Most of the algal cultivation techniques and systems created in collaboration with the *ASP, including raceway ponds and *PBRs, were specifically designed for a few species of microalgae, the majority of which are high in lipid content and an important source of bio-fuels, as a result, much of the study on algal cultivation systems was sparked by the objectives of the *ASP, which was financed by the United States Department of Energy between 1978 and 1996 with its main focus being to develop renewable transportation fuels (Karimi, et al., 2021). Since algae are able to produce about thirty times the amount of oil per hectare than corn and soybean crops, algae biodiesel is relatively safe for the environment and is non-toxic, sulfur-free, highly biodegradable, and very biodegradable (Ghasemi, et al., 2012). When employing the semi-continuous mode, biomass production could be kept at a high level during the cultivation process, but it dropped significantly in batch cultures (Zhou, et al., 2013). Despite the fact that water is one of the most important factors influencing the sustainable development of algal bio-fuels, open pond systems typically

consume much more water (216 to 2000 gal/gal) than photo-bioreactors (25 to 72 gal/gal), with algae growth using the most water (165 to 2000 gal/gal) in the system (Tu, et al., (2016). Aside from bio-fuel, value-added bio-products can be produced through the conversion of algae, which would increase the economic viability of algal bio-refineries. However, various arguments contest the economically and energetically feasible of algal culture, harvesting, and processing methods (Zhou, et al., (2022).

Table 1: Comparison of the diesel and biodiesel specifications depending upon the European Standard (EN) as well as the American Society for Testing and Materials (ASTM) (Ramchandran, et. Al., 2013).

Properties	Units	Diesel		Biodiesel	
Details		ASTM D975	EN590	ASTM D6751	EN14214
Used in		Diesel	Diesel	FAAE	FAME
Density	g/cm ³	0.85	0.82-0.845		0.86-0.9
Viscosity	mm ² /s	2.6	2-4.5	1.9-6	3.5-5
Focal point	C	Min 59	Min 55	Min 93	Min 120
Sulfur	mg/kg	Max 50	Max 350	Max 15	Max 10
Water	mg/kg	Max 50	Max 200	Max 500	Max 500
Acidity level	mgKOH/g	~	~	Max 0.5	Max 0.5
Methanol	%mass	~	~	Max 0.2	Max 0.2
Ash containing sulfur	%mass	~	~	0.02	0.02
Number of Cetane	Minutes	48	51	47	51
Corrosion	3 h/50C	1	Max 1	Max 3	Max 1

2. Classification of Algae

Many species of algae are capable of absorbing organic pollutants from seawater, and they serve as refuge areas and environments for thousands of species, algae have also been used by humans for many centuries as a source of food, fertilizer, and fodder as well as for the production of molecules with antifungal, antiviral, anticancer, and other medicinal properties. They also serve as the foundation of ecological webs, generate the oxygen that allows the respiration of many aquatic organisms, and absorb CO₂ (Gonzalez Fernandez, et al., 2023). Among the still-evolving bio-fuel technologies, the production of biodiesel from algae offers an improved possibility for large-scale practical use because algae are capable of yielding much more yield than other bio-fuels; bio-fuels are seen as intriguing substitutes to existing energy sources because they can eradicate major environmental problems caused by fossil fuels (Show, et al., 2013). Microalgae have recently been reclassified as one of the most likely candidates due to their rapid development (with low use of land as well as not participating with food crops), excellent resistance to nutrient and salt stresses, and survival in biological composition, in turn, enabling for the production of a plethora of potential bio-based goods such as feed for animals, chemicals, and bio-fuels (Catone, et al., 2021).

Algal cells can photosyse macroalgae or microalgae developing in aquatic environments. Macroalgae are classified into three general groups based on their pigmentations: diatoms, green algae, blue-green algae, and golden algae; there are two main communities of algae: filamentous and phytoplankton algae, which are characterized into four primary categories: diatoms, green algae, blue-green algae, and golden algae and there are three types of seaweed: brown (Phaeophyceae), red (Rhodophyceae), and green (Chlorophyceae) (Demirbas, 2010).

The five monophyletic eukaryotic subgroups that now include algae on the Tree of Life are Archaeplastida (Glaucocystophyta, Rhodophyta, Prasinodermophyta, Chlorophyta, and Charophyta), TSAT (Ochrophyta, Dinophyta, Chlorarachniophyta, and photosynthetic species of the genera Chrome (Gololobova, et al., 2022).

Zygnematophyceae (conjugatophyceae), a group of conjugating green algae with a large number of species, has long piqued the interest of phycologists it is now generally acknowledged that this group of charophyte algae occupies an important place in the evolutionary tree of streptophytes, where it stands as the closest relative of all land plants (embryophytes) (Zhou, et al., 2020). The three sub-orders of the Bryopsidales are Ostreobineae, Bryopsidineae, and Halimedineae and the Bryopsidales are a morphologically diversified group of primarily marine green macroalgae that are known for their siphonous structure (Ceremen, et al., 2019). One of the 17 classes of the enormous algal phylum Ochrophyta, Eustigmatophyceae contains the diverse eustigmatophytes, which have expanded from the few initially recognized species to nearly over 200 genetically different organisms (potential species in general) as the majority of eustigs continue to be characterized by unidentified strains or even only by metabarcoding sequences derived from environmental samples (Barcyte, et al., 2022). Phylogenetic data suggest that the transition from simple to highly distinguished cellular diversity was not a peculiar development in the

development of life, but rather a relatively common event; the development of cellular diversity, the differentiation of the germline cells from asexual somatic tissues, and the creation of male and female dichotomy are unquestionably among the most significant achievements of eukaryotes, such as the circular green algae *Volvox* and its closest relatives, the volvocine algae, exhibit the full spectrum of organizational complexity, ranging from single-cell and genera to cellular genera with an entire germ-soma dividing of labor and a male-female dichotomy; as a result, these algae are excellent examples of model organisms for solving fundamental questions regarding the conversion to multicellularity and for identifying universal rules that characterize this transition (Hallmann, 2011). The superphylum Archaeplastida's red algae, or Rhodophyta, is a species-rich group with a variety of morphologies; the classes Porphyridiophyceae, Bangiophyceae, and Florideophyceae all underwent genome expansions at the same time as increased in morphological complexity, and TAPs control transcription, display lineage-specific patterns and are associated with organismal complexity (Petroll, et al., 2021). The fossil record of dinophytes is one of the finest amongst protists and suggests that the group has a geologically old beginning that dates back to the Triassic; unicellular dinophytes are a significant portion of toxic algae and powerful makers of toxins (Chacon, et al., 2020). Most of the freshwater algae in the class Eustigmatophyceae are coccoid, while some genera are also prevalent in terrestrial settings and two are exclusively marine (Amaral, et al., 2020).

Table 2: Classification of different types of algae based on their reserved good, types of pigment present, and mode of reproduction

Classification	Common name	Reserve food	Pigment	Reproduction	Example	References
Cyanophyceae or Myxophyceae	Blue-green algae	Cyanophyceae, Starch, and Protein	C-Phycocyanin	Vegetative & asexual means	Nostoc, Anabeana, etc.	(Haselcorn, 2009).
Chlorophyceae	Green algae	Starch, amylase, etc.	Chlorophyll a & b	Sexual reproduction	Marimo, Chlorella, etc.	(Fucikova, et al., 2019).
Phaeophyceae	Brown algae	Laminarian, Mannitol, etc.	Fucoxanthin	Vegetative, asexual & sexual	Sargassum, Kelp, etc.	(Li, et al., 2021).

Rhodophyceae	Red algae	Floridian starch	r-Phycoerythrin	Vegetative, asexual & sexual	Gracilaria, Rhodella, etc.	(Osako, et al., 2013).
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3. Algae Cultivation

Algal technology has the potential to combat the world's energy crisis, and stunted growth, and produce several value-added goods that are beneficial to humanity, the basis for achieving this goal is a cost-effective cultivation system, and raceway pond microalgal production appears to be the most promising, especially at a large scale where various environmental factors (cultivation system location, precipitation, radiation from the sun, etc.), technology (pond depth, CO₂ transport system, metallogenesis, etc.), the biomass production in the open pond system is impacted by biological (light, pH, oxygenation, salinity, and other factors) factors (Kumar, et al., 2015). Although many photo-bioreactors have been suggested, only a few of them can be used for the large-scale cultivation of algae, which is one of the main factors that limit the uses they have in algal cultivation in mass transfer; algae are attracting a lot of attention for the manufacturing of foods, bioactive compounds, and also for their value in cleaning the environment, so to grow as well as tap the endless possibilities of algae, efficient photo-bioreactors are required (Ugwu, et al., 2008). A growing area of research involves using algae to eliminate wastewater nutrients as a treatment option and using sewage for algal cultivation to produce biomass and bio-products; however, wastewaters come from a variety of sources and as a result, have a variety of properties, synthetic wastewaters with different nutrient profiles are frequently used, probably to normalize experimental results, algal cultivation would benefit from an in-depth knowledge of both sewage parameters and algal demands, both of which are kinetic features, this is because an algal species' ability to use minerals in any particular chemical form and at particular concentrations and proportions may vary (Monfet, et al., 2017). In essentially pure D2O, strains of the green algae *Scenedesmus obliquus*, *Chlorella vulgaris*, and *C. pyrenoidosa*, as well as the blue-green algae *Fremyella diplosiphon*, *Plectonema calothricoides*, *Phormidium luridum*, and *Synechococcus lividus*, have been mass cultured, closed leucite rocking-box units holding 5 liters of culture media (Daboll, et al., 1962). Algal bio-film technology has recently been hailed as a promising way to generate algae biomass as the fuel for the manufacture of bio-fuels; nevertheless, the carrier content right now utilized for creating algal bio-film has become either challenging to find at a low cost or unreliable, and commercializing the technology for the production of algal biomass urgently needs new and inexpensive components as bio-film recipients with high production of biomass performances (Zhang, et al., 2017). The green alga known as *Botryococcus braunii* was grown as a bio-film because this system produced the most biomass harvest concentration, 96.4 kg/m³, with a total fat amount of 26.8% by dry weight and productivity of 0.71 g/m² day, representing light to biomass production conversion rate for the energy of

2.02%; additionally, it used 45% less water than open ponds to produce a kilogram of algal biomass and 99.7% less energy to dewater it (Ozkan, et al., 2012). The most effective culture medium was the strong base medium, combined with a combination of vitamin thiamine, cyanocobalamin, and soil extract; the most favorable environmental factors for algae growth were daylight, a temperature of 25 C, and rotation at a speed of 100 rpm (Allaguvatova, et al., 2019). Algal biomass can be harvested, treated, and transformed into the desired biofuels in closed, open, and hybrid frameworks, various closed systems can be used for the cultivation of algae, including stirred tank photo-bioreactors, vertical columns photo-bioreactors, bubble column photo-bioreactors, and horizontal tubular photo-bioreactors; the kind of cultivating structure along with various factors, such as the amount of sunlight exposure, can also affect how the algae are grown (Mahmood, et al., 2022). Microalgae and bacterial co-culture systems have recently come to light as an intriguing alternative that can lower the high risk of contamination related to axenic cultures and, as a result, boost biomass yield and the synthesis of active chemicals (Perkovic, et al., 2022). For determining the financial viability and long-term viability of large-scale outdoor algae production, the capacity to simulate algal productivity under changing temperatures and light conditions is essential, and different models are used i.e., Type I, Type II, and Type III, Type I models predict the rate of the photosynthesis process of the whole culture as an indicator of the actual or typical light intensity accomplishing the culture, whereas Type II models compute efficiency as a total of local productivity inside the medium of cultivation broth (depending on the light magnitude locally encountered by individual cells), without taking into account short light phases, while Type III models then consider account of short light cycles (Bechet, et al., 2013).

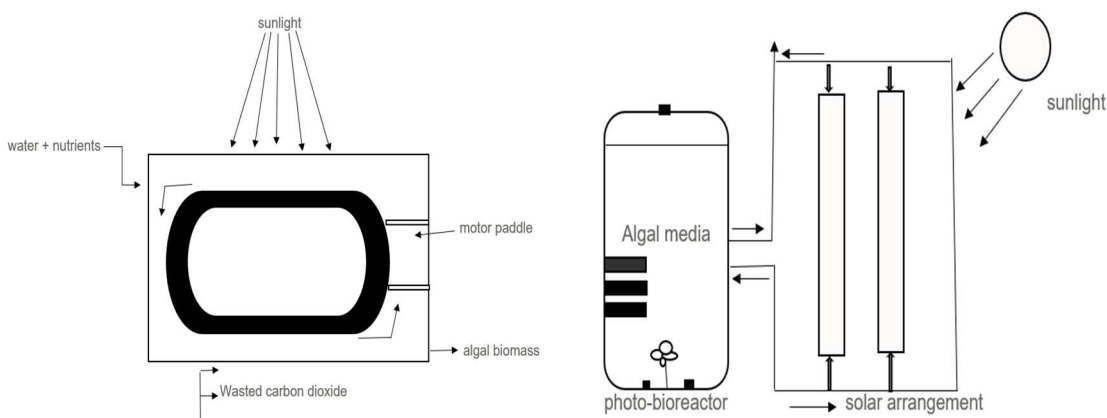


Fig. 1. Open Pond System for algal biomass cultivation and a photo-bioreactor with a horizontal tube system.

The most significant cost factors for fourth-generation bio-fuel (FGB) synthesis are the oil content and biomass yield of algal strains because genetic modification holds the key to increasing the accumulation of oil and biomass yield, which will subsequently spur economic growth (Shokravi, et al., 2022). Algae have been genetically modified to produce a wide range of industrially and commercially important compounds, such as biofuels, materials, and food products (Sebesta, et al., 2022). The recent emergence of several transgenic algal strains with altered photosynthesis,

recombinant protein production, and improved metabolism encourages the possibility of designer microalgae (Rosenberg, et al., 2008). Omics techniques are currently being developed for algae because transgenic algae have a very bright future due to their higher biomass production, carbon dioxide acceptance rate, accumulation of high-value substances, reduction in cultivation and production costs, and achievement of the target in the global algal marketplace and capital flow (Fayyaz, et al., 2020). The field of algal glycol-biotechnology was essentially created by the association of microalgal glycobiology with Omics approaches; recently, a lot of research has been done to improve the strain of different microalgae, which include *Chlorella*, *Chlamydomonas reinhardtii*, *Botryococcus braunii*, etc., employing genome sequencing and metabolic engineering with a major focus on significantly increasing the productivity of biofuels, biopolymers, pigments, and other products (Sirohi, et al., 2021).

Table 4: Advantages and disadvantages of open pond system and photo-bioreactor closed system of algal cultivation.

S no.	Phototrophic cultivation system	Advantages	Disadvantages	References
1.	Open-pond system	<ul style="list-style-type: none"> • Temperature is maintained through evaporation. • Capital cost is lower. 	<ul style="list-style-type: none"> • Dependent on daily and seasonal variations in humidity and temperature. • Monocultures are inherently difficult to maintain. • Need the most exposure to light. 	(Zhou, et al., 2021).
2.	Photo-bioreactor closed system	<ul style="list-style-type: none"> • Compared to open ponds, less water is lost. • Superior long-term maintenance of culture. • High volumetric cell densities can be 	<ul style="list-style-type: none"> • Scalability difficulties. • Since they lack evaporative cooling, they necessitate temperature maintenance. • Due to bio-film buildup, occasional 	(Arias, et al., 2017).

		supported by higher surface-to-volume ratios.	cleaning may be necessary. • Need the most exposure to light.	
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4. Development of algal bio-fuel

Bio-fuel's initial byproducts can be gaseous, liquid, or solid, these byproducts can then be further converted through biological, physical, and thermo-chemical processes to form two distinct groups: primary and secondary bio-fuel, where primary bio-fuel is made directly from igniting fibrous or cellulosic plant-based substances and dry animal waste, while secondary bio-fuel is divided into three different types that are each indirectly produced from both animal and plant materials - the earliest bio-fuels are ethanol produced from starch-rich food crops or biodiesel made from animal-derived waste products like cooking grease, the subsequent generations of bio-ethanol are produced from additional cellulosic material, and the fuel from oil-rich plant seeds like soybean or jatropha, and finally, bio-fuels of the third generation are produced from organisms like blue-green algae and microalgae, which is a particularly promising method of meeting the world's energy needs (Rodionova, et al., 2017). According to studies, algal-bacterial synergy promotes the capture of carbon in wastewater bioremediation and, as a result, results in the production of bio-fuels from algal-bacterial biomass (Yong, et al., 2021). Algal bio-fuels have thus become one of the most popular subjects in the field of renewable energy in the twenty-first century; particularly in the past ten years, in the period between 2007-2017, studies concerning algae bio-fuels encountered a dramatic, three-stage growth, rising, growing rapidly, and then decreasing. It has been suggested that future research attempts ought to concentrate on basic studies associated with algae-derived renewable energy sources and different high-value biological products for the profitable production of algal bio-fuels (Chen, et al., 2019).

Bacillus sp. strain RP1137 is a bacteria that can quickly assemble several algae that are potential sources of bio-fuel, including a *Nannochloropsis* species, the capacity of this bacteria to assemble algae in a pH-dependent, reversible way after paraformaldehyde fixation allows for the possibility of recycling the cells (Powell, et al., 2013). To maximize production and ensure that the large-scale cultivation of microalgae is both energetically and economically sustainable, it is necessary to utilize available radiation as well as possible (Simionato, et al., 2013).

4.1 Liquefaction of algal cells

As the feedstock for liquefaction is typically a wet matter that breaks down into small molecules, the biomass undergoes conversion to liquified products through an intricate series of chemical and physical alterations during the liquefaction process. A wide range of biomass, including municipal and agricultural wastes, can be partially converted into a heavy-oil product by reacting with water

as well as carbon monoxide/hydrogen with the help of sodium carbonate (Demirbas, 2010). The hydrolysis-produced micellar-like broken-down fragments undergo dehydration, which dehydrogenates oxygen deprivation and decarboxylation to break down into smaller molecules during the liquefaction process. After being created, these molecules reorganize through the polymerization process cyclization, and condensation to create new molecules (Demirbas, 2000). Both direct and indirect liquefaction are possible; direct liquefaction uses rapid pyrolysis to create fluid tars, oils, and/or condensable organic vapors, whereas indirect liquefaction uses the catalysts to turn gaseous, non-condensable byproducts of gasification or pyrolysis into liquid byproducts. Alkali salts, such as potassium carbonate and sodium carbonate, can hydrolyze hemicellulose as well as cellulose into smaller pieces. Depolymerization and deoxygenation are the primary processes that break down cellulose into smaller components (Demirbas, 2010).

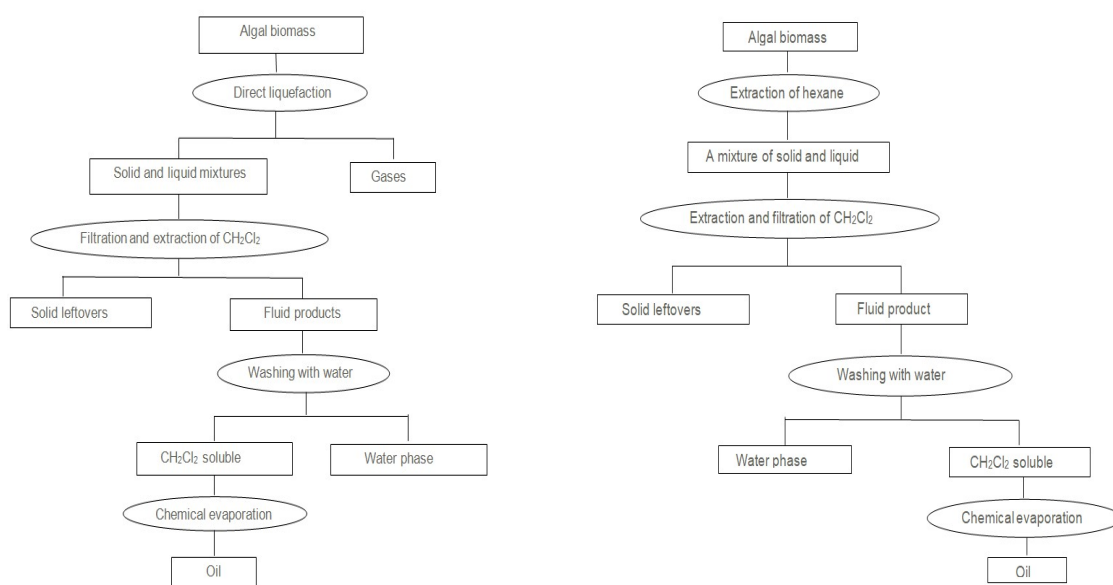


Fig. 3. Direct liquefaction of algae and extraction of oil using CH_2Cl_2 from liquefaction product and Primary oil is obtained from cells of algae by hexane extraction liquefaction.

4.2 Pyrolysis of algal cells

Pyrolysis is defined as the processes by which biomass is broken down by heat without the presence of oxygen to produce the charcoal, fluid, and gaseous products, three subcategories of pyrolysis—conventional, rapid, and flash pyrolysis (Demirbas, et. al., 2002). The chemical makeup of biomass's major constituents—cellulose, hemicelluloses, and lignin—as well as its minor constituents, which include extractives and inorganic elements, determines the biomass's pyrolytic capabilities (Shafizadeh, 1982). Hemicelluloses decompose in this process at temperatures between 470 and 530 K, cellulose does so in the range of 510 to 620 K, and lignin pyrolyzes last, at temperatures between 550 and 770 K. A low temperature, high heating range, and short gas period of residence procedure would be needed to maximize the number of liquid products produced by biomass pyrolysis; on the other hand, a low rate of the heating process would

be selected to produce a high amount of char; however, an elevated temperature, low heating and cooling rate, and lengthy gas residency time process would be more appropriate if the goal was to maximize the yield of fuel gas produced by pyrolysis (Demirbas, et al., 2002). A combination of low molecular weight, gaseous, and volatile products are produced by the anhydrous sugar compounds' splitting, water loss, dispersion, and decarboxylation reactions at even higher temperatures (Shafizadeh, 1982).

4.3 Direct Transesterification

4.3.1 Conventional transesterification process

It has proven possible to produce biodiesel by trans-esterifying microalgal cell oils through heterogeneous and homogeneous catalysis - the most popular method for producing biodiesel is homogeneous alkaline catalysis, which can catalyze the process at low temperatures and air pressure while quickly producing a high conversion yield, alkaline catalysts such as potassium hydroxide (KOH) and sodium hydroxide (NaOH) are commonly utilized; however, because microalgal oils have a high liberated fatty acid content, alkaline catalysts are not appropriate for producing microalgal biodiesel and instead stimulate the unsaturated fatty acids in oils to form soap. When the quantity of fatty acids that are free is more than 1%, acid catalysts are usually employed to overcome the drawback of high liberated fatty acid content, hydrochloric acid (HCl) and sulfuric acid (H₂SO₄) are the most commonly utilized acid catalysts. Compared with alkaline catalysts, they require greater temperatures and longer reaction durations (Hidalgo, et. al., 2013). To begin with, free fatty acid is trans-esterified into Esters using an acid catalyst. Using an alkaline catalyst, the oils go through a second transesterification stage until their free fatty acid level is less than 1%. Because heterogeneous catalysts have their benefits of recovery and reuse, it is anticipated that their usage will become more significant in the future. This is because, even with high conversion yields achieved by homogeneous catalysts, catalyst loss occurs after the reaction (Park, 2015). Metallic oxides consisting of ZrO, TiO, and Al₂O₃ concurrently esterified trans-esterified free saturated fats and triglycerides under supercritical conditions, converting algae-based oils obtained from *Dunaliella tertiolecta* and *Nannochloropsis oculata* to biodiesel and the final conversion yield was 85% (Krohn, et. al., 2011).

4.3.2 Direct transesterification process

Dry microalgal biomass has been used in several experiments to study direct transesterification processes. Methanol serves as both an esterification reagent and an extraction solvent when dry biomass combines with sulphuric acid. The facile extraction of oils from microalgal cells and the improved interaction of algae-based hydrocarbons are facilitated by the inclusion of a second solvent, which could be hexane or chloroform (Cao, et. al., 2013). *Chlorella* sp. along with *Nannochloropsis oculata* were trans-esterified at water concentrations of 0%, 1.5%, and 10% with the use of alkaline and acid catalysts (sodium hydroxide, sodium methoxide, and sulfuric acid). When used as a catalyst, sulfuric acid increased the FAME yield by seventy-three percent for

Nannochloropsis oculata and ninety-two percent for *Chlorella* sp. The yield was not affected by the biomass's salinity and declined as moisture increased (Velasquez-Orta, et. al., 2013). For direct transesterification, a high level of methanol and sulfuric acid is required compared with a commercial biodiesel process, the amount of methanol and sulfuric acid should be reduced to avoid the need for a large reactor and reactor corrosion by sulfuric acid; solvents such as pentane and diethyl ether have been used to reduce the volume of methanol by enhancing the reaction yield. These solvents assist in the extraction of microalgal oils in conjugation with methanol by improving the diffusion of the microalgal oils across the cell walls. This is facilitated by increasing the selectivity and solubility of the extraction media, thereby providing greater availability of the oils for the transesterification process (Ehimen, 2012).

5. Conclusion:-

This review investigated categorization and algal production technologies, including open pond systems, photo-bioreactor closed systems, and genetic engineering, or hybrid systems. Also covered were various applications, such as food, fuel & energy, combustion, and the development and use of algal biofuel.

Algae belong to the plants with the highest rate of growth, and nearly half of their total weight is made up of oil. Microalgae grow far more quickly than terrestrial crops do. The oil yield from algae is thought to range from 20,000 to 80,000 per acre per year, which is 7–31 times greater than the yield from palm oil, the next best crop. Algae are very significant as a biomass source, and they will eventually be competitive as a source of biofuel. Various kinds of algae may be better suited for various kinds of fuels. Industrial reactors for algal cultivation include open ponds, photo-bioreactors, and closed systems. Algae can be cultivated practically anywhere, including in salt water and sewage, and it doesn't need fertile soil to grow food crops. Processing algae also uses less energy than algae itself.

The majority of the most recent oil extraction research is concentrated on microalgae to create biodiesel from algal oil. Algal oil may be converted into biodiesel with the same efficiency as oil from crops grown on land. Shortly, algae biomass may be crucial in resolving the conflict between the manufacturing of food and biofuels.

It has been reviewed how microalgae are converted into energy via thermochemical, chemical, and biological mechanisms. Bio-oil, biodiesel, and ethanol, as well as methane and hydrogen, are the products of energy conversion via thermochemical, chemical, and biological processes, respectively.

6. References:-

(1) Anto, S., Mukherjee, S. S., Muthappa, R., Mathimani, T., Deviram, G., Kumar, S. S., Verma, T. N., & Pugazhendhi, A. (2020). "Algae as green energy reserve: Technological outlook on biofuel production". *Chemosphere*, 242, 125079.

- (2) Menetrez, M.Y., (2012). "An overview of Algae Biofuel production and potential environmental impact". *Environmental science and technology*, 46, 7073-7085.
- (3) Adeniyi, O.M., Azimov, U., Burluka, A., (2018). "Algal Biofuel: current status and future applications". *Renewable and sustainable energy reviews*, 90, 316-335.
- (4) Guiry, M.D., (2012). "How many species of algae are there?". *Journal of Phycology*, 48(5).
- (5) Pittman, J.K., Dean, A P., Osubdeko, O., (2011). "The potential of sustainable algal biofuel production using wastewater resources". *Bioresource Technology*, 102(1), 17-25.
- (6) Demirbas, A., (2010). "Use of algae as biofuel sources". *Energy conversion and management*, 51(12), 2738-2749.
- (7) Hannon, M., Impel, J., Tran, M., Rasala, B., Mayfield, S., (2010). "Biofuels from Algae: challenges and potential". *Biofuels*, 1(5), 763-784.
- (8) Adenle, A.A., Haslam, E., G., Lee, L. (2013). "Global assessment of research and development for algae biofuel production and its potential role for sustainable development in developing countries". *Energy policy*, 61, 182-195.
- (9) Ruffing, A. M., Davis, R. W., & Lane, T. W. (2022). "Advances in engineering algae for biofuel production". *Current opinion in biotechnology*, 78, 102830.
- (10) Chia, S. R., Nomanbhay, S. B. H. M., Chew, K. W., Munawaroh, H. S. H., Shamsuddin, A. H., & Show, P. L. (2022). "Algae as potential feedstock for various bioenergy production". *Chemosphere*, 287(Pt 1), 131944.
- (11) Zhang, W., Zhao, Y., Cui, B., Wang, H., & Liu, T. (2016). "Evaluation of filamentous green algae as feedstocks for biofuel production". *Bioresource Technology*, 220, 407–413
- (12) Yao, S., Lyu, S., An, Y., Lu, J., Gjermansen, C., & Schramm, A. (2019). "Microalgae-bacteria symbiosis in microalgal growth and biofuel production: a review". *Journal of applied microbiology*, 126(2), 359–368.
- (13) Singh, A. K., & Singh, M. P. (2014). "Importance of algae as a potential source of biofuel". *Cellular and molecular biology (Noisy-le-Grand, France)*, 60(5), 106–109.
- (14) Puri, M., Gupta, A., McKinnon, R. A., & Abraham, R. E. (2022). "Marine bioactive: from energy to nutrition". *Trends in biotechnology*, 40(3), 271–280.
- (15) Medipally, S. R., Yusoff, F. M., Banerjee, S., & Shariff, M. (2015). "Microalgae as sustainable renewable energy feedstock for biofuel production". *BioMed research international*, 2015, 519513.

- (16) Vo Hoang Nhat, P., Ngo, H. H., Guo, W. S., Chang, S. W., Nguyen, D. D., Nguyen, P. D., Bui, X. T., Zhang, X. B., & Guo, J. B. (2018). "Can algae-based technologies be an affordable green process for biofuel production and wastewater remediation?". *Bioresource Technology*, 256, 491–501.
- (17) Razeghifard R. (2013). "Algal biofuels". *Photosynthesis Research*, 117(1-3), 207–219.
- (18) Leong, Y. K., Chew, K. W., Chen, W. H., Chang, J. S., & Show, P. L. (2021). "Reuniting the Biogeochemistry of Algae for a Low-Carbon Circular Bioeconomy". *Trends in plant science*, 26(7), 729–740.
- (19) Park, S., Van Ginkel, S. W., Pradeep, P., Igou, T., Yi, C., Snell, T., & Chen, Y. (2016). "The Selective Use of Hypochlorite to Prevent Pond Crashes for Algae-Biofuel Production". *Water environment research: a research publication of the Water Environment Federation*, 88(1), 70–78.
- (20) Duffy, J. E., Canuel, E. A., Adey, W., & Swaddle, J. P. (2009). "Biofuels: algae". *Science (New York, N.Y.)*, 326(5958), 1345–1346.
- (21) Chang, J. S., Yang, J. W., Lee, D. J., & Hallenbeck, P. C. (2015). Editorial. "Advances in biofuels and chemicals from algae". *Bioresource Technology*, 184, 1.
- (22) Karimi, Z., Laughinghouse, H. D., 4th, Davis, V. A., & Blersch, D. M. (2021). "Substrate properties as controlling parameters in attached algal cultivation". *Applied microbiology and biotechnology*, 105(5), 1823–1835.
- (23) Ghasemi, Y., Rasoul-Amini, S., Naseri, A. T., Montazeri-Najafabady, N., Mobasher, M. A., & Dabbagh, F. (2012). "Microalgae biofuel potentials (review)". *Prikladnaia biokhimiia i mikrobiologiia*, 48(2), 150–168.
- (24) Zhou, X., Ge, H., Xia, L., Zhang, D., & Hu, C. (2013). "Evaluation of oil-producing algae as potential biodiesel feedstock". *Bioresource Technology*, 134, 24–29.
- (25) Tu, Q., Lu, M., Thiansathit, W., & Keener, T. C. (2016). "Review of Water Consumption and Water Conservation Technologies in the Algal Biofuel Process". *Water environment research: a research publication of the Water Environment Federation*, 88(1), 21–28.
- (26) Zhou, Y., Liu, L., Li, M., & Hu, C. (2022). "Algal biomass valorization to high-value chemicals and bioproducts: Recent advances, opportunities, and challenges". *Bioresource technology*, 344(Pt B), 126371.
- (27) Ramachandran, K., Suganya, T., Gandhi, N. N., & Renganathan, S. (2013). Recent developments for biodiesel production by ultrasonic assist transesterification using different heterogeneous catalyst: A review. *Renewable and Sustainable Energy Reviews*, 22, 410-418.

- (28) González Fernández, L. A., Castillo Ramos, V., Sánchez Polo, M., & Medellín Castillo, N. A. (2023). "Fundamentals in applications of algae biomass: A review". *Journal of Environmental Management*, 338, 117830.
- (29) Show, K. Y., Lee, D. J., & Chang, J. S. (2013). "Algal biomass dehydration". *Bioresource Technology*, 135, 720–729.
- (30) Catone, C. M., Ripa, M., Geremia, E., & Ulgiati, S. (2021). "Bio-products from algae-based biorefinery on wastewater: A review". *Journal of Environmental Management*, 293, 112792.
- (31) Gololobova, M. A., & Belyakova, G. A. (2022). "Position of Algae on the Tree of Life". *Doklady Biological Sciences: Proceedings of the Academy of Sciences of the USSR, Biological Sciences sections*, 507(1), 312–326.
- (32) Zhou, H., & von Schwartzberg, K. (2020). "Zygnematophyceae: from living algae collections to the establishment of future models". *Journal of experimental botany*, 71(11), 3296–3304.
- (33) Cremen, M. C. M., Leliaert, F., West, J., Lam, D. W., Shimada, S., Lopez-Bautista, J. M., & Verbruggen, H. (2019). "Reassessment of Bryopsidales (Chlorophyta) classification based on chloroplast phylogenomic analyses". *Molecular phylogenetics and evolution*, 130, 397–405.
- (34) Barcytė, D., Zátopková, M., Němcová, Y., Richtář, M., Yurchenko, T., Jaške, K., Fawley, K. P., Škaloud, P., Ševčíková, T., Fawley, M. W., & Eliáš, M. (2022). "Redefining Chlorobotryaceae as one of the principals and most diverse lineages of eustigmatophyte algae". *Molecular phylogenetics and evolution*, 177, 107607.
- (35) Hallmann A. (2011). "Evolution of reproductive development in the volvocine algae". *Sexual plant reproduction*, 24(2), 97–112.
- (36) Petroll, R., Schreiber, M., Finke, H., Cock, J. M., Gould, S. B., & Rensing, S. A. (2021). "Signatures of Transcription Factor Evolution and the Secondary Gain of Red Algae Complexity". *Genes*, 12(7), 1055.
- (37) Chacón, J., & Gottschling, M. (2020). "Dawn of the dinophytes: A first attempt to date origin and diversification of harmful algae". *Harmful algae*, 97, 101871.
- (38) Amaral, R., Fawley, K. P., Němcová, Y., Ševčíková, T., Lukešová, A., Fawley, M. W., Santos, L. M. A., & Eliáš, M. (2020). "Toward Modern Classification of Eustigmatophytes, Including the Description of Neomonodaceae Fam. Nov. and Three New Genera". *Journal of phycology*, 56(3), 630–648.
- (39) Haselkorn R. (2009). "Cyanobacteria". *Current biology: CB*, 19(7), R277–R278.

- (40) Fučíková, K., Lewis, P. O., Neupane, S., Karol, K. G., & Lewis, L. A. (2019). "Order, please! Uncertainty in the ordinal-level classification of Chlorophyceae". *PeerJ*, 7, e6899.
- (41) Li, Y., Zheng, Y., Zhang, Y., Yang, Y., Wang, P., Imre, B., Wong, A. C. Y., Hsieh, Y. S. Y., & Wang, D. (2021). "Brown Algae Carbohydrates: Structures, Pharmaceutical Properties, and Research Challenges". *Marine drugs*, 19(11), 620.
- (42) Osako, K., & Teixeira, V. L. (2013). "Natural products from marine algae of the genus *Osmundaria* (Rhodophyceae, Ceramiales)". *Natural product communications*, 8(4), 533–538.
- (43) Kumar, K., Mishra, S. K., Shrivastav, A., Park, M. S., & Yang, J. W. (2015). "Recent trends in the mass cultivation of algae in raceway ponds". *Renewable and Sustainable Energy Reviews*, 51, 875-885.
- (44) Ugwu, C. U., Aoyagi, H., & Uchiyama, H. (2008). "Photobioreactors for mass cultivation of algae". *Bioresource Technology*, 99(10), 4021-4028.
- (45) Monfet, E., & Unc, A. (2017). "Defining wastewaters used for cultivation of algae". *Algal Research*, 24, 520-526.
- (46) Daboll, H. F., Crespi, H. L., & Katz, J. J. (1962). "Mass cultivation of algae in pure heavy water". *Biotechnology and Bioengineering*, 4(3), 281-297.
- (47) Zhang, Q., Liu, C., Li, Y., Yu, Z., Chen, Z., Ye, T., Wang, X., Hu, Z., Liu, S., Xiao, B. & Jin, S. (2017). "Cultivation of algal biofilm using different lignocellulosic materials as carriers". *Biotechnology for biofuels*, 10(1), 1-16.
- (48) Ozkan, A., Kinney, K., Katz, L., & Berberoglu, H. "Reduction of water and energy requirement of algae cultivation using an algae cultivation using an algae biofilm photobioreactor". *Bioresource Technology*, 114, 542-548.
- (49) Allaguvatova, R., Myasina, Y., Zakharenko, V., & Gaysina, L. (2019, November). "A simple method for the cultivation of algae *Chlorella vulgaris* Beijerinck". In *IOP Conference Series: Earth and Environmental Science* (Vol. 390, No. 1, p. 012020). IOP Publishing.
- (50) Mahmood, T., Hussain, N., Shahbaz, A., Mulla, S. I., Iqbal, H. M. N., & Bilal, M. (2022). "Sustainable production of biofuels from the algae-derived biomass". *Bioprocess and biosystems engineering*, 10.1007/s00449-022-02796-8. Advanced online publication.
- (51) Perković, L., Djedović, E., Vujović, T., Baković, M., Paradžik, T., & Čož-Rakovac, R. (2022). "Biotechnological Enhancement of Probiotics through Co-Cultivation with Algae: Future or a Trend?". *Marine drugs*, 20(2), 142.

- (52) Béchet, Q., Shilton, A., & Guieysse, B. (2013). "Modeling the effects of light and temperature on algae growth: state of the art and critical assessment for productivity prediction during outdoor cultivation". *Biotechnology advances*, 31(8), 1648–1663.
- (53) Shokravi, H., Heidarrezaei, M., Shokravi, Z., Ong, H. C., Lau, W. J., Din, M. F. M., & Ismail, A. F. (2022). "Fourth generation biofuel from genetically modified algal biomass for bioeconomic development". *Journal of Biotechnology*, 360, 23–36.
- (54) Sebesta, J., Xiong, W., Guarnieri, M. T., & Yu, J. (2022). "Biocontainment of Genetically Engineered Algae". *Frontiers in plant science*, 13, 839446.
- (55) Rosenberg, J. N., Oyler, G. A., Wilkinson, L., & Betenbaugh, M. J. (2008). "A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution". *Current opinion in biotechnology*, 19(5), 430–436.
- (56) Fayyaz, M., Chew, K. W., Show, P. L., Ling, T. C., Ng, I. S., & Chang, J. S. (2020). "Genetic engineering of microalgae for enhanced biorefinery capabilities". *Biotechnology advances*, 43, 107554.
- (57) Sirohi, R., Joun, J., Choi, H. I., Gaur, V. K., & Sim, S. J. (2021). "Algal glycobiochemistry: omics approaches for strain improvement". *Microbial cell factories*, 20(1), 163.
- (58) Zhou, Z., Li, Q., Song, K., Wang, R., Wen, S., Zhang, D., & Cong, W. (2021). "Exploration of applying growth-promotion bacteria of *Chlorella sorokiniana* to open cultivation systems". *Bioprocess and biosystems engineering*, 44(7), 1567–1576.
- (59) Arias, D. M., Uggetti, E., García-Galán, M. J., & García, J. (2017). "Cultivation and selection of cyanobacteria in a closed photobioreactor used for secondary effluent and digestate treatment". *The Science of the total environment*, 587-588, 157–167.
- (60) Rodionova, M. V., Poudyal, R. S., Tiwari, I., Voloshin, R. A., Zharmukhamedov, S. K., Nam, H. G., ... & Allakhverdiev, S. I. (2017). Biofuel production: challenges and opportunities. *International Journal of Hydrogen Energy*, 42(12), 8450-8461.
- (61) Yong, J. J. J. Y., Chew, K. W., Khoo, K. S., Show, P. L., & Chang, J. S. (2021). "Prospects and development of algal-bacterial biotechnology in environmental management and protection". *Biotechnology advances*, 47, 107684.
- (62) Chen, H., Li, T., & Wang, Q. (2019). "Ten years of algal biofuel and bioproducts: gains and pains". *Planta*, 249(1), 195–219.
- (63) Powell, R. J., & Hill, R. T. (2013). "Rapid aggregation of biofuel-producing algae by the bacterium *Bacillus* sp. strain RP1137". *Applied and environmental microbiology*, 79(19), 6093–6101.

- (64) Simionato, D., Basso, S., Giacometti, G. M., & Morosinotto, T. (2013). "Optimization of light use efficiency for biofuel production in algae". *Biophysical chemistry*, 182, 71–78.
- (65) Demirbaş, A. (2000). Mechanisms of liquefaction and pyrolysis reactions of biomass. *Energy conversion and management*, 41(6), 633-646.
- (66) Demirbas, A., & Arin, G. (2002). An overview of biomass pyrolysis. *Energy sources*, 24(5), 471-482.
- (67) Shafizadeh, F. (1982). Introduction to pyrolysis of biomass. *Journal of analytical and applied pyrolysis*, 3(4), 283-305.
- (68) Hidalgo, P., Toro, C., Ciudad, G., Navia, R., (2013). Advances in direct transesterification of microalgal biomass for biodiesel production. *Review Environmental Science Biotechnology*, 12, 179-199.
- (69) Park, J. Y., Park, M. S., Lee, Y. C., & Yang, J. W. (2015). Advances in direct transesterification of algal oils from wet biomass. *Bioresource technology*, 184, 267-275.
- (70) Krohn, B.J., McNeff, C.V., Yan, B., Nowlan, D., (2011). Production of algae-based biodiesel using the continuous catalytic Mcgyan Processes *Bioresource Technology*, 102, 94-100.
- (71) Cao, H., Zhang, Z., Wu, X., Miao, X., (2013). Direct biodiesel production from wet microalgae biomass of *Chlorella pyrenoidosa* through in situ transesterification. *MioMed Res. Int.* 1-6.
- (72) Velasquez-Orta, S.B., Lee, J.G.M., Harvey, A.P., (2013). Evaluation of FAME production from wet marine and freshwater microalgae by in situ transesterification. *Biochemistry Engineering Journal*, 76, 83-89.
- (73) Ehimen, E.A., Sun, Z., Carrington, G.C., (2012). Use of ultrasound and co-solvents to improve the in-situ transesterification of microalgae biomass. *Procedia Environmental Science*. 15, 47-55.